NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

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FY 1986 ANNUAL REPORT OF INTRAMURAL RESEARCH PROGRAMS

October 1, 1985 through September 30, 1986

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As this Annual Report is in preparation, the National Institutes of Health is preparing to celebrate its Centennial, and simultaneous with its participation in the observance of the 100th year of NIH's service, the National Institute of Environmental Health Sciences will observe the twentieth anniversary of NIEHS' establishment as a division within NIH, November 1, 1966. Appropriately, as the Institute looks back on its productive early years, it will also take careful account of its present and its future, through a special symposium December 2 and 3, 1986, which will feature scientific presentations and poster sessions highlighting our ongoing research.

In 1986, the Report of the Third Task Force for Research Planning in Environmental Health Sciences was published in book form, titled Human Health and the Environment: Some Research Needs. This task force study by more than 85 science, medical and public health professionals, reflects the development of the NIEHS, lending a sharpened sense of its potential and direction as a force in the environmental health science field as we approach the Twenty-first Century.

In short, the NIEHS role is a unique and special one, serving as the principal federal agency for biomedical research on the health effects of environmental agents, supplying to regulatory agencies and other public and private institutions and organizations the data of basic science that discerns and interprets the mechanisms by which toxic agents act at the molecular and cellular level to give rise to disease. Especially important are studies related to longterm, low-dose exposures that may trigger diseases over years or decades, and may act only in combination with other exposures or upon certain genetically vulnerable individuals. Over its brief 20-year existence, NIEHS has been able to play a central leadership role in establishing and expanding the significant knowledge base upon which private industry, regulators, the medical community and public health officials depend in making decisions.

As well as playing its integral role within the NIH structure, NIEHS serves as one key agency within the Department of Health and Human Services' National Toxicology Program, coordinating closely with the Centers for Disease Control's National Institute for Occupational Safety and Health (NIOSH) and the Food and Drug Administration's, National Center for Toxicological Research (NCTR). Heads of four major health regulatory agencies -- the Environmental Protection Agency, the Occupational Safety and Health Administration, the Consumer Product Safety Commission, and the Food and Drug Administration -- serve on the NTP Executive Committee with heads of key research agencies -- the National Institutes of Health, the National Cancer Institute, NIOSH, and NIEHS. The forum provided by the NTP Executive Committee provides a close communications link between regulatory agencies and NIEHS. Hence, while maintaining a rigorous program at the frontiers of basic research, the NIEHS is also in tune with those environmental health science questions and needs that are of immediate concern to public health and appropriate regulatory and research agencies.

This application of state-of-the-art science to real-life problems is well-illustrated by the inhalation studies recently performed at the NIEHS facilities. The toxicity of methyl isocyanate, the chemical responsible for the tragedy in Bhopal, India, was comprehensively studied in laboratory animals, and

the results were provided to medical staffs in India treating survivors of the accident. Scientists from NIEHS went to India in February 1986 to acquaint the Indian government and concerned scientists there with the results of these studies. In addition, in March 1986, the Institute sponsored a conference which brought together investigators from NIEHS and other research facilities in the U.S. and Canada, at which studies on the effects of MIC in animals were presented. The Institute's MIC studies were also presented at the annual national meeting of the Society of Toxicology in March 1986.

Although budget constraints may not always allow such prompt responses to emergent environmental questions, the Institute strives to anticipate the best opportunities for scientific advancement, and to utilize these in our ongoing programs. An example of this is our planned expansion of our Nuclear Magnetic Resonance facilities. NMR provides a technology that is unique in its applications for spectroscopy as well as imaging of organs/lesions in vivo. NMR spectroscopy allows the investigator to determine the presence and level of a particular chemical in specified organ tissues of a living animal, without surgery, thus enabling researchers to obtain unprecedentedly precise data on the uptake and metabolism of toxins. Also, NMR technology coupled with imaging techniques can provide exquisitely detailed images of lesions such as tumors, which allow one to monitor the development, progression or regression in a living animal over an extended period of time. Previously this data was available only by sacrificing groups of animals periodically during the course of an experiment. Therefore, in addition to enhancing the understanding of the biology of a given lesion, NMR technology may mean fewer animals will be needed in studies.

The Institute has achieved the critical mass of scientific expertise, and has established the specificity of focus to continue its crucial work in studying the mechanisms at the cellular and molecular level by which contaminants promote and/or cause disease. The tools of genetics, immunology, biostatistics, pharmacokinetics, and related fields extend the research of investigators almost daily. Program directors at the Institute encourage an active exchange of information and views between the programs, laboratories, and branches to take full advantage of the rapidly widening horizons of technology and science.

On this the Centennial birthday of NIH, and the twentieth anniversary of the founding of NIEHS as a Division within NIH, it can truly be said that biomedical research in the United States is one of the crowning achievements of our national life, and as an international endeavor is one of the great attainments of human civilization. This heartening view can give scientists and other research professionals encouragement as they confront the difficult challenges of the future.

OFFICE OF THE ASSOCIATE DIRECTOR FOR GENETICS
Summary Statement

During FY 1986 the Office of the Associate Director for Genetics (OADG) continued to fulfill its role in the Genetic Toxicology programs of the Institute by serving as an expert consultant to the Director and the intramural research staff and by developing programs in the areas of genetics and environmental mutagenesis. The OADG has provided a focal point as well as planning and coordination functions in a number of areas of genetic toxicology including (1) international programs, (2) national programs, (3) committees, (4) collaborative studies and (5) collaborative research programs.

International Programs

US-Japan

The Associate Director for Genetics (ADG) is Chairman of the U.S. Panel on Environmental Mutagenesis and Carcinogenesis in the U.S.-Japan Cooperative Medical Science Program. The 13th Joint Conference of the Panel was held at N.I.E.H.S., Research Triangle Park, North Carolina, October 21-23, 1985, on the topic "The Development and Utilization of In-Vivo Systems". The 14th Joint Conference was held in Nikko, Japan, May 27-30, 1986, and addressed "Deployment of Short-Term Tests for Environmental Mutagens and Carcinogens.

ICPEMC

The Associate Director for Genetics attended the 17th Commission meeting of the International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) held in Thonon, France, October 14-17, 1985, to review the work of the various Task Groups and Committees as well as to identify future projects and activities.

5th International Conference on Environmental Mutagens

As chairman of the organizing committee, the ADG presided over a meeting in Cleveland, Ohio, February 24-25, 1986, to advance plans for the Conference and Satellite meetings which are to be held in 1989. An additional meeting was held in Cleveland, Ohio, June 23-25, 1986, in order to evaluate the facilities at the site of the Conference.

National Programs

EPA Gene-Tox Program

The ADG has participated in several periodically-held meetings of the Coordinating Committee during the second phase of the program. The purpose of this phase is for the various Assessment Panels to evaluate the utility of the various test systems, to cross-index the data and to make recommendations for appropriate batteries of tests for mass screening. The Coordinating Committee reviews the reports of the Panels and reviews the feasibility of panel activities in terms of the computerized data base.

Collaborative Studies

WHO-International Program for Chemical Safety

The ADG is chairman of a working group of the International Program for Chemical Safety (IPCS) sponsored by the World Health Organization, the United Nations Environmental Program and International Labor Organization. The Report of the IPCS Collaborative Study on *in vivo* assays is in the final stages of preparation for publication. The ADG is one of the editors.

Collaborative Research Programs

Illinois State University

The data generated during the period that the contract was in force are being utilized as the basis for scientific reports which continue to be prepared for publication.

Public Lectures

F. J. de Serres

1. 17th Annual Meeting of Environmental Mutagen Society, Baltimore, Maryland, April 9-13, 1986, "Heterozygous Loci in Two-component Heterokaryons of Neurospora Mimic Heterozygous Loci of Mammalian Cells Thus Permitting the Recovery of Both Point Mutations and Multilocus Deletions."

2. 17th Annual Meeting of Environmental Mutagen Society, Baltimore, Maryland, April 9-13, 1986, "The Spectrum of AD-3 Mutations Induced By Methyl Methanesulfonate in the Nucleotide Repair-Deficient Two-component Heterokaryon H59 of *Neurospora crassa* is Different from that Induced in the "Wild Type" Repair-Proficient Heterokaryon H12."

3. 191st Meeting of the American Chemical Society, New York, New York, April 13-18, 1986, "New Approaches for the Use of Short-Term Tests for Genotoxicity to Evaluate Mutagenic and Carcinogenic Potential."

4. Workshop "Ecotoxicology for Illinois", Urbana, Illinois, May 15-16, 1986, "Use of Short-Term Tests for Genotoxicity to Evaluate Mutagenic and Carcinogenic Potential of Environmental Chemicals."

5. 14th Joint Conference of the US-Japan Panel on Environmental Mutagenesis and Carcinogenesis, Nikko, Japan, May 27-30, 1986, "New Approaches for the Use of Short-Term Tests for Genotoxicity for Evaluation of the Mutagenic and Carcinogenic Potential of Environmental Chemicals."

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

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

Cooperation with the World Health Organization (WHO)

NIEHS has been designated by WHO as a Collaborating Center for Environmental Health Effects since 1975. As a Collaborating Center, NIEHS provides advice and scientific expertise to WHO headquarters and WHO Regional Offices, and assists them in formulating research programs related to the biomedical aspects of environmental pollution. During FY 1986, the Director, NIEHS, attended a special consultation to meet with the Director-General of WHO to discuss WHO's role and issues in environmental health and chemical safety.

In 1979, WHO established the International Programme on Chemical Safety (IPCS) a cooperative undertaking involving WHO, the United Nations Environmental Programme, the International Labor Organization, and their Member States. In October 1980, a cooperative agreement was signed between NIEHS and WHO, and NIEHS assumed the function of a Participating Institution (PI) within the IPCS for such activities as international evaluation of the biological effects of chemicals and health hazard assessments, and review and/or validation of methods for testing of mutagenicity, carcinogenicity, neurobehavioral toxicity, and toxicity to reproductive function. In September 1983 the Agreement was extended for another three years. A WHO Interregional Research Unit (IRRU), housed at NIEHS was established in 1981 to assist the Central Unit established at WHO headquarters in coordinating the activities of the IPCS/PI's. Since the inception of the Programme, numerous scientific experts from NIEHS have participated on IPCS committees, special consultations, conferences, and technical working groups. The Director, NIEHS, serves as a member of the IPCS Programme Advisory Committee (PAC) and chaired the PAC during the Programme's first two years of operation. This Committee, composed of members designated by the Director-General of WHO, is the general oversight body providing advice on the policies and priorities of the IPCS. A number of NIEHS scientists continue to play a leading role in the IPCS Collaborative Study on Short-Term Tests for Genotoxicity and Carcinogenicity. Institute scientists participated in the preparation of the monographs on (1) Principles and Methods for Evaluating the Neurotoxicity of Chemicals; (2) Toxicokinetics; (3) Principles and Methods for Evaluating the Toxicity of Chemicals - Part II; (4) Manual on Epidemiological Methods; and (5) Approaches Needed to Establish the Role of Chemical Agents in the Etiopathogenesis of Certain Non-Communicable Diseases. Also, during 1986, NIEHS staff continued to review IPCS criteria documents, working papers, and proposed projects, and the Deputy Director, NIEHS, chaired several working group meetings to review the draft IPCS criteria document on cadmium.

The objectives of the IPCS are: (1) to encourage international cooperation in the evaluation of the effects of chemicals on human health and on the quality of the environment; (2) to coordinate chemical testing and toxicological research to eliminate unnecessary duplication of efforts; (3) to develop international protocols for laboratory testing, epidemiological studies, and

risk assessment; (4) to develop international guidelines and exposure limits for chemicals in air, water, and food and limits for hazardous chemicals in workplaces; (5) to develop response mechanisms for coping with chemical emergencies which may be international in scope; and (6) to promote training and development of manpower in areas of specialties necessary for the achievement of program goals. The IPCS provides an effective multilateral mechanism for dealing with global environmental health issues and avoiding costly duplication of national efforts to test and assess chemicals.

During FY 1986, NIEHS staff also participated in the International Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) dealing with methods for the assessment of exposure to chemicals of both human and non-human biota. SGOMSEC is an IPCS activity sponsored jointly with the Scientific Committee on Problems of the Environment of the International Council of Scientific Unions.

NIEHS also collaborates with the WHO International Agency for Research on Cancer (IARC). Collaborative efforts include the establishment of a registry of workers exposed to particular pesticides which contain dioxin contaminants. During FY 1986, scientists from NIEHS and the U.S. national Toxicology Program (NTP) participated in a number of IARC sponsored expert working groups to prepare "IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans." These monographs collect all available relevant experimental and epidemiological data about a chemical or groups of chemicals to which humans are known to be exposed, and summarize the evidence for the carcinogenicity of chemicals and other relevant information.

US-China (Mainland) Cooperation

Cooperation between the United States and the People's Republic of China in the area of environmental health was initiated during 1980 under the US-PRC Agreement for Cooperation in the Science and Technology of Medicine and Public Health. NIEHS is a participant in the topic on public health and health services research, which includes concerns relating to environmental and occupational health. Exploratory discussions between both sides have been held during exchange visits with initial discussions centering around cooperation in the following areas: reproductive and developmental toxicology, validation of short-term test methods to detect and assess carcinogens, mutagens, and teratogens in the environment; and the extrapolation of laboratory animal data to man. NIEHS scientists have given research seminars at various institutions in the People's Republic of China, and have hosted the visits of numerous Chinese scientists to discuss research programs of mutual interest.

US-China (Taiwan) Cooperation

Collaborative studies between U.S. and Taiwanese scientists are carried out through a Cooperative Program in the Biomedical Sciences between the American Institute in Taiwan and the Coordination Council for North American Affairs. For the past four years, NIEHS scientists from the Biometry and Risk Assessment Program have collaborated with Taiwanese scientists in studies investigating the effects of accidental human exposure to polychlorinated biphenyls (PCBs) in Taiwan. Studies on the effects of PCB exposure on enzymatic activity in human tissues suggest a potential for substantial and

persistent effects of these pollutants on human metabolism. NIEHS epidemiologists in collaboration with Taiwanese scientists are conducting a clinical evaluation of a cohort of children exposed transplacentally to PCBs and their thermal degradation products.

US-Egypt Cooperation

Cooperation between American and Egyptian environmental health scientists is being carried out under the auspices of a U.S.-Egypt Joint Working Group on Health Cooperation (JWGHC), supported by the U.S. Agency for International Development. NIEHS has been assigned responsibility for the U.S. Subcommittee on Environmental and Occupational Health of the JWGHC. Efforts are continuing in the establishment of an Information Unit for Environmental Impact Assessment. This Information Unit, located at the Environmental Health Center in Cairo, will provide the Egyptian Ministry of Health and Egyptian institutions and universities with access to U.S. information on environmental and occupational health hazards, thus strengthening the scientific base for research and regulatory decision making.

US-Finland Cooperation

A Memorandum of Understanding on Collaboration between NIEHS and the Finnish Institute of Occupational Health, Helsinki, was formalized in November 1982. Since then a number of exchange visits have taken place between U.S. and Finnish scientists to exchange information on pharmacokinetics, reproductive toxicology, neurobehavioral toxicology, genetic toxicology, epidemiology and risk assessment, and strategies for toxicological research priority settings. NIEHS and the Finnish Institute of Occupational Health also co-sponsored an "International Workshop on Occupational Hazards Caused by Polychlorinated Biphenyls and Chlorobenzenes in Capacitors and Transformers."

US-India Cooperation

NIEHS and NTP scientists are cooperating with India by conducting experimental animal studies on the toxicity of methyl isocyanate, the chemical responsible for the world's worst industrial disaster which occurred in Bhopal, India in December 1984. During FY 1986, several NIEHS scientists visited research and government organizations in India to discuss NTP's research findings on the toxicity of methyl isocyanate. NIEHS also sponsored and hosted a conference on the "Toxicity of Methyl Isocyanate" in March, 1986; and hosted the visits of a number of scientists from India to discuss the research on methyl isocyanate.

US-Italy Cooperation

Cooperation in environmental health research between American and Italian health scientists has taken place since 1977 under a Memorandum of Understanding between the U.S. Department of Health and Human Services and the Italian Ministry of Health. Since then a number of exchange visits have taken place to exchange information on the bioavailability of 2,3,7,8-tetrachloro-dibenzodioxin, studies on the chemical contamination of drinking water, chemical selection procedures, and the design of two-year toxicity studies. During FY 1986, the Director, NIEHS visited Italy to discuss potential future

US-Italy collaborative activities, and NIEHS co-sponsored two international conferences, held in Italy, on "Occupational and Environmental Significance of Industrial Carcinogens," and "Biochemical and Cellular Indices of Human Toxicity."

US-Japan Cooperation

Cooperation between American and Japanese scientists on environmental health problems takes place under two formal agreements: The US-Japan Cooperative Medical Sciences Program and the Agreement on US-Japan Cooperation in Research and Development in Science and Technology. Under the US-Japan Cooperative Medical Sciences Program, American environmental health scientists participate in the Panel on Environmental Mutagenesis and Carcinogenesis chaired by the Associate Director for Genetics, NIEHS. Joint areas of research focus on the detection of mutagenic and carcinogenic chemicals using both in vitro and in vivo test systems, and on monitoring human populations for evidence of exposure to mutagenic and carcinogenic chemicals. The Director, NIEHS, serves as a member of the Joint Committee which oversees the overall activities of the US-Japan Cooperative Medical Sciences Program. During FY 1986, NIEHS hosted the 13th US-Japan Joint Environmental Panel Conference on "The Development and Utilization of in vivo systems;" and the Associate Director for Genetics, and the Assistant to the Director for International Programs participated in the 14th US-Japan Joint Environmental Panel Conference on "Short-Term Test Method Development and Deployment," held in Nikko, Japan. Also, during 1986, the Director, NIEHS, participated in the US-Japan Joint Subcommittee Meeting on Program Review and Planning, and the 22nd US-Japan Joint Committee Meeting.

Under the US-Japan Agreement on Cooperation in Research and Development in Science and Technology, NIEHS participates in the toxicology program area in the counterpart working group on health. Cooperative activities under this agreement focus on the testing of chemicals for mutagenic, carcinogenic, and other toxic effects. In FY 1986, annual exchange visits between Japanese and NIEHS scientists continued, and both sides have shared information on test method development, which chemicals will be tested, and test results. Several test systems are being reviewed and validated in both U.S. and Japanese laboratories, and collaborative activities on oncogenes are being planned.

US-Sweden Cooperation

During FY 1986, NIEHS and the Swedish Work Environment Fund co-sponsored a "US-Sweden Collaborative Workshop in Toxicology." Particular areas of interest included genetic toxicology, neurotoxicology, and allergy/hypersensitivity.

US-USSR Cooperation

Collaboration between Soviet and American environmental health scientists is carried out under the auspices of two cooperative agreements between the United States and the Soviet Union. Under the Medical Science and Public Health Cooperative Agreement, scientists from both countries are conducting joint research on the effects of physical and chemical environmental agents on human health. 1986 was the fourteenth year of formal collaboration in environmental health research between the U.S. and U.S.S.R. Cooperative research efforts have involved formal workshops and exchange visits between

scientists of both countries. A duplicate collaborative experiment has been initiated aimed at evaluating sensitive tests for determining the biological effects of electromagnetic fields on the nervous system and to validate research results obtained in the U.S. and the Soviet Union. A large amount of scientific information has been exchanged during this collaboration.

NIEHS also participates in the US-USSR Agreement on Cooperation in the Field of Environmental Protection which is administered for the United States by the Environmental Protection Agency. The Director, NIEHS, serves as DHHS representative to the Environmental Protection Agreement and co-chairman of the working group concerned with the biological and genetic effect of pollution. Exchange visits under this Agreement have been conducted in research areas concerned with the mutagenic effects of environmental contaminants.

US-Yugoslavia Cooperation

Under the auspices of the US-Yugoslavia Joint Board for Scientific and Technological Cooperation, NIEHS scientists continued collaborative studies in 1986 on the evaluation of the genetic effects of low levels of environmental chemical mutagens in bacterial systems, and comparison with eukaryotic cells.

Interagency Coordination

A number of federal and state agencies are involved in collaborative efforts to establish integrated systems for gathering, evaluating, and disseminating information on the health and environmental effects of chemical substances. The Assistant to the Director for International programs represents NIEHS on the Subcommittee on Information Coordination of the DHHS Committee to Coordinate Environmental Health and Related Programs (CCEHRP). This committee identifies the needs and establishes the mechanisms for the collection, storage, and dissemination of toxicologic information within DHHS.

The Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA), provides for several Federal organizations to participate in a coordinated response to provide information and advice on health hazards resulting from chemicals released into the environment and from the cleanup of hazardous waste disposal sites. In order to provide for the effective coordination of the collection, development and evaluation of the information necessary to determine the potential health hazards associated with such chemicals, the DHHS Committee to Coordinate Environmental Health and Related Programs established a Hazardous Waste Information Evaluation Subcommittee (HWIES). The Assistant to the Director for International Programs represents NIEHS on this Subcommittee. The HWIES, composed of technical experts from various DHHS agencies, evaluates the available information on a number of chemicals frequently found in waste dumps and makes recommendations concerning the testing of these chemicals by the National Toxicology Program, and structured data record creation by the National Library of Medicine. The Assistant to the Director for International Programs is also a member of the Technical Advisory Committee of the Governor's Waste Management Board of the State of North Carolina.

OFFICE OF FACILITIES ENGINEERING

SUMMARY STATEMENT

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

As directed, OFE performs other mission support activities including, but not limited to, security and the design, fabrication, alteration and repair of scientific instrumentation.

Office Functional Sections:
OFE is divided into six functional, cohesive sections under the Office of the Chief.

(1) The Facilities Management Section is the coordination point for all service requests/work orders providing planning, estimating, scheduling, expediting, storage of shops materials and parts and contracts management.

(2) The Engineering Design Section (EDS) provides architectural and engineering support required for planning new facilities and improvements, major alterations and repairs to existing facilities through either in-house design or contract with Architectural/Engineering firms. EDS also administers and inspects construction performed under contract.

(3) The Facilities Operations Section operates and maintains the power plant and building mechanical systems on South Campus. The power plant houses two 40 million BTUH boilers and two 2500 ton chillers. These systems are in continuous operation and deliver the environmental control (heating, cooling, and humidity) to all South Campus facilities. The Facilities Operations Section also provides project officer support for similar operations on North Campus.

(4) The Maintenance Section provides construction, renovation, maintenance, and repair service for buildings and installed equipment. Trades include carpentry, locksmithing, masonry, plumbing, sheet metal, steamfitting, welding, painting, and electrical.

(5) The Instrumentation Section provides instrumentation fabrication services and also supports repair and maintenance of scientific equipment.

(6) The Special Projects Section coordinates planning, design, and construction of an addition to Building 101 and related site improvements and utilities systems expansion on South Campus, as well as other special design and construction projects.

Goals and Accomplishments:
On North Campus, alterations are continuing in Building 6 to house the mass

spectrometry group of the Laboratory of Molecular Biophysics (LMB). Alterations are underway in Building 2 to accommodate the Extramural Program. Construction has begun on a temporary building annex to Building 5 to house the Magnetic Resonance Imaging Group of the LMB. Renovations are underway in Building 4 to house other LMB Laboratories.

On South Campus, construction has been completed to replace the patio tile at Building 101. Construction is underway to repair faulty flashing and roofing on Building 101. Studies are underway to correct disturbing air-conditioning system sound level problems in the laboratories; and air-conditioning system control problems for the Administrative areas. Design has begun to correct some major flow problems in the high temperature hot water and chilled water distribution system on South Campus which will also provide significant energy savings. A second primary electrical feeder has been installed to assure uninterrupted electrical service to South Campus.

Other projects include additional site signage to aid delivery personnel, contractors and visitors; and the addition of a sound system in A and B Modules of Building 101 to mask or dampen conversational tones in open office areas.

OFE continues to improve and expand its automated systems. Areas affected include planning/estimating, Records of Call, work order tracking, etc.

A Program of Requirements for major additions to Building 101 and support facilities on South Campus has been completed.

Future Branch Objectives:

During Fiscal Year 1987, OFE will focus efforts in the following areas: (1) Continue alterations in buildings on North Campus, (2) Concepts predesign phase to program and support facilities (Building 101), (3) Construction of offices in Module A Basement (Building 101), (4) Installation of a new general waste incinerator in Building 105, (5) Continuation of a contract effort to provide a comprehensive preventive maintenance program for all physical facilities and equipment on South Campus, (6) Provision of additional laboratory space in the high bay area of Module E, Building 101.

The NIEHS Health and Safety Office is administratively located within the Office of the Deputy Director and has broad responsibility for chemical and radiation safety, physical safety, fire protection, emergency preparedness, environmental protection and occupational health surveillance. The Health and Safety Office conducts research studies and special investigations relative to specific safety concerns.

Toxicological research laboratories pose unique health and safety problems due to the great variety of chemicals and radioisotopes that may be used and the non-routine nature of many laboratory procedures. The primary emphasis of the NIEHS Health and Safety Office is to minimize exposures and unsafe conditions through utilization of containment equipment, following appropriate work practices and procedures and use of personal protective equipment. The primary tools for accomplishing this objective are the required hazardous agent safety protocol, employee training and programs for information dissemination.

Chemical Safety/Industrial Hygiene

Programs for safe use of hazardous chemical agents are high priority at NIEHS. There were approximately 173 active protocols for use of hazardous chemicals in force during FY85. An important component of the Health and Safety Program is routine surveys of all laboratories, shops and warehouses on a quarterly basis. These surveys serve to identify potential hazards and to initiate preventive actions as well as to maintain an awareness of potential hazards. Use of primary containment devices and other engineering control measures is the preferred means of minimizing occupational exposures. All laboratory hoods, a total of 141, are inspected and their performance measured on a quarterly basis. Information concerning deficient hoods is immediately referred to the Office of Facilities Engineering for corrective action. In addition to fume hoods, a total of 49 biological safety cabinets are in use at NIEHS. All biological safety cabinets are tested and certified annually by an independent testing firm.

During FY86 the Health and Safety Office continued to expand and improve programs for monitoring occupational exposures. Workplace air samples for evaluating a variety of potential exposures such as organic vapors, wood dust, formaldehyde and nuisance dust were collected as part of routine sampling programs and in response to specific requests. A special sampling program for evaluating exposures to acrylamide was initiated and included an initial questionnaire to identify heavy users followed by workplace sampling. Special air sampling studies were completed to evaluate wood dust exposures among animal bedding handlers. An initial industrial hygiene study of exposures experienced by animal handlers was completed in conjunction with a ongoing surveillance program for laboratory animal allergies. These samples will be further analyzed for specific animal antigens through an interagency agreement which was established with the National Institute for Occupational Safety and Health. This study will continue during FY87.

During March 1985, selected North Campus buildings were sandblasted and painted by a contractor hired by the building owner. The Health and Safety Office followed this process closely including collection of respirable dust samples inside of occupied buildings in order to evaluate potential exposures and containment. Results of this sampling were used to modify work practices used by the contractor in order to minimize exposures and potential disruption of research activities.

A quality control program for the industrial hygiene laboratory was expanded in FY86. This program includes written procedures, preventive maintenance schedules for instrumentation, calibration procedures and recordkeeping requirements. As a part of the quality control program, NIEHS applied and was accepted as a participant in the NIOSH Proficiency Analytical Testing Program (PAT) for organic solvents and asbestos. The PAT program provides a good means of quality control for analytical procedures.

The primary emphasis of NIEHS health and safety programs is exposure prevention through proper experimental design and use of laboratory containment equipment. Personnel protective equipment is used to supplement other preventive measures. A written respiratory protection program has been developed which includes initial selection criteria, qualitative fit testing, training of new users and annual maintenance checks by the Health and Safety Office. There were approximately 85 occasional users of respirators at NIEHS during FY85. In addition to qualititative fit testing, respirator users are given a complete overview of the respiratory protection program. Inspection and maintenance of respiratory protection equipment is conducted by the Health and Safety Office to insure that broken, worn, or deteriorated parts are replaced and filters and/or cartridges are changed as indicated.

Federal OSHA adopted the Hazard Communication Standard on November 25, 1983; for the purpose of ensuring that chemicals are evaluated for their hazards by chemical manufacturers and importers and that these hazards are communicated to employers and employees in the manufacturing sector. The State of North Carolina has adopted the federal standards and in addition has added significant amendments. These amendments are included in the N. C. Occupational Safety and Health Hazard Communication Standard and the N. C. Hazardous Chemicals Right to Know Act and went into effect on June 27, 1985. In compliance with these regulations NIEHS supplied the local fire marshal with a list of chemicals stored on-site in quantities greater than 55 gallons or 500 lbs. A detailed analysis of these regulations as well as options for NIEHS compliance was completed during FY86.

Radiation Protection

Use of radioisotopes and radiation sources has become an integral part of biomedical research. The Institutes' use of radioisotopes continues to increase with over 200 active protocols for the use of radioactive material. Approximately 1200 shipments of radioactive material were received in FY86. The Institutes' use of ^{32}P has continued to increase while the use of other isotopes remained about the same as in FY85.

Routine duties of the radiation protection program include monthly laboratory surveys, surveys of sealed sources, checking for contamination in cases of suspected spills, receiving and distributing incoming isotopes, calibration of radiation detection instruments, disposal of radioactive wastes, bioassay procedures, monitoring of personnel exposures and keeping an inventory of all radioisotopes at the Institute. In addition to these routine duties, special investigations are conducted to address specific issues relative to NIEHS radiation safety programs. A two year study on the fate of incinerated radionuclides was completed in FY85 and a manuscript accepted in FY86 for publication. New studies were initiated in FY85 on the dosimetry and calibration of beta emitting radioactive material and continued in FY86. An extrapolation chamber was used in conjunction with National Bureau of Standards traceable beta sources to calibrate dosimeters and radiation detection equipment. This study has been useful in evaluating exposures of personnel handling beta emitters such as ^{32}P in various laboratory procedures. In conjunction with this study a finger tip dosimeter has been developed which is both accurate and convenient. Further studies using this dosimeter are underway.

Safety and Health Training

Safety and health training is an important component of the Institutes' safety program. The Health and Safety Office offers a number of courses for laboratory personnel including "General Laboratory Safety," and "Introduction to Radiation Safety". Approximately 100 employees attend each of these courses per year. In addition to the above laboratory safety courses, routinely scheduled courses in CPR, First Aid, and " Fire Extinguisher Use" are made available to all interested NIEHS employees. The Health and Safety Office also provides other special training as necessary. Other special training provided in FY85 included instruction in proper lifting techniques for Comparative Medicine Branch personnel and use and care of compressed gas cylinders for warehouse personnel. Hazard Communication training was introduced into routine safety training meetings of the Office of Facilities Engineering personnel. In FY86 a certification program was established and implemented for forklift operators in the Material Storage and Distribution Section of the warehouse. This program consists of on-going training sessions and safety classes as well as a practical examination required every 18 months.

Safety and Health programs and training for NIEHS employees other than laboratory workers, such as shops and maintenance personnel, is an important area of emphasis. Major new programs including confined space entry and lockout/tagout procedures were developed and fully implemented during FY85. Among other requirements, the confined space program requires Health and Safety Office monitoring of all confined areas such as valve pits, electrical pits and tanks for hazardous conditions on a routine basis and before entry by maintenance personnel. Monthly safety training meetings for Office of Facilities Engineering personnel continued in FY86 covering a variety of topics.

Fire Protection/Emergency Preparedness

Fire prevention and emergency preparedness continue to receive considerable attention. As North Campus buildings were renovated in FY86 fire protection features were upgraded. The Durham County Fire Marshall began a program of annual fire inspections for all NIEHS properties. Suggestions made by the Fire Marshall were implemented. During FY86 an datailed Bomb Threat Plan was developed as a result of several bomb threats received by the Institute. The Bomb Threat Plan was incorporated into the NIEHS Occupant Emergency Plan.

Occupational Medicine Programs

The Health and Safety Office has responsibility for providing occupational health services for NIEHS employees. Services are currently provided through an interagency agreement with the PHS, Division of Federal Employee Occupational Health. Services provided include emergency treatment, periodic occupational health surveillance programs, preventive health programs, health promotion and education programs. A special project to develop a surveillance program for laboratory animal allergies was initiated during FY85 and continued in FY86. The initial implementation included a questionnaire to determine allergy prevalence among NIEHS employees and initiation of an industrial hygiene study to identify potential exposures to airborne allergens during animal handling. In FY85 an Employee Assistance Program was developed and implemented in FY86 including employee orientation sessions. This program provides employees with assistance in dealing with psychological or emotional problems potentially affecting work performance. During FY86 the decision was made to contract directly for occupational health services in FY87 and a detailed scope of work developed.

Workmen's Compensation

The Health and Safety Office is responsible for maintaining NIEHS illness and injury statistics and for managing claims submitted to the Federal Office of Workmen's Compensation Programs. During FY86 NIEHS injury and illness data for fiscal years 83-85 were analyzed. NIEHS injury and illness incidence rates were found to be approximately one third those for all private sectors and half those for all Federal programs. Results of these analyses will be used to direct future prevention programs at the Institute.

Hazardous Waste Management

The Health and Safety Office continues to seek ways of improving the Institutes' hazardous waste management programs. During FY85, the design of a new 5000 square foot waste processing facility was completed as well as an environmental impact assessment. However, it now appears that construction funds will not be available as soon as planned; therefore, alternate short

term solutions to the space problem are being sought. On possible alternative is to construct storage space for flammable chemicals in the existing warehouse thus freeing space in Building 103 for waste processing. This alternative was researched and design requirements identified. An expanded scope of work was developed for the new hazardous waste contract to be effective in FY87.

Environmental Protection

Responsibility for the Institutes' environmental protection programs reside with the Health and Safety Office. Responsibilities include Federal, State and County contact, maintaining environmental permits required by requlatory agencies for compliance, and advising other components at NIEHS on changes in requirements for regulatory compliance. During FY86 these programs received considerable attention. Protocols for a detailed environmental audits were developed and reviewed. These protocols will provide a suitable framework for evaluating and documenting compliance with the wide range of environmental regulations with which NIEHS must comply.

In order to comply with 40 CFR Part 761.40, regarding marking of PCBs and PCB items, the Health and Safety Office sampled and had analyzed the dielectric fluid in seven transformers owned and serviced by NIEHS. The remaining transformers(2) on North and South Campus are owned and serviced by Duke Power. In response to an inquiry sent to Duke Power, Duke Power indicated that both transformers were classified as "Non-PCB". In addition Duke Power acknowledged the presence of 24 capacitor cans that contain 2.7 gallons per can of PCB oil. Duke Power also accepted responsibility for the cleanup of any spills that may occur from their equipment, as well as the transportation of waste products, and the final disposal of the PCB material.

In July 1983, the Health and Safety Office completed an Industrial Waste Survey as required by the City of Durham . The survey involved identifying a list of chemical compounds that could potentially be discharged into the sanitary sewer. To ascertain whether these chemical compounds (priority pollutants which comprise the list of toxic pollutants in 40 CFR Part 401.15) were present in NIEHS waste streams in detectable quantities and to verify that NIEHS is in compliance with waste stream characteristics specified in Chapter 23 of the Durham City Code, the sanitary sewers serving North and South Campuses were monitored for a two week period. The analyses indicated that NIEHS is in compliance with Chapter 23 of the Durham City Code. In addition, no priority pollutants were found above detectable limits. Although self-monitoring is not required, self-monitoring on an annual basis is encouraged by the City of Durham. Therefore an annual sampling schedule will be implemented by the Health and Safety Office.

The U.S. Environmental Protection Agency is concerned with the health and environmental problems caused by leaking underground and above-ground storage tanks and spills associated with these tanks. The EPA requires any facility that has over 42,000 gallons of below-ground or 1,320 of above-ground oil storage capacity to prepare and implement a Spill Prevention and Countermeasure Plan (40 CFR Part 112). NIEHS exceeds the below-ground

capacity, therefore, compliance with the EPA regulation is necessary. During FY86 a spill prevention, control and countermeasure plan was prepared. The plan describes the responsibilities of each operational unit as well as procedures and actions that will be taken regarding oil operations.

In an effort to control and prevent releases from underground storage tanks, Congress passed amendments to RCRA (the Hazardous and Solid Waste Amendments of 1984). As part of this new RCRA provision, Congress included requirements for owners of underground storage tanks to notify designated state or local agencies. The notification requirements apply to underground storage tanks that contain regulated substances defined as hazardous under Superfund legislation and underground storage tanks that contain petroleum products. The notification forms for NIEHS storage tanks were completed in February of FY86 and sent to the designated state agency.

In addition to the above requirements for underground storage tanks, pending EPA legislation will require that all existing underground storage tanks be tested for integrity. While not currently required by regulations, NIEHS will complete initial testing of all underground storage tanks during FY87.

LIBRARY AND INFORMATION SERVICES OFFICE
Summary Statement

The NIEHS Library is the principal science reference resource for the Institute. Library and information services include reference services, computerized literature searching of bibliographic and scientific databases, maintenance of a collection of 700 periodical titles and 16,000 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 1,600 new books for the Library and the laboratories, and publication of a monthly newsletter and the annual bibliography of publications by NIEHS personnel. Microcomputers and large automated systems now play an integral role in every functional area in the NIEHS Library.

Reference/Literature Searching: The Library maintains one of the most advanced computerized literature searching capabilities in the world, with access to more than 600 databases covering subjects from toxicology to public administration. During FY86, Library personnel performed comprehensive multi-database searches on some 2,500 topics for Institute investigators and administrators. This was more than double the number the year before due primarily to the addition of Larry Wright to the staff as Reference Librarian. Besides online searches, 4,700 other reference questions were answered. The most heavily used online databases continued to be TOXLINE, MEDLINE, Toxicology Data Bank, Biological Abstracts, and Chemical Abstracts. Examples of search requests include the following:

- What articles have been published that discuss the benefit of animals in biomedical research? (For IRP)
- What biographical and bibliographical information can you find on Otis Bowen, the new Secretary of DHHS? (for Dr. Rall)
- Has work done at NIEHS (e.g., the NTP bioassay reports) been cited in support of regulations promulgated by other agencies? (For OPPE)
- Can you give me a bibliography on the environmental fate and toxicology of ergot alkaloids and trichothecenes? (For OD)
- What references can you find on the metabolism of chemicals by lung and the interaction of drugs in the lung? (For IRP)
- What are some articles on zoonoses in laboratory animals? (For TRTP)

The Library staff began the second year of training investigators to do their own online literature searches. The number of trainees increased from eleven to forty and the preferred database changed from TOXLINE to MEDLINE through the use of a user-friendly program called PaperChase. Now, NIEHS scientists can choose between having searches done by Library Search Analysts or doing the online searches themselves.

Journal Collection: The medium of the journal continues to be the primary means of disseminating scientific information. The Library subscribed to approximately 700 periodicals during FY86 and ordered 400 subscriptions for the various laboratories. Issues were checked in and missing ones claimed on an automated system. The Library continued to bind journals selectively or replace them with microfilm to save space. The collection now includes 20,000 journal volumes and 1,578 microfilm reels. The Library joined with a dozen other biomedical libraries to form a North Carolina Union List of Health Sciences Serials which will expedite interlibrary loans.

Book Collection: Continuing the development of the book collection, the Library ordered 1,600 books in FY86, of which 38% were ordered for the Library and 62% for the laboratories. The Library also ordered more than 1,000 government reports.

Computer Catalog: FY86 was the fourth year of operation for the C.L. Systems LIBS 100 computerized catalog and circulation system. This computer supports an online catalog of the books in the Library and in the Labs and is searchable by author, title, or subject using terminals in the Library or any terminal in the labs or offices. This makes it much easier for Institute scientists to find out what books are in the Library. The system also speeds up the check-out procedure, produces overdue notices, and provides statistical reports for management purposes. During FY86 the system was used to check-out 300 books per month. An acquisitions software module was used for book-ordering.

The Library continued using the automated cataloging system, OCLC, a computerized union catalog of books held by more than 3,500 libraries nationwide. The NIEHS Library has experienced a tremendous savings in time owing to the 95% hit rate for new books which already have cataloging data on OCLC. Through an interface, catalog records are transmitted from the OCLC computer in Ohio to the LIBS 100 computer in the NIEHS Library where they are immediately integrated into the public catalog.

Interlibrary Loan: The number of photocopy and loan requests increased by 10% in FY86, the total being 21,320. For the fourth year in a row, more of the requests were filled from the Library collection (57%) than from other libraries through interlibrary loan (43%). This reflects the ongoing improvement in the NIEHS collection. In addition, the Library made "vendacards" available which could be used by Institute employees who wanted to do their own photocopying at the UNC, Duke and N.C. State libraries. The OCLC computerized catalog also proved useful for verifying titles for interlibrary loan and for locating libraries from which to borrow books throughout the U.S. The NIEHS Library provided 575 loans or photocopies to other libraries or to individuals in the Research Triangle Park area. This 25% increase in the last two years reflects the growing importance of the NIEHS Library as a national resource.

Institute Manuscripts and Bibliography: The Library continued to maintain the NIEHS archives of manuscripts submitted for publication and to list them in the monthly newsletter. More than 750 manuscripts were written by NIEHS scientists during the year, up 50% over last year. The Library published the 1985 NIEHS Bibliography, a catalog of the papers published by Institute personnel since 1966.

Experimental Data Repository: The Library continued the project of having laboratory notebooks microfilmed for archival purposes.

Library Interns: NIEHS, through an Interagency Agreement with EPA, obtained assistance from graduate students in the UNC School of Library Science. Three students worked half-time for the NIEHS Library as interns in the one-year appointments. They performed services in cataloging and serials management, and in a new project managing the BRAP branch library.

Offices and Meetings: Dav Robertson maintained close contact with various library and information organizations in FY86. Mr. Robertson was elected President of a new organization, TRI-LIBS, the RTP Association of Librarians and Information Specialists. He also served as Secretary of the N. C. Chapter of the Special Libraries Association. He was appointed to the Networking Advisory Committee for the State Library of N. C. and was instrumental in the establishment of an automated statewide Union List of Monographs and Serials. Mr. Robertson also was appointed to the Advisory Council for the N. C. Library Staff Development Program centered at N. C. Central University. He represented NIEHS at the national meeting of the Special Libraries Association in Boston and the quarterly state chapter meetings in North Carolina. He also represented NIEHS at the C. L. Systems User Group Meeting in Baltimore.

INTRAMURAL RESEARCH PROGRAM

INTRAMURAL RESEARCH PROGRAM
Summary Statement

Scientists in the Intramural Research Program (IRP) of the National Institute of Environmental Health Sciences (NIEHS) have the primary mission of investigating the mechanisms by which our environment affects the structure/function of biological systems. The ultimate goal is to apply such information towards a better understanding of how the environment impinges on human health. In focusing on this goal, IRP scientists examine the biological basis of toxicology which includes the effects of man-made chemicals, sound and light waves, and natural products. Such efforts necessitate a broad spectrum of scientific disciplines, including biochemistry, molecular biology, pharmacology, genetics, cell biology, physics, and both organic and inorganic chemistry. Organizationally, these disciplines have been incorporated into six laboratories designated Laboratory of Behavioral and Neurological Toxicology (LBNT), Genetics (LG), Molecular Biophysics (LMB), Pharmacology (LP), Pulmonary Pathobiology (LPP), and Reproductive and Developmental Toxicology (LRDT). A separate Branch in IRP designated Comparative Medicine (CMB) is primarily a technical-administrative service function whose principal activities involve animal procurement; animal facilities management; animal health diagnosis and measurement; glassware and media service, and miscellaneous functions relating to legal and policy requirements for the use of animals. As such, CMB is an asset not only to the Intramural Program but also to the other divisions or programs in NIEHS (TRTP and BRAP). CMB has proven to be a particularly important asset because of its renowned quality of animal care which is, with few exceptions, unmatched by other scientific institutions.

The summary reports provided by the Laboratory and Branch Chiefs of IRP are but a capsule overview of the substantive research efforts. In broad terms the research tends toward understanding the mechanisms that govern the regulation of cellular metabolism, growth, and differentiation, and the interplay between these fundamental processes. Because a multitude of toxic chemicals and carcinogens induce modification of the DNA in genes, a large portion of the research effort is targeted to understanding what happens to the structure of DNA and what elements dictate the course of events that lead to permanent damage and the resultant deleterious effects on cells and organisms.

Research in the biomedical field has revealed that without intimate knowledge of such mechanisms, efforts to understand the effects of the environment on biological systems and on human health will bear little fruit. In the same vein, by knowing the underlying mechanisms it should be possible to develop better means of examining and quantifying the potential deleterious effects of noxious chemicals and other environmental factors. Hence, IRP is considered an essential resource of the other divisions or programs (TRTP and BRAP) within the Institute. This can be appreciated from the fact that scientists from all three programs continue to form productive collaborative projects as new ideas and procedures come forth.

Much of the scientific efforts have been directed towards developing or improving techniques that permit investigations at the cellular and molecular level. During this past year there has been a surge of activity using probes for detecting and quantifying levels of gene products such as messenger RNA (mRNA), the essential material for translating gene information into useful proteins. For example, mRNA for naturally occurring opiates that regulate information transfer in neuronal tissue dramatically increases in turnover when animals are subjected to a variety of toxic chemicals. Such findings suggest that the implied increased production of opiates is involved perhaps in defense mechanisms designed to alert the organism to adverse changes in the environment. Probes to detect RNA and selected protein molecules are increasingly used in other fields such as reproductive biology. In this case, it has been demonstrated by IRP scientists that estrogens induce the release of a growth promoting factor belonging to a family of proteins called transferrin. Cloning of the gene for this protein has been achieved making it now possible to utilize this gene probe as a marker for uterine development and function. Genes for specific oxidative enzymes (P450 class) in liver that metabolize sex steroid hormone have been cloned and will be utilized to determine that nature and actions of growth factors that control the production of selective forms of these enzymes in male and female animals.

Cloning of specific genes has permitted a relatively new means of deducing sequences of proteins. With such knowledge it is now possible to synthesize appropriate epitopes for preparation of monoclonal antibodies. In turn, the antibodies are powerful tools for detecting and quantitating growth factors secreted by cells, surface antigens involved in cellular development, reproductive hormones secreted from pituitary in response to neurotransmitters, and GTP-binding hormones critically involved in membrane signal transduction processes. Monoclonal antibodies against various types or classes of oncogenes are being developed to examine the concentrations and actions of these substances under the influence of tumor promoters and other agents that affect the growth of cells in culture. These are but a few examples of the increased usage of monoclonal antibodies and reasons why many of the scientists in IRP are requesting tissue culture facilities.

With the ever-growing demand for instruments and facilities necessary for sequencing and synthesizing DNA and protein molecules, it is clear that an Institute-wide common facility must be considered to accommodate the needs of our scientists. Alternatively, efforts will be made to set-up collaborative relationships with the Chemistry Department at the University of North Carolina where equipment and the required scientific expertise could be made available.

Fortunately, IRP has invested heavily in nuclear magnetic resonance (NMR) facilities and mass spectrometry. These state-of-the-art facilities and the excellent leadership in NMR have provided notable advances this past year. Because of the non-invasive detection characteristics of NMR, it is possible to monitor the levels of biological substances both in vivo and with fluorine-containing calcium chelators that allow sensitive measurements by NMR

regulation of cellular metabolism, growth, and differentiation. IRP scientists are finding that numerous hormones or neurotransmitters act by promoting changes in intracellular calcium. These changes are often reflected in stimulation of cellular growth and in secretion of hormones and neurotransmitters from the pituitary gland and from reproductive tissues such as the uterus. Several laboratories are now engaged in understanding how hormones act on calcium-mediated processes. Dr. James Putney has recently joined IRP. Recognized internationally as an eminent scientist in the field of calcium regulation, he will be a great asset in stimulating and coordinating the efforts of other scientists in IRP working along similar lines of investigation.

Some organizational changes have been made and more are contemplated by the Scientific Director in order to focus research on the regulatory processes involved in cellular growth and differentiation. IRP has an excellent staff with expertise in this area that hitherto has been dispersed or not coordinated to yield maximal creative activity. As an effort to promote communication between scientists, a one-day retreat was organized with participation by the laboratory chiefs. The success of this venture has encouraged more frequent use of one- or two-day retreats with emphasis on scientific discussions. As another means of increasing scientific intercourse among the staff, both senior and junior staff scientist now present 30-minute talks before the Laboratory Chief Meetings. Finally, as an effort to increase interactions between other institutions in the Research Triangle area and NIEHS, a biannual lecture series has been inaugurated in honor of the late Hans Falk, a noted scientist in the Institute who contributed heavily to the inception and operation of this Institution. The Series was inaugurated with Professor Bruce Ames as the first honored lecturer and was attended by a large audience.

The Board of Scientific Counselors met in November and in May to review the programs of the Laboratories of Genetics and Molecular Biophysics. The reports of the Advisory Board were highly complementary to the scientists in these Laboratories and incorporated suggestions for improvements in certain programs.

Drs. Drake and Judd were given bonuses for their outstanding contributions to the Genetics program in IRP. Dr. McLachlan was promoted and given a Superior Service Award. Dr. Bend was awarded the Director's Award for his many contributions to the Laboratory of Pharmacology. Our scientists continue to publish at a high rate and are recognized in the national and international community based on the large number of invitations to lecture at symposia and in universities and other institutions in the U.S. and abroad.

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY
Summary Statement

The 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 goal of the Laboratory is to obtain a better understanding of neuronal plasticity and nervous system mechanisms responsible for adaption. General approaches include the following: (1) investigations of the biophysical and molecular biological properties of excitable cells, (2) studies on afferent and feedback mechanisms which influence neuronal plasticity of selected functional systems, (3) studies on the neurobehavioral mechanisms involved in plasticity and adaptive responses to environmental agents, (4) studies on peptides and their effects on biological systems, (5) identification of vulnerable neural circuits by study of neurotransmitter translocations and enzyme analysis, and (6) studies on the functional roles of neurotransmitters or neuromodulators (such as brain peptides) in CNS adaptation following chemical or physical insult.

OVERVIEW OF CURRENT RESEARCH EFFORTS

Neural Mechanisms Group

The Neurobehavioral Section conducts research to understand at the neurological, neurochemical and anatomical levels the compensatory or adaptive changes activated by neurodegenerative processes and exposure to neuroactive chemicals. Many disease states in humans such as Alzheimer's disease and senile dementia involve some degree of cognitive impairment due to progressive neurological degeneration. The cognitive deficits are characterized as loss of memory capability and have been associated with a breakdown of central cholinergic transmission. Deterioration of specific regions of the central nervous system such as the cerebral cortex and hippocampus has been noted in affected individuals. Current research is dedicated to the development of animal models to mimic cognitive deficits due to cholinergic dysfunction or deterioration of the neocortex and/or hippocampus. Future studies are aimed at determining strategies to treat such deficits either pharmacologically or with agents that retard or repair the degenerative process. Other studies are designed to study the role various components of the extrapyramidal system play in the expression of various signs of motor dysfunction, including tremor and myoclonia, that are often associated with progressive neurological disease.

Degeneration of the limbic system can be induced experimentally in animals by systemic or direct intracerebral administration of cytotoxicants. Specific behavioral tests, including assessments of reference and working memory, for limbic dysfunction are used to measure degenerative process. Neurochemical and/or histopathological procedures are used to determine the specificity of the lesion. Intracerebral administration of AF64-A, a cholinergic cytoxicant, causes behavioral impairments indicative of limbic deterioration, including impaired working memory. Pharmacological studies show that rats given AF64-A have increased sensitivity to cholinergic agents. However, some data suggest that AF64-A may have some degree of nonspecificity as determined by neurochemical and histopathological endpoints. Colchicine

applied directly into the dentate gyrus of the hippocampus caused preferential destruction of granule cells and mossy fibers, with minimal effects on other components of the hippocampus. Intrahippocampal colchicine has been found to produce deficits in learning and memory. Pharmacological agents are currently being investigated in an attempt to alter these behavioral deficits. Preliminary studies have indicated that pretreatment with GM1, a purported neuronotrophic factor, can protect against the limbic damage produced by colchicine. Future studies will address the possible mechanism by which this protective effect occurs and will determine other models of cognitive dysfunction.

Tremor and aberrations in reflex modulation have been studied in animals using chemical probes. Activation of the brainstem and spinal cord with an agent believed to produce repetitive firing of axons can be antagonized by phenytoin, which holds sodium channels in the inactive state, and by mephenesin, which blocks polysynaptic reflexes. However, pretreatment with phenoxybenzamine or prazoin to block adrenergic receptors only modulates tremor activity. Similar effects have been observed for chemical-induced hyperreflexia. These data support the interpretation that there is a descending noradrenergic pathway that modulates motor outflow and that the a-1-adrenergic receptor subpopulation acts to enhance the efferent activity. Future studies will examine this possibility using direct intrathecal administration and will address the question concerning the modulatory role of the serotonergic pathways that descend from the brainstem and terminate in the ventral and dorsal horn of the spinal cord.

Neurophysiology of Adaptive Mechanisms Group

Research in the Physiology of Adaptive Mechanisms Workgroup seeks to understand basic mechanisms underlying normal and abnormal modulation of neuronal function. The hippocampal formation has been chosen as a "model" for these studies since it contains many of the general features found throughout the cerebral cortex, yet has been rather well defined anatomically, physiologically and neurochemically. Moreover, it exhibits exquisite neuronal plasticity. The research focuses on two major topics: (1) Modulation of neuronal function by neuropeptides (especially opioid peptides) and steriod hormones (especially glucocorticoids); and (2) the role of zinc in the mossy fibers of the hippocampal formation.

These studies utilize in vivo models for elicitation of seizure activity, the in vitro hippocampal slice technique and primary cultures of rat hippocampal cells. Each has its own advantages and disadvantages; together, however, they form the ideal complement. Two general approaches are used. These are (1) investigation of the biophysics and molecular biology of excitable cells, and (2) studies on the feedforward and feedback mechanism influencing neuronal plasticity in the three major fiber tracts (perforant path, mossy fibers and Schaffer collaterals within the hippocampal formation.

Our interest in the neuropeptides and steroid hormones stems from their known ability to alter neuronal excitability and the accumulating evidence that steriod hormones can alter the sensitivity to neuropeptides. Yet, the nature and mechanisms for these effects are not known. Our interest in zinc stems from the evidence suggesting that endogenous opioids and zinc may also interact to regulate neuronal excitability within the hippocampal formation.

Current research has succeeded in developing a method for electrical stimulation of the perforant path within the hippocampal formation which will elicit "wet dog

shakes" (WDS) consistently and repeatedly in the absense of an overt seizure. Moreover, we have demonstrated that naloxone is an effective antagonist for this effect thus implicating mu or delta receptor opioid agonists as an important factor in the elicitation of WDS. Studies in progress are examining the working hypothesis that release of enkephalin from the perforant path terminals causes a loss of recurrent inhibition in the dentate granule cells; further that this loss of recurrent inhibition is a requirement for the elicitation of WDS.

Current research has also demonstrated that dithizone, a chelator of zinc, has a profound and dose related effect on the toxicity of kianic acid. Work in progress involves (1) quantitation of changes in zinc levels in the hippocampus as a result of dithizone administration, (2) examination of changes in hippocampal levels of enkephalin and dynorphin, and (3) examination of the electrophysiological alterations in the hippocampus induced by dithizone administration.

Non-Ionizing Radiation Group

Research to investigate the interaction of microwave radiation with neural tissue has shown that electromagnetic fields do alter the response of isolated frog sciatic nerves. The threshold for the effect (a reduction in the compound action potential, CAP) occurred at an energy absorption of approximately 4.5 mW/g and the magnitude of the alteration increased with increased energy absorption. Although a decrease in CAP began to occur shortly after initiation of exposure, a significant decrease is not observed until approximately 50 minutes after exposure. Latency, time for the nerve to respond after stimulation, and refractory, the ability of the nerve to respond to a second stimulation, were not affected. These results indicated that the alterations produced by the electromagnetic fields are due to changes in the slow processes such as slow sodium conductance inactivation, potassium conductance inactivation, the activity of Na^+ K^+-ATPase or other activities that lead to the accumulation or depletion of ions with time constants of the order of 100 milliseconds to seconds rather than fast processes such as fast sodium conductance inactivation with time constants in the order of a few milliseconds. These results plus results from experiments varying duration of exposure, varying temperature of the nerves, and blocking K^+ channels using TEA (Tetraethylammonium), indicate that electromagnetic fields interact with voltage sensitive membranes by altering Na^+ conductance and/or Na^+ K^+-ATPase activity. Patch clamp techniques for measuring single channel activity in membranes are being developed and will be applied to study cellular response to biological signals and outside environmental factors.

Neurotransmitter Mechanisms Group

Research in the Neurotransmitter Mechanism Group focuses on two major areas: 1) Molecular mechanisms underlying the regulation of brain opioid peptides and tachykinins by neurotransmitters or psychoactive compounds. This was achieved by determining levels of peptides, precursor molecules and mRNA coding for a particular peptide precursor after different treatments. This type of approach not only provides basic information regarding the metabolism of peptides, but also helps elucidate the interaction between peptides and neurotransmitters. 2) Functional roles of brain opioid peptides and tachykinins in mediating certain neurological dysfunction states, such as epilepsy, Parkinsonism, etc. For this purpose, different animal models mimicking the disease states in humans have been employed to examine: (a) how brain peptides are perturbed in different kinds of experimental models, and (b) how peptide treatment affects the disease states.

One of the major efforts in the last year is the final development of a method for determining turnover of opioid peptides (enkephalin and dynorphin) and substance P in the central nervous system. Research concerning the dynamic changes of peptides was hampered for the last few years because of the lack of methods. Development of blot hybridization methods using cDNA coding for the above-mentioned peptides, in conjunction with radioimmunoassay of both peptides and precursor molecules, allow for the determination of the biosynthesis, processing, or release of peptides. This represents an important step for further understanding of the molecular mechanisms of peptide regulation.

Using these newly developed methods, we have studied the regulation of enkephalin, dynorphin and substance P in the basal ganglia by different neurotransmitters, such as dopamine (DA), serotonin and acetylcholine. We have obtained several lines of evidence suggesting that the nigrostriatal DA pathway exerts a potent influence on the metabolism of these three neuropeptides. Long-term blockade of DA receptors with a DA antagonist, haloperidol (an antipsychotic drug which produces tardive dyskinesia after long-term medication in patients) or specific lesion of DA neurons by 6-hydroxdopamine leads to an increase in the expression of enkephalin biosynthetic process. This finding not only suggests the physiological regulation of the enkephalin system by DA, but also raises an important concept that gene expression of neuropeptides may be an important site of action for psychoactive compounds. Further, these results may also have relevance to the adaptive processes in some neurological diseases such as Parkinson's disease or Lesch-Nyhan syndrome. Further studies have shown that DA exerts an opposite effect on dynorphin compared with that of enkephalin. The reciprocal interaction with enkephalin and dynorphin by the DA system suggests a delicate modulation of the activity of the basal ganglia by these two opioid peptides.

Another major effort was to study the relationship between opioid peptides and seizures. Seizure activity induced by different experimental procedures, such as electroconvulsive shock, amygdaloid kindling or kainic acid, causes robust perturbations of the metabolism of opioid peptides in the limbic-basal ganglia regions. We have obtained evidence suggesting that the change of opioid peptide may be related to both preseizure behavior such as "wet-dog shakes" or seizure-related behaviors, such as change of seizure threshold or loss of memory. This study not only suggests the possible physiological function of opioid peptides in regulating brain excitability, but also provides additional avenues for the therapy of epilepsy.

Peptide Neurochemistry Group

Research in the Peptide Neurochemistry Group focuses on three major themes: (1) Biochemistry of neuropeptides involves the identification in and isolation of peptides from mammalian tissues and preparation of antibodies directed against them. Studies include their tissue distribution and the purification of new peptide hormones. Research also involves the interaction of peptides with neuronal receptors and their release from brain tissue; (2) Pharmacology of neuropeptides studies the spectrum of bioactivity of neuropeptides using isolated tissue preparations in order to differentiate between various peptide groups. Research is designed to understand the correlation between immunoreactive and bioactive peptides of unknown structure; and (3) Physiology of neuropeptides involves a study on the central mechanism of action of peptides using the regulation of gastric secretion as a model system. Major efforts evaluate the role of prostaglandins, biogenic amines and lithium on peptide neuromodulation in brain tissue.

Results obtained in the last year include the development of a protocol to purify microgram quantities of the immunoreactive bombesin-related peptide from milk. Antisera were raised and characterized against amphibian tryptophyllin-13 and neuromedin B, a mammalian spinal cord peptide structurally related to the bombesin/ranatensin group; its distribution in neuronal and peripheral tissue in rats has begun. Receptor studies using iodinated [Tyr^0]-neuromedin B were initiated in rat brain synaptosomes, however, without substantial results to date.

Pharmacological assessment of fractions in the purification of immunoreactive milk bombesin from fresh milk reveal the presence of a peptide(s) exhibiting bioactivity differing from that of bombesin. Partially purified fractions from powdered milk had a spectrum of activity similar to bombesin. The action of PHLIP-8 differs from that of physalaemin on several tissues from various species, including blood pressure tests.

The neuromodulatory effect of bombesin on gastric secretion involves arachidonate metabolites as shown through the prevention of bombesin-induced changes by indomethacin, in particular changes involving pH and [H+] concentration. Dermorphin administered icv at doses below that to produce analgesia or catalepsy modified gastric secretion primarily the volume of gastric juice. These data suggest the involvement of mu receptors in brain tissues in the neuroregulation of gastric secretion.

Developmental Neurobiology Workgroup

The maturation of the central nervous system and its functional output, behavior, relect a series of highly ordered and precisely timed events. The maturation of the central nervous system may be further characterized as reflecting a dynamic balance between the greater susceptibility of the immature organism to environmental and chemical insult and the greater capacity for plasticity and reorganization in the immature nervous system. Our rationale is that the study of perturbations of this balance and how they are reflected in recovery or sparing of function are important to elucidation of the processes underlying appropriate behavioral and neural development. We have specifically focused on perturbations of the developing hypothalamic-pituitary-adrenal axis and the hippocampus induced by early exposure to various classes of environmental agents (chlordecone, triethyl lead, and carbon monoxide).

Neonatal exposure to the organochlorine insecticide, chlordecone, produced functional imbalances in circulating and adrenal steroids which persist well into adulthood. Extremely rapid and persistent changes in adrenal morphology were also noted. These steroid hormone alterations induced by chlordecone, accompanied by it's inherent but weak estrogenicity, were associated with behavioral impairments indicative of alterations in the sexual differentiation of hypothalamic nuclei. In each case these behavioral deficits were not attributable to the persistence of chlordecone in neural tissue.

Our previous studies have shown that neonatal exposure to triethyl lead, the active metabolite of leaded gasoline, produces preferential and permanent damage to specific hippocampal cell fields. This cell loss is reflected in behavioral dysfunction characterized by marked hyperreactivity. Our continuation studies have indicated that 1) the acute behavioral manifestations of toxicity were associated with linear dose-dependent blood and brain levels of lead; 2) although all brain regions examined contained significant amounts of lead, the limbic system was particularly

affected; and 3) lead was not detectable in the brains of animals upon maturation to adulthood. Thus, the neural and behavioral damage is attributable to an early "pharmacological" effect of the lead, which results in an apparently permanent neuronal rearrangement.

Prenatal exposure to carbon monoxide, with carboxyhemoglobin levels within the range experienced by cigarette smokers, was used to induce mild tissue hypoxia. The animals were evaluated in a test of cognitive function particularly sensitive to limbic system damage. Our longitudinal studies indicated that upon maturation to adulthood the prenatal carbon monoxide exposed animals learned an avoidance task as well as controls. Although a subtle memory deficit was suggested 24 hours later, extending the retention interval to 28 days produced a pronounced retention deficit. An additional study, in which animals matured to one year of age prior to psychological testing, the prenatal carbon monoxide exposed animals showed pronounced deficits in both learning and 24-hour memory. Indeed, the deficits in this aging population were similar to that of the juvenile-aged animals reported last year.

It is now our plan to use this information concerning the disruption of the hypothalamic-pituitary-adrenal axis and hippocampus to attempt some prophylactic treatments. To the extent that we can mitigate or block the neurotoxicant-induced damage, we will gain vital information concerning the mechanism of action of these compounds which will also be of use for developing post-exposure therapeutic strategies.

Neurochemistry Group

Research in the Neurochemistry Group focuses on the effects of organochlorine insecticides on the intracellular calcium levels. A method that has recently been developed was applied to the determination of levels of free ionic calcium within synaptosomes. This procedure involves loading the synaptosomes with an ester of a tetracarboxylic acid which is hydrolysed within cells to yield the free acid. The ionic acid is trapped within the cell and gives a fluorescent signal when complexed with calcium.

Initial studies demonstrated that the synaptosome was not adversely affected by the dyes used. The membrane potential and responses of synaptosomes to pharmacological agents was normal and levels of ATP were maintained.

Chlordecone caused a dose- and time-dependent increase of intracellular calcium while the related non-neurotoxic mirex had no such effect. This effect was attributed to damage to the plasma membrane. Both membrane "leakiness" and specific calcium channels allowed a greater penetration of entracellular calcium. The increase in intracellular calcium was not attributable to mitochondrial damage or to failure of oxidative phosphorylation. Parallel studies with radioactive ^{45}calcium gave results that initially seemed anomalous: chlordecone appeared to inhibit synaptosoma ^{45}Ca uptake. Further study of this phenomenon revealed that this was due to chlordecone-induced lysis of a proportion of synaptosomes, which were then not available for ^{45}Ca uptake. This illustrates that the measurement of calcium concentration within cells or synaptosomes can yield data that would not be apparent with only ^{45}Ca-based experiments.

PERSONNEL

Additions to the Laboratory were: Senior Staff Fellow - Dr. Michal Stachowiak; Visiting Fellow - Dr. Paul Lee; Visiting Fellow - Dr. Dayho Zhad. Individuals leaving the Laboratory were: Supervisory Research Chemist - Dr. Stephen C. Bondy; Visiting Associate - Dr. Katsunori Saitoh; Visiting Fellow - Dr. Hannu Komulainen.

OTHER ACTIVITIES

Dr. S. C. Bondy: Adjunct Associate Professor, Department of Pharmacology, University of North Carolina, School of Medicine; Member, Editorial Board, Environmental Health Perspectives; Member, Editorial board, International Journal of Developmental Neuroscience; Member, Editorial Board, Neurotoxicology; Member, Editorial Board, Neurochemical Pathology; Member, Organizing Committee of the Winter Conference on Brain Research; Board Member, Neurological Research Foundation; Ad Hoc Reviewer, National Science Foundation (Neurobiology Program), Brain Research, Journal of Neurochemistry, Proceedings National Academy of Science.

Dr. J. S. Hong: Adjunct Associate Professor, Department of Psychiatry, Duke University Medical School; Adjunct Associate Professor, Toxicology Curriculum, University of North Carolina; Adjunct Associate Professor, Department of Pharmacology, Medical College of Virginia; Member, Editorial Board, Neurotoxicology; Invited speaker of the Symposium entitled "Modulation of Brain Opioid Peptides by Antipsychotic Drugs and Electroconvulsive Shock" Taiwan; Invited speaker of the Symposium entitled "Intrastriatal Injection of Kainic Acid Increases the Abundance of mRNA Coding for Preproenkephalin A in Rat Hippocampus" in First SCBA International Symposium; Invited speaker of the Symposium entitled "Use of Cytotoxicants to Study the Metabolism of Opioid Peptides," ASPET Meeting; Invited speaker of Seminars entitled "Modulation of Brain Opioid Peptides by Neuroleptics and ECS," University of Toledo; "Brain Opioid Peptides and Seizure Activities," University of Kansas; "Modulation of Brain Opioid Peptides by ECS, Kaninic Acid," University of Wyoming; Ad Hoc Reviewer for Brain Research, Science, Biochemical Pharmacology, Neuropharmacology, Neurotoxicology, Toxicology and Applied Pharmacology, Journal of Pharmacology and Experimental Therapeutics, grant proposal from National Science Foundation (Neurobiology Program).

Dr. L. H. Lazarus: Adjunct Associate Professor, Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill; Adjunct Member Lineberger Cancer Research Center, University of North Carolina, Chapel Hill; Ad Hoc reviewer for Analytical Biochemistry, Cancer Research, Chemico-Biological Interactions, Journal of Biological Chemistry, Proceedings of the National Academy of Science, Regulatory Peptides, Science, and Winter Neuropeptide Conference; Invited guest of Fidia Research Foundation, Georgetown University, Washington, D.C.; Trainee in Recombinant DNA and DNA Sequencing Methodologies, Molecular Biology Program, University of North Carolina, Chapel Hill; Hosted internationally prominant guest speakers at NIEHS, including A. Kastin, M.D. (Editor, Peptides), V. Erspamer, M.D. (discoverer of serotonin, peptide hormones and vertebrate alkaloids; Nobel nominee), and A. C. Hollinshead, Ph.D.

Dr. C. L. Mactutus: Membership accepted to Western Pharmacology Society; Elected Council Member in Behavioral Teratology Society; Invited participant to the Fourth International Symposium on Neurotoxicity of Selected Chemical Agents: The Fetus and Child, Presenting paper entitled "The Influence of Early Steroidal Alterations

on Neural and Nonreproductive Behavioral Function"; Invited participant to the Symposium on the Collaborative Behavioral Teratology Study, presenting a paper entitled "Assessment of Learning and Memory Dysfunction: Ontogenetic Considerations"; Invited seminar entitled "Preweaning Evaluation of Neurotoxicant-induced Memory Dysfunction: Implications of a Multidimensional Analysis of Behavior," U.S. Environmental Protection Agency; Invited seminar entitled "Conceptual and Procedural Considerations for the Assessment of Memory (Dys)Function Following Early Neurotoxicant Exposure," The DuPont Corporation; Invited seminar entitled "Neonatal Triethyl Lead Neurotoxicity: Persistent, and Apparently Permanent, Behavioral and Pathological Sequela," The Jefferson Medical College; Ad Hoc reviewer for Developmental Psychobiology; Obstetrics and Gynecology; Pharmacology, Biochemistry and Behavior; Physiology and Behavior; Physiological Psychology; Neurobehavioral Toxicology and Teratology.

Dr. D. I. McRee: Adjunct Professor, NCSU; Coordinator U.S.-U.S.S.R. Cooperative Program on Health Effects of Physical Environmental Factors; Invited participant on Delegation to Soviet Union by National Bureau of Standards; Hosted Soviet delegations to the U.S.; Representative for DHHS on Interagency Advisory Committee on Electric Field Effects from High Voltage Transmission Lines; Member of American National Standards Institute C95 Committee on Safety Standards for Non-Ionizing Radiation; Appointed member of IEEE's Committee on Man and Radiation (COMAR); Elected to membership on USA Commission A (Electromagnetic Metrology) of the International Union Radio Science; Editoral Review Board of Environmental Health Perspectives; Appointed to National Research Council Commission on Physical Sciences, Mathematics, and Resources; Invited to serve on WHO Working Group on Non-Ionizing Radiation; Invited reviewer of WHO document, "Environmental Health Criteria Document - Magnetic Fields"; Invited participant on Subpanel of the Committee on Interagency Radiation Research and Policy Coordination to write document on "Future Research Needs in Health Related Effects of Non-Ionizing Radiation"; Served as Acting Editor of the Bioelectromagnetics Journal; Reviewer of Contract Proposals for EPA; Reviewer of manuscripts for Bioelectromagnetics, Radiation Research, and Health Physics; Invited Session Chairman for the Bioelectromagnetics Symposium.

Dr. C. L. Mitchell: Adjunct Professor, Department of Pharmacology and the Neurobiology Program, University of North Carolina, lectures presented to medical graduate and undergraduate students of the University of North Carolina; Member, Editorial Board, Environmental Health Perspectives; Member, Editorial Board, Neurotoxicology; Chairman, Committee on Methods in Neurobehavioral Toxicology, International Programme on Chemical Safety, World Health Organization; Review of manuscripts for Toxicology and Applied Pharmacology, Journal of Pharmacology and Experimental Therapy, and Journal of Medicinal Chemistry; Participant in a U.S.-U.S.S.R. Workshop in the Soviet Union.

Dr. H. A. Tilson: Adjunct Associate Professor, Department of Zoology, North Carolina State University; Adjunct Associate Professor, Toxicology Curriculum, University of North Carolina; Associate Editor, Neurotoxicology; Member, Editorial Board, Neurobehavioral Toxicology and Teratology; Member, Editorial Board, Toxicology and Applied Pharmacology; Member, Animal Care Committee, NIEHS; Ad Hoc Reviewer for Brain Research, Psychopharmacology, Pharmacology, Biochemistry and Behavior, Journal of Pharmacology and Experimental Therapeutics, Fundamental and Applied Pharmacology and Teratology; President, Neurotoxicology Specialty Section, Society of Toxicology: Invited participant, Symposium and Workshop on Design

Consideration in Screening for Behavioral Teratogens, Cincinnati, Ohio, September 3-6, 1985; Invited speaker, Symposium on Predicting Neurotoxicity and Behavioral Dysfunction from Preclinical Toxicologic Data, Washington, DC, September 30 - October 1, 1985; Invited participant, Symposium on Neurobehavioral Methods in Safety Assessment of Chemicals and Drugs, Dusseldorf, Federal Republic of Germany, December 16-19, 1985; Invited speaker, National Food Institute, Institute of Toxicology, Søborg, Denmark, December 14, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50015-12 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effects of Microwaves on Neural Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donald I. McRee	Research Physicist	LBNT	NIEHS
Others:	L. Lee	Visiting Fellow	LBNT	NIEHS
	C. L. Mitchell	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Non-Ionizing Radiation

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Research to investigate the interaction of microwave radiation with neural tissue has shown that electromagnetic fields do alter the response of isolated frog sciatic nerves. The threshold for the effect (a reduction in the compound action potential, CAP) occurred at an energy absorption of approximately 4.5 mW/g and the magnitude of the alteration increased with increase energy absorption. Although a decrease in CAP began to occur shortly after initiation of exposure, a significant decrease is not observed until approximately 50 minutes after exposure. Latency, time for the nerve to respond after stimulation, and refractory, the ability of the nerve to respond to a second stimulation, were not affected. These results indicated that the alterations produced by the electromagnetic fields are due to changes in the slow processes such as slow sodium conductance inactivation, potassium conductance inactivation, the activity of Na^+K^+-ATPase or other activities that lead to the accumulation or depletion of ions with time constants of the order of 100 milliseconds to seconds rather than fast processes such as fast sodium conductance inactivation with time constants in the order of a few milliseconds. These results plus results from experiments varying duration of exposure, varying temperature of the nerves and blocking K^+ channels using TEA (tetraethylammonium), indicate that electromagnetic fields interact with membranes by altering Na^+ conductance and/or Na^+K^+-ATPase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90030-06-LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effects of Toxicants on Membrane-Related Neurochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen C. Bondy	Research Chemist	LBNT	NIEHS
Others:	H. Komulainen	Visiting Fellow	LBNT	NIEHS
	H. A. Tilson	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

U.S. Environmental Protection Agency

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

One purpose of membrane-related studies has been to delineate injury to discrete neuronal tracts. This has the goal of identification of damage to specific circuitry which is especially vulnerable to a given neurotoxic agent. This project has involved study of the effect of various neurotoxic agents upon neurotransmitter translocations in the synaptic region. High-affinity uptake systems, calcium stimulated neurotransmitter release and assay of receptor binding sites have been carried out in animals treated with organometals. The differential susceptibility of various brain regions to trimethyltin and triethyl tin has been studied in this manner and data related to morphological findings. In the case of triethyl lead studies, biochemical behavioral correlations between analgesia and benzodiazepine binding sites have been made.

A second goal is to study less selective damage to cerebral membranes. Such general effects may still present as specifically damaging certain nerve pathways, perhaps because of their intrinsic sensitivity to insult. This work focuses on the effect of agents upon levels of free calcium within the synaptosome and on depolarization-induced calcium fluxes across the synaptosomal membrane. Novel methods of assaying these parameters have been adapted for our studies. This project is to be terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90031-05 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Assessment of Neurophysiological Effects of Organometals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Clifford L. Mitchell Pharmacologist LBNT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Membrane Physiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The organometals have numerous applications in industrial and occupational settings. The neurotoxicity of these agents, particularly organoleads and tins, is well known. However, their precise sites and mechanisms of action are poorly understood. The purpose of these studies is to characterize the neurophysiological effects of relevant organometals in an attempt to determine the site of action and aid in determining the mechanism of action of selected organometals. We have found that triethyl lead (TEL), but not trimethyl lead, triethyl tin or trimethyl tin markedly increases the sensitivity to pentylenetetrazol induced seizures. This increase in sensitivity is most pronounced in animals receiving multiple pentylenetetrazol injections. There is little, if any, change in sensitivity to a single dose of pentylenetetrazol. In addition, we have found that TEL accelerates amygdaloid kindling. On the other hand, it reduces the severity of seizures induced by electroconvulsive shock delivered via ear electrodes. These results indicate that (1) TEL produces a clear increase in seizure susceptibility, (2) the mode of eliciting the seizures is critical for observing the effect, and (3) suggest possible involvement of the limbic system in the toxicity of the compound. This project is being terminated.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90033-04 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LBNT	NIEHS
Others:	W. E. Wilson	Research Chemist	LBNT	NIEHS
	B. J. Irons	Biological Lab. Technician	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*
University of Rome, Italy
University of Kyoto, Japan
University of North Carolina, Chapel Hill

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Peptide Neurochemistry

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

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

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

The milk bombesin (MB) composition of bovine milk has been extensively investigated from the points of view of obtaining pure peptides and of attempting to characterized the biological properties of the milk extract. The purification efforts involved several manipulations of an acid extract of milk including: differential solubilization, ammonium sulfate precipitation, gel filtration, chromatography on ion exchange media, and reverse phase HPLC using a variety of columns and conditions. Smooth muscle contractility studies have suggested that one of the MB components of bovine milk may have biological properties similar to those of amphibian bombesin. However, there are multiple forms of MB in bovine milk extracts, and some of these do not appear to be biologically active. Furthermore, there are biologically active milk extract peptides which do not cross-react with the antiserum which is used to recognize MB; that antiserum recognizes the peptide sequence -Gly-Asp-Leu-Trp- (residues 5-8 of bombesin). Efforts are continuing to characterize the smooth muscle contractile profiles of milk extracts and to achieve final purification of several of the low-molecular weight range-MBs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90034-03 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Rabbit Stomach Peptide [Physalaemin-like Material (PLIM)] in Mammalian Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Wilson	Research Chemist	LBNT	NIEHS
Others:	L. H. Lazarus	Research Chemist	LBNT	NIEHS
	B. H. Irons	Biological Technician	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS
	C. Hamm	Electronics Technician	LBNT	NIEHS
	D. Harvan	Chemist	(formerly with LMB, NIEHS)	

COOPERATING UNITS *(if any)*

University of Kyoto, Kyoto, Japan
University of Rome, Rome, Italy
University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.	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 *(Use standard unreduced type. Do not exceed the space provided.)*

The rabbit stomach metabolic precursor of PHLIP-8, a physalaemin-like octapeptide with the amino acid sequence <Glu-Val-Asp-Pro-Asn-Ile-Gln-Ala, is apparently a glycoprotein, however, precise structural characterization has not been performed. In order to determine the nature of the precursor(s) in nerve and lung tissue, attempts have been made to raise antisera to thyroglobulin-PHLIP-8 complexes; characterization of the antisera has yet to be completed. Bioassays have indicated that, while PHLIP-8 does not possess typical tachykinin properties, relatively large concentrations of this peptide will facilitate enhanced contractile responsiveness in several smooth muscle preparations and, in the rabbit, it lowers blood pressure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90035-03 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Use of Ornithine Decarboxylase in the Detection of Tissue Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen C. Bondy	Research Chemist	LBNT	NIEHS
Others:	J. S. Hong	Pharmacologist	LBNT	NIEHS
	C. L. Mitchell	Pharmacologist	LBNT	NIEHS
	H. A. Tilson	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this project is to account for the rapid changes in levels of ornithine decarboxylase (ODC) in tissues that respond to damage by regenerative or adaptive changes. The role of ODC is somewhat understood in relation to cell division but the function of this enzyme in non-proliferating tissues is unknown. Chlordecone administration to rats, at levels causing tremor (40 mg/kg body weight) causes a 21-24-fold increase in levels of adrenal ODC. This is a rapidly occurring, reversible event of much greater magnitude than any other biochemical response to chlordecone hitherto reported. The onset of chlordecone induced tremor can be prevented by pretreatment with an irreversible inhibitor of ODC, difluoromethylornithine (DFMO). This implies a relation between polyamines and behavioral responses. However, cerebral ODC levels are not markedly elevated in chlordecone-treated rats. Cerebral ODC is dramatically elevated following electroconvulsive shock or after intracerebral injections of colchicine or kainic acid. The regional specificity and time course of these effects are currently under study. This project is to be terminated.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90036-03 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	T. J. Walsh	Staff Fellow	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS
	R. L. McLamb	Technician	LBNT	NIEHS
	S. C. Bondy	Research Chemist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neurobehavioral

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

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

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

Because of their purported specificity for the nervous system, the neurotoxic effects of triethyllead, trimethyllead, triethyltin, and trimethyltin were studied in rats. Initial experiments found that these metals produced dose- and time-related antinociception. Subsequent studies found that triethyl and trimethyl lead produce behavioral effects that are similar, but not identical. Histological assessment of rats 7 or 28 days after a single injection of these agents indicated that triethyllead caused structural abnormalities in the hippocampus and dorsal root ganglion, while trimethyl lead produced changes primarily in the spinal cord and brainstem. Camparison of the neurochemical effects of these four alkylmetals indicated that triethyltin, triethyllead and trimethyllead produce somewhat nonspecific changes in regional brain levels of biogenic amines and amino acid transmitters. However, trimethyltin produced relatively specific decreases in inhibitory transmitters in the hippocampus and frontal cortex. Because of its environmental relevance, further studies characterized the effects of triethyllead. These experiments found that the anitinociception produced by this metal is probably not associated with an alteration in opiate systems. Subsequent experiments suggested that acute exposure to triethyllead enhances the responsiveness of dopaminergic processes which contribute to locomotor activity; selective depletion of brain dopamine with 6-hydroxydopamine was found to block the antinociceptive effects of triethyllead. Acute and short-term repeated exposure to triethyl alters reactivity, locomotor activity and avoidance learning. Other experiments indicated that triethyllead-induced behavioral changes may be related to alterations in reactivity to stress and damage to the hippocampus. However, other studies found that triethyllead had no significant effect on spatial learning in rats, indicating a lack of specificity for the limbic system. This is a final report for this program and no further work in this area is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90037-03 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Toxicological Perturbations of Behavioral and Neural Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles F. Mactutus	Senior Staff Fellow	LBNT	NIEHS
Others:	M. Bonner	Biological Lab Technician	LBNT	NIEHS
	D. Murray	Biological Aid	LBNT	NIEHS
	R. M. Booze	Neuropharmacologist	Dept. of Pharmacology	Duke Univ.
	L.D. Fechter	Neurotoxicologist	Environ. Neurobiology	Johns Hopkins University

COOPERATING UNITS *(if any)*

Duke University
The Johns Hopkins University

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Behavioral/Developmental Neurobiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

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

The functional outcome of the dynamic interplay between the greater susceptibility to disruption and the greater capacity for reorganization and recovery (or sparing) of function of the immature central nervous system was examined with respect to maturation of the hypothalamic-pituitary-adrenal axis and the hippocampus. Various toxicants were used to perturb these systems. Neonatal exposure to chlordecone, an organochlorine insecticide, produced functional imbalances in circulating and adrenal steroids as well as extremely rapid and apparently permanent changes in adrenal morphology. These changes were mirrored in behavioral dysfunction indicative of alterations in the sexual differentiation of hypothalamic nuclei and were not attributable to the long-term presence of chlordecone in neural tissue. Neonatal exposure to triethyl lead (TEL), the active metabolite of leaded gasoline, produced a preferential and permanent destruction of hippocampal pyramidal cell fields as indicated by quantitative neuromorphometry. Distributional and pharmacokinetic studies indicate this permanent brain damage was highly related to the early accumulation of lead in specific limbic regions of the central nervous system, but not to its persistence through adulthood. Prenatal exposure to carbon monoxide, with carboxyhemoglobin levels within the range experienced by cigarette smokers, was used to induce mild tissue hypoxia. The disruption of hippocampal function previously suggested by impairment in acquisition and retention of a two-way avoidance task in juvenile-aged offspring was substantially attenuated upon maturation to adulthood. However, a marked exacerbation of learning and memory dysfunction was noted with continued aging.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90038-03 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Animal Model of Organochlorine Neurotoxicity

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	J. S. Hong	Pharmacologist	LBNT	NIEHS
	D. Herr	Technician	LBNT	NIEHS
	K. Nanry	Technician	LBNT	NIEHS
	L. Cook	Guest Worker	LBNT	NIEHS
	S. Shaw	Biological Aid	LBNT	NIEHS
	S. Bondy	Research Chemist	LBNT	NIEHS
	K. Saitoh	Visiting Associate	LBNT	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina, Toxicology Curriculum
North Carolina State University, Zoology Department

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	1.0	2.25

CHECK APPROPRIATE BOX(ES)

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

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

The neuropharmacological basis for tremor and hyperexcitability produced by p,p'-DDT and chlordecone was compared in a series of experiments. Pretreatment with phenytoin, an anticonvulsant that decreases membrane excitability by holding sodium channels in the inactivation state, was used to study the functional significance of brain neurochemical changes produced by p,p'-DDT and chlordecone. Phenytoin selectively enhanced chlordecone-induced tremor and significantly increased chlordecone-induced changes in brainstem MHPG, the major metabolite of the neurotransmitter norepinephrine. Regional levels of norepinephrine were not affected by p,p'-DDT or chlordecone. Phenytoin blocked tremor induced by p,p'-DDT, as well as increases in brainstem MHPG. Subsequent experiments found that systemic administration of phenoxybenzamine attenuated the tremor and hyperexcitability produced by chlordecone and p,p'-DDT. These data suggest that tremor and hyperexcitability produced by p,p'-DDT and chlordecone are produced by different mechanisms, but are mediated by a common neural pathway. A facilitatory noradrenergic influence, perhaps from pathways descending from the locus coeruleus to the ventral horn, appears to be activated by both agents. Subsequent studies found that systemic administration of alpha-1-noradrenergic receptor antagonists attenuated the tremorigenic effects of p,p'-DDT, while yohimbine, an alpha-2-receptor antagonist, exacerbated the effects of p,p'-DDT. That p,p'-DDT and chlordecone act by different mechanisms is supported by experiments showing that intraventricular administration of calcium into the lateral cerebroventricles exacerbated the effects of chlordecone, but decreased those produced by p,p'-DDT. Furthermore, pretreatment with difluromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, attenuated the effects of chlordecone, but had no effect in rats treated with p,p'-DDT. Putrescine, the product formed by ornithine decarboxylase, reversed the effects of DFMO on chlordecone. Future studies will emphasize the neural substrate responsible for the modification of tremor and startle reflexes mediated at the level of the brainstem.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90039-03 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Modulation of Brain Opioids and Tachykinins by Psychoactive Drugs and Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Subbiah P. Sivam	Senior Staff Fellow	LBNT	NIEHS
Others:	J. S. Hong	Pharmacologist	LBNT	NIEHS
	S. Li	Visiting Fellow	LBNT	NIEHS
	P. H. Hudson	Biological Lab Technician	LBNT	NIEHS
	H. A. Tilson	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neuropharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project were: a) to understand the basic biosynthetic process of opioid and tachykinin systems by employing molecular biology and immunochemical techniques, b) to elucidate how the classical neurotransmitter systems such as dopamine (DA) interact with neuropeptide systems, c) to understand whether pharmacological agents such as haloperidol, lithium, apomorphine or physiological stress, such as insulin-shock, would perturb the biosynthetic processes of neuropeptides. The methods to study the biosynthetic steps include the quantitation of specific mRNAs, measurement of the precursor content as well as the steady-state peptide concentration. Long-term blockade of DA receptors with DA antagonist haloperidol (an antipsychotic drug) or specific lesion of DA neurons leads to an increase in the expression of Met^5-enkephalin (ME) biosynthetic process. This finding not only suggests the physiological regulation of metabolism of the ME system by the DA system but also raises important concept that gene expression of neuropeptide systems may be an important site of action for antipsychotic drugs. Further, these results may also have relevance to the adaptive processes in the pathological states such as Parkinson's disease and Lesch-Nyhan syndrome. Apomorphine, a dopamine agonist induced a selective increase in dynorphin (DY) level which could be blocked by haloperidol; the latter drug on the other hand, increases ME level without affecting DY level. These results demonstrate a reciprocal regulation of ME and DY systems by DA system. The antimanic drug lithium appears to increase the gene expression of ME, DY as well as substance P systems. This suggests that lithium may stimulate genetic expression by a common mechanism at the transcription level. These and other *in vitro* studies with adrenal chromaffin cells provide a strategy to determine the dynamic state of a given neuropeptide system. In conclusion, the approaches used not only provide information on the biosynthetic pathways of neuropeptide systems and their interactions with neurotransmitter systems, but also gives impetus to the development of new strategies for pharmacological manipulation of pathological states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90040-03 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
On the Possible Mechanism of Chlordecone-Elicited Tremor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jau-Shyong Hong	Pharmacologist	LBNT	NIEHS
Others:	P. Chen	Expert	LBNT	NIEHS
	P. M. Hudson	Biological Lab Technician	LBNT	NIEHS
	J. Obie	Biological Lab Technician	LBNT	NIEHS
	H. A. Tilson	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neuropharmacology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

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

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

1. To evaluate the possible role of sodium channel in the neurotoxicity of the organochlorine insecticides in vivo in rats.

p,p'-DDT and related agents act to hold the sodium channel open once opened and this effect is believed to be responsible for neurological effects of tremor and hyperexcitability in vivo. Tremor was almost completely blocked in rats pretreated with hydantoin, an anticonvulsant believed to block repetitive firing of nerves by interfacing with the inactivation gates of sodium. A similar antagonism was observed for permethrin, a Type I pyrethroid believed to have a mechanism equal to that of DDT. However, hydantoin increased the tremorigenic effects of chlordecone, an organochlorine whose mechanism has not been linked to the sodium channel. Our data are consistent with the hypothesis that in vivo neurotoxicity of some organochlorine insecticides is related to their effects on the sodium channel.

2. To characterize neurochemical effects of chlordecone on the hypothalamo-pituitary-adrenal axis.

A single injection of a tremorigenic dose of chlordecone (75 mg/kg, i.p.) increased the levels of plasma and adrenal corticosterone and the plasma level of ACTH. The increase in the pituitary-adrenal activity is consistent with morphological observations which indicate that adrenal cortical cells and corticotrophs of the pituitary hypertrophy after chlordecone exposure. Pretreatment with phenoxybenzamine and pizotifen (a 5-HT receptor blocker) completely prevented chlordecone-elicited increase in plasma levels of ACTH and corticosterone suggesting that monoamine mechanisms within the hypothalamus may mediate the neuroendocrine effects of chlordecone on the pituitary-adrenal axis. This project will be terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90041-02 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Sensitivity to Amino Acids in Mouse Spinal Cord Neuron Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Clifford L. Mitchell	Pharmacologist	LBNT	NIEHS
Others:	G. Westbrook	Senior Staff Fellow	LDN	NICHD
	P. G. Nelson	Chief	LDN	NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Membrane Physiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

L-glutamate (GLU) and L-aspartate (ASP) are neurotransmitter candidates at primary afferent and intraspinal synapses in the mammalian spinal cord. Several receptor types exist for the excitatory amino acids based on selective activation by kainate (KA), quisqualate (QA) and N-methyl-D-aspartate (NMDA), whereas GLU can activate both NMDA and non-NMDA (i.e. either QA or KA) receptors. Recent evidence in culture suggests that a non-NMDA receptor mediates monosynaptic EPSPs formed between dorsal root ganglion and dorsal horn neurons as well as between spinal cord neurons. The purpose of the present study is to test the chemosensitivity of spinal cord neurons to the above mentioned amino acids during the first week in culture. The results indicate that at day 2-3, most spinal cord neurons are sensitive to QA (10 μM) and GLU (100 μM) as well as γ-aminobutyric acid (GABA, 100 μM). Responses to KA (10 μM) are small or absent. QA and GABA responses are associated with conductance increases while GLU responses result in little or no apparent conductance change. Under voltage clamp GLU-activated currents have a region of zero or negative slope conductance consistent with a mixed agonist action on both NMDA and non-NMDA receptors. Both QA and GLU-activated currents have reversal potentials near 0 mV. GABA responses reverse at -50 mV with $KMeSO_4$ and at 0 mV with CsCl solutions in the patch electrode, consistent with a chloride conductance. By day 7, spinal cord neurons are highly sentitive to all agonists tested. These results suggest that both NMDA and non-NMDA receptors are present before detectable synaptic activity, but that sensitivity increases during a period of rapid synapse formation. This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90042-01 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Models of Neurogenerative Processes Involving Cognitive and Motor Dysfunction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	R. McLamb	Technician	LBNT	NIEHS
	J. Peterson	Technician	LBNT	NIEHS
	G. Harry	Guest Worker	LBNT	NIEHS
	J. Hong	Pharmacologist	LBNT	NIEHS
	C. Hamm	Technician	LBNT	NIEHS
	W. Wilson	Chemist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

Biological Science Research Center, University of North Carolina

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research program is to develop: 1) animal models of cognitive dysfunction using neurotoxicants as tools to mimic specific aspects of neurological diseases known to exist in humans, and 2) strategies for the treatment and/or prevention of neurodegenerative processes. Cognitive dysfunction is frequently associated with cholinergic hypofunction or damage to the limbic forebrain; cytotoxicants were administered directly into the brain to mimic these processes. Injection of AF64-A, a purported cholinergic cytotoxicant, produced behavioral effects indicative of cholinergic dysfunction in rats, including hyperactivity and deficits in spatial learning. However, other studies indicated that this agent may have direct effects on other neurotransmitter or neuromodulator systems. However, direct administration of colchicine into the hippocampus caused preferential damage to the granule cells and mossy fibers of the dentate gyrus. These alterations were associated with deficits in spatial learning and acquisition and retention of an avoidance task and increased sensitivity to challenge with scopolamine, a muscarinic receptor antagonist. Preliminary studies showed that behavioral and histopathological damage produced by colchicine was attenuated by pretreatment with gangliosides. Future studies will: 1) replicate and extend the finding that gangliosides protect against the effects produced by colchicine, as well as those produced by another cytotoxicant such as 6-hydroxydopamine, 2) determine possible restorative effects of gangliosides, 3) investigate other models of cognitive dysfunction such as the effects of administration of ibotinec acid into the nucleus basalis of Meynert, and 4) evaluate pharmacological agents such as physostigmine and analogues of arecoline as to their potential activity against colchicine-induced degeneration of the hippocampus.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90043-01 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Zinc in Synaptic Transmission in the Hippocampal Formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Clifford L. Mitchell	Pharmacologist	LBNT	NIEHS
Others:	L. M. Grimes	Guest Worker	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

Curriculum in Toxicology, University of North Carolina

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Membrane Physiology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

Several pieces of evidence suggest that endogenous opioids and zinc may interact to regulate neuronal excitability within the hippocampal formation. The purpose of this project is to conduct a systematic investigation into the effects of zinc on hippocampal neuronal excitability, with an emphasis on its interaction with enkephalin. The goal is to explain the nature of the effects of zinc and the mechanism(s) for its interaction with enkephalin. First it was necessary to determine the manner in which zinc levels were to be altered and the model for equating the significance of these changes to the functioning of the organism. As an initial approach we chose to attempt to alter zinc levels by systemic administration of zinc chloride or the intraviral zinc chelator, dithizone. The biological assay used was occurrence of wet dog shakes and seizures following subcutaneous administration of kainic acid (KA). We were unable to confirm the report of Porsche (IRCS Med. Sci. 11: 599, 1983) that subcutaneously administered $ZnCl_2$ prevents KA induced seizures in rats. Instead, we found no effect of $ZnCl_2$ in doses up to and including 100 mg/kg. This was true whether zinc was given before or after KA. In contrast, intraperitoneal injection of dithizone (12.5-100 mg/kg) has a profound and dose related effect on the toxicity of KA. When given 15 minutes after the subcutaneous injection of KA, it markedly potentiates KA toxicity. However, when KA is given 24 hours after dithizone, the toxicity of KA is reduced. It appears, then, that dithizone may prove to be extremely useful as a tool for exploring the actions of zinc on the hippocampus. Work in progress involves: 1) quantitation of changes in zinc levels in the hippocampus as a result of the various manipulations, examination of changes in hippocampal levels of enkephalin and dynorphin and examination of electrophysiological effects of dithizone in the hippocampus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90044-01 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Modulation of Neuronal Function by Neuropeptides and Steroid Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Clifford L. Mitchell	Pharmacologist	LBNT	NIEHS
Others:	L. M. Grimes	Guest Worker	LBNT	NIEHS
	J. S. Hong	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

Curriculum in Toxicology, University of North Carolina

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Membrane Physiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	0.5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Considerable work in the Laboratory of Behavioral and Neurological Toxicology has focused on the role of enkephalin and dynorphine in seizure activity and related sequelae. Among other things, this work has implicated enkephalin as playing a major role in the elucidation of a phenomenon in rats known as "wet dog shakes" (WDS). This work, as well as other sources, has also implicated the dentate granule cells (DGCs) as being necessary for the elicitation of WDS, at least with respect to indution by kainic acid or by stimulation of the perforant path (PP). Our first objective of this project is to develop a method for electrical stimulation of the PP which will elicit WDS consistently and repeatedly in the absence of an overt seizure. To date we have succeeded in eliciting WDS in the absence of overt seizures but cannot do so after the animals have received several stimulations. We have reached a stage where we can elicit enough WDS in the absence of overt seizures to effectively determine whether or not changes in hippocampal enkephalin content, but not dynorphin, are correlated with the number of WDS. During this year we also plan to determine whether or not opioid delta receptor antagonists will block the occurrence of WDS and whether they are specific among the opioid receptors in so doing. In addition, preliminary experiments suggest that dexamethazone increases the number of WDS following PP stimulation. Thus a possible interaction between glucocorticoids and enkephalin is suggested and will be pursued. Other studies in progress concern whether or not there is a loss of recurrent inhibition on the DGC before WDS can be elicited. If such is the case we will determine whether or not delta receptor antagonists can restore this recurrent inhibition. The effect of enkephalin on dentate granule cells and basket cells (inhibitory internuncial neurons) will be examined in hippocampal slices and in hippocampal cells grown in tissue culture in order to characterize the mechanism by which enkephalin affects these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90045-01 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Studies on the Relationship Between Opioid Peptides and Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jau-Shyong Hong	Pharmacologist	LBNT	NIEHS
Others:	L. Grimes	Guest Worker	LBNT	NIEHS
	P. Lee	Visiting Fellow	LBNT	NIEHS
	J. Obie	Biological Lab Technician	LBNT	NIEHS
	C. Mitchell	Pharmacologist	LBNT	NIEHS
	H. Tilson	Pharmacologist	LBNT	NIEHS
	T. Kanamatsu	Visiting Fellow	LBNT	NIEHS
	K. Takeuchi	Guest Worker	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neuropharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purposes of this project were: 1) to determine alterations of the metabolism of enkephalin and dynorphin in the limbic-basal ganglia regions after electrical stimulation-induced [electroconvulsive shock (ECS), or amygdaloid kindling] or chemical-induced [kainic acid (KA)] seizures; 2) to study the possible roles of brain opioid peptides in the expression of preseizure behaviors, such as wet dog shakes (WDS) or seizure activities after KA administration. Both repeated ECS and amygdaloid kindling produced a profound alteration of the metabolism of both enkephalin and dynorphin in different brain regions. Repeated ECS caused an increase in the level of [Met^5]-enkephalin-like immunoreactivity (ME-LI) in the limbic-basal ganglia regions. Measurement of the abundance of mRNA coding for enkephalin suggested that the increase in ME-LI after repeated ECS is the result of an increased biosynthesis of this peptide. KA produces WDS and behavioral convulsions in the experimental animals. We have attempted to illucidate the possible relationship between hippocampal opioid peptides and KA-induced seizure-related behaviors. KA caused an initial release of both ME-LI and DN-LI during the period of recurrent convulsions which was followed by a large rebound in ME-LI and a modest increase in DN-LI 2 days later. We have obtained the following evidence suggesting that the profound change in the metabolism of enkephalin may be related to KA-induced WDS. (1) Naloxone, an opiate antagonist, caused a dose-dependent attenuation of KA-induced WDS. (2) Intraventricular injection of antibodies against [Met^5]-enkephalin, but not dynorphin A (1-8) caused a significant reduction in KA-induced WDS. (3) Colchicine lesions of ME and DYN containing granule cells eliminate KA-induced WDS. These results support the concept that enkephalin may mediate KA-induced WDS. We plan to employ more specific opiate agonists and ontagonists injected to different brain regions in KA-treated rats in an attempt to further understand the roles of opioid peptides in seizures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90046-01 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nervous System Effects of Microwave Radiation - U.S.-U.S.S.R. Duplicate Projects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donald I. McRee	Research Physicist	LBNT	NIEHS
Others:	C. L. Mitchell	Pharmacologist	LBNT	NIEHS
	H. A. Tilson	Pharmacologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

A. N. Marzeev Institute of General and Communal Hygiene, Kiev, U.S.S.R.

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Non-Ionizing Radiation

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

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

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

A duplicate project was jointly planned by U.S. and U.S.S.R. scientists to study the effects of microwave radiation on the nervous system. The purpose of the duplicate project was to compare experimental methodologies and to determine response indicators which would be sensitive to exposure to microwave radiation. Fischer-344 rats were exposed for 7 hours for one day to 2.45-GHz microwave radiation at an incident power density of 10 mW/cm^2 after eight days of adaptation to the exposure cages and EEG connections. EEG measurements before, during and after exposure were made and light evoke potential measurements were also recorded before and after exposure. The behavioral test used to evaluate changes were passive avoidance to foot shock and activity in an open field. Biochemical measurements were made immediately after exposure in the rat cerebral cortex. Na^+K^+-ATPase, Mg^{2+}-ATPase, $Mg^{2+}Ca^{2+}$-ATPase activities and alkaline phosphatase were measured. Results of the EEGs showed a shift in frequencies at various times during the exposure period. The shifts were in the direction of a greater percentage of the spectrum being in the lower frequencies of the EEG. The exposed and control animals did not respond differently to the light evoke stimulation of the cortex. No differences were observed in the passive avoidance response and open field activity. The data from the biochemical measurements showed that Na^+K^+-ATPase was the only biochemical parameter significantly changed by the exposure. A lower Na^+K^+-ATPase activity was observed in the synaptosomes of the cortex in the exposed animals.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90047-01 LBNT

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LBNT	NIEHS
Others:	W. E. Wilson	Research Chemist	LBNT	NIEHS
	B. J. Irons	Biological Lab Technician	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina, Chapel Hill
University of Kyoto, Japan

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Peptide Neurochemistry

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

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Neuromedin B is a neuropeptide originally isolated from spinal cord and belongs to the ranatensin/litorin family of peptides which are related to bombesin. Heterologous antisera against neuromedin B was raised in rabbits and exhibited a wide range of specificities: one antiserum had an absolute specificity for the carboxy terminal tetrapeptide, thus cross-reacting equally well with ranatensin and litorin, but not bombesin; other antisera had slight recognition (0.1-2%) of these peptides. None of the antisera cross-reacted with substance P which shares the carboxy terminal Met-NH_2 residue. The appropriate extraction conditions for neuromedin B-related peptides revealed that either acidic or basic conditions yielded the highest concentrations from spinal cord, whereas the incorporation of organic solvents in the extraction method markedly reduced the values obtained.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90048-01 LBNT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Physiology and Pharmacology of Neuropeptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence H. Lazarus	Research Chemist	LBNT	NIEHS
Others:	W. E. Wilson	Research Chemist	LBNT	NIEHS
	B. J. Irons	Biological Lab Technician	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

University of Rome, Italy
University of North Carolina, Chapel Hill

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

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

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

The central neuromodulation of gastric secretion by bombesin (and bombesin-related peptides) and the new morphinomimetic peptide dermorphin were investigated using the laboratory rat as the model system. The intracerebral ventricular (icv) administration of bombesin regulates gastric secretion in pylorus-ligated rats: neutralizes gastric acidity, reduces hydrogen ion concentration, and decreases the volume of secretion. Only peptides closely resembling bombesin, such as alytesin and GRP (mammalian heptacosapeptide resembling bombesin) had comparable bioactivity. Indomethacin, a potent inhibitor of prostaglandin biosynthesis, prevented the bombesin-mediated effects when administered icv. These results suggest that the neuromediation of gastric secretion by bombesin involves the release of prostaglandins in brain. Dermorphin, an amphibian opiate-like heptapeptide containing a natural D-amino acid, effectively reduces gastric secretion when administered icv. The analysis of 19 homologues and analogues of dermorphin provided evidence on the sequence specificity of the neuroregulation of gastric acid output. The amino-terminal tetrapeptide amide provided the smallest biological active fragment (similar to the limit digestion product of dermorphin in a brain homogenate). The D-isomer of Ala residue at position 2 is indispensible for the retention of bioactivity. The dosages of dermorphin utilized to suppress gastric secretion were not only without effect on analgesia or catalepsy, but also the analogues displayed differential effects in respect to these physiological parameters. Thus, dermorphin acts centrally in the brain, presumably interacting with either mu receptors, or subtype thereof, to regulate gastric secretion.

LABORATORY OF GENETICS
Summary Statement

Eukaryotic Gene Structure: Members of this section are studying the nature of genes and how they function. Selected genes are being studied genetically and molecularly to gain information about their organization, function, regulation, mutation and evolution. Particular emphasis is focused on 1) the molecular structure of genes and how they are regulated in development, 2) the role of transposable elements in mutation and 3) the genetic diversity that exists in populations of organisms, the nature and amount of that diversity and the factors that influence it.

As the number of genes in Drosophila melanogaster that are molecularly cloned and characterized increases, it is evident that a major fraction of spontaneous mutations are caused by the insertion/excision of transposable elements. Laboratory members are investigating several aspects of transposon-induced genetic change. First, a number of transposon-induced mutations alter the regulation of the gene into which the transposon has inserted. These present an opportunity to study mechanisms of gene control as well as transposon-mediated mutation. Such a series of mutations at the white locus is being investigated by the Judd group. The retrovirus-like transposon BEL inserted into an intron of the white gene produces the mutation w^{zm}, which gives no change in white locus phenotype unless combined with a mutation of the zeste locus, z^1. In that combination the eye color of the fly becomes mosaic for mutant (yellow) and wild-type (red) eye color. The w^{zm} mutation is mutationally unstable and has produced a family of derivatives some of which are the null class with bleached white eyes; others are similar to w^{zm} and have mosaic phenotypes where the pattern of expression is cell autonomous and clonal. Another group of w^{zm} derivatives is associated with the transposition of the entire white region and some adjoining genes into the third chromosome. These derivatives also show mosaic expression of white locus when homozygous and combined with z^1. The mosaicism in these, however, is non-autonomous and non-clonal in origin. Both classes of w^{zm} derivatives in their interaction with z^1 show a proximity or pairing effect known as transvection. This interesting aspect of gene regulation is being investigated at the molecular level to try to understand the mechanism of regulators for the loci.

Second, the Judd group has been studying a set of asymmetrical exchange products generated by recombination between transposons located at different places in homologous chromosomes. A number of years ago Judd reported that the expected recombination products from heterozygotes for the white locus alleles w^{bf}/w^a were not recovered but four classes of unexpected recombinants (thought to be two sets of reciprocal duplication and deficiency chromosomes) occurred with regularity. Those recombinant and parental chromosomes have now been examined at the molecular level and the basis for the unusual recombination events has become clear. In the w^{bf} chromosome two copies of the transposon roo are located about 32 kb apart, one within the white locus (to cause the w^{bf} mutation) and the other located proximal to white. In the w^a chromosome the transposon copia is inserted in the white locus to cause the w^a mutation. It was discovered that in the w^a strain used in these experiments roo had invaded copia, to create a new allele, w^{ric}, but had not changed the phenotype from that of the original w^a significantly. In the heterozygous condition the three

copies of roo can pair in either of two patterns, each pattern producing duplication and deficiency products by crossingover. The remarkable aspect is that transposons located at such distant positions can still establish pairing and undergo exchange. Considering the large number of families of transposons and their high copy numbers, the question is what prevents extremely high frequencies of asymmetrical exchanges. The products of such exchanges would often be lethals, resulting in a high mutational burden to the population.

Third, the Voelker group has been examining a gene, su(s), that when mutant suppresses specific mutations at any of several loci that are due to insertions of a transposon. Among the mutations suppressed by su(s) are several alleles of vermilion, which has been cloned and characterized by the Searles group. The mobile elements 412 and B104 have been determined to be responsible for the vermilion suppressible mutations. Dr. Voelker has cloned and characterized the su(s) DNA and a large number of mutant alleles. All of the spontaneous mutations contain non-su(s) DNA at the locus, apparently inserted transposable elements. The locus produces a 5 kb poly-A^+ mRNA spanning about 8 kb of genomic DNA. S1 protection mapping shows at least 5 exons. The su(s) region has been placed in an expression vector to produce a part of the protein product that can be used to raise antibodies against the su(s) product. This step will allow Dr. Voelker to gather information about the normal function of the gene and possibly provide clues to the mechanism of suppression of transposon-induced gene changes.

Fourth, Dr. Johng Lim of the University of Wisconsin, Eau Claire is a collaborator with Drs. Judd and Jack in the investigation of a highly mutable system involving the mobilization and amplification of the retrovirus-like transposon gypsy. In certain crosses, the offspring-of F_1 females show high levels of mutation associated with the insertion of gypsy into new chromosomal sites. F_1 males on the other hand are stable. Gypsy is mobilized in somatic cells to produce mosaic somatic mutations and clusters of mutations that are transmitted through the germ line. Reciprocal crosses produce similar results, suggesting that cytoplasmic factors are not playing prominant roles in the activation of the transposon. Genetic and molecular studies of the transposon and the mutable strains are being carried out to determine the conditions that raise the insertion/excision of gypsy to very high levels.

Fifth, the Langley group is studying a novel mutator found by Dr. Claude Hinton at Wooster College. The mutator is found in D. ananassae where is causes an abnormal eye phenotype, Om. The curious aspect is that 21 different loci produce essentially the same phenotype. Dr. Langley was able to identify a 6.5 kb insert as the element responsible for the mutator effect. Four different Om loci, studied by recombination, in situ hybridization and molecular analysis showed complete linkage of the inserted element and the Om phenotype. The element, tom is like the copia family of transposons with large direct repeat sequences at the termini. Restriction enzyme maps of several Om mutations indicate that at some sites a single tom element is present while at others multiple copies are found. These studies are continuing to try to discover the basis for the rather specific morphological changes imposed by this transposon inserted at any of a large number of chromosomal sites.

Sixth, at the population level, the Langley group is investigating the deleterious effects of individual transposable elements inserted into chromosomes of Drosophila. Insertions of elements often have striking morphological or viability effects, but a majority of them are not associated with any known effect. Dr. Langley reasoned that if natural selection is acting to remove individual insertions because of their deleterious effect on adjacent genes, then X chromosomes, because they are hemizygous in males, would be expected to have fewer elements than autosomes. A survey of the distribution of a set of retrovirus-like transposons in Drosophila polytene chromosomes by in situ hybridization produced no evidence for selection against those transposons in X chromosomes. The control of transposon copy number appears then to depend on forces other than natural selection against individual insertions. A model based on removal of transposons through creation of aneuploid chromosomal rearrangements by recombination between transposons at non-allelic positions in the genome is being tested.

A number of projects by investigators in the Eukaryotic Gene Structure Section focus on the organization and function of specific genes and how they are regulated in development. Dr. Boswell, who has accepted a faculty position at the University of Colorado, has been investigating genes that function in germ cell determination and differentiation. Operating under the concept that the fate of embryonic cells is regulated by morphogenetic determinants localized in ooplasm, Dr. Boswell has identified mutants in Drosophila that have a maternal effect on germ cell formation. Such mutants are grandchildless because both male and female offspring from mutant females lack germ cells and are thus sterile. He has focused on one such locus, tudor, and found that different mutant strains contain reduced and varying amounts of polar granule material in posterior pole plasm of the eggs produced by homozygous mutant females. The ability or inability of the developing embryo to form germ cells is correlated directly with the amount of assembled polar granule material seen in the germ plasm. Dr. Boswell has shown that normal assembly of these granules is disrupted in embryos from tudor mothers. A number of X-ray induced alleles of tudor are chromosomal rearrangements that have facilitated the cytological mapping of the locus and have helped in the molecular cloning of tudor DNA. The molecular analysis of the gene's organization, function and its developmental regulation is continuing in the Boswell laboratory at the University of Colorado.

Another developmentally regulated gene of great interest is the cut locus, studied by Dr. Jack. This is a very large gene, extending over more than 0.5 map unit and about 200 kb of DNA. Mutations clustered in the most centromere-distal region cause developmental abnormalities primarily in the legs of adult Drosophila. Mutations in the central portion of the gene cause wing defects and a cluster of lesions about 70 kb more proximal to those affects wings, head and thorax and causes lethality. A cluster of mutant sites near the most proximal border of the gene causes lethality and lacks all cut locus function. Dr. Jack has characterized at the molecular level a very large number of alleles and is now studying the RNA transcripts produced by the gene. Drs. Lily and Yuh Nung Jan at the University of California, San Francisco, have discovered that in embryos homozygous for cut lethal mutations, a particular set of sensory neurons is transformed into neurons of another type. In addition the sensory organs innervated by the neurons are also transformed. Dr. Jack has accepted a position at the Sloane-Kettering Cancer Center where he will continue the analysis of the cut locus and his collaboration with the Jans.

The work on the vermilion (v) locus carried out by Dr. Searles was mentioned previously in conjunction with the su(s) locus because some alleles of v are suppressible by su(s). The vermilion gene product, tryptophan oxygenase, is required for the first step in the synthesis of brown pigments in Drosophila. Dr. Searles has cloned the v locus DNA and determined the structure of the gene and a number of its alleles. It is a small gene about 2 kb in length that produces a 1.4 kb transcript. There are three spontaneous mutations that are suppressible by su(s), all of which result from the insertion of the transposon 412 into the locus. There are no detectable v transcripts in poly-A^+ RNA isolated from these three mutants but in combination with su(s) there is partial restoration of wild-type size v transcript. It appears then that the insertion of the transposon disrupts transcription of v and that somehow the su(s) mutation restores a low level of normal transcription. The mechanism for this disruption and its restoration is not understood. Dr. Searles will continue the study of the v locus function and the mechanism of transposon suppression at the University of North Carolina, Chapel Hill where she has taken a faculty position.

Drs. Voelker and Searles have now completed their collaboration with Dr. Arno Greenleaf, Duke University on the study of genes encoding components of the RNA polymerase II transcription complex. They identified a locus, RPII215, that encodes the 215,000 dalton subunit of the polymerase complex, mutant forms of which can confirm α-amanitin resistance to RNA polymerase II. The locus was molecularly cloned, using P-element induced mutants of the gene. RNA transcripts were identified and studied relative to the complementation groups that are detected genetically. Some correlation between complementation pattern and transcription has been established, but there are more transcripts than complementation groups. All transcripts appear to relate only to the 215,000 dalton subunit, however. This work is continuing in Dr. Greenleaf's laboratory.

Dr. Li's group is analyzing the structure, function and regulation of the genes encoding mammalian lactate dehydrogenases. Five isozymes of LDH are found in various proportions of A and B subunits among different somatic tissues. The homotetrameric LDH-C4 is found primarily in testes and sperm. The complete amino acid sequences and nucleotide sequences of LDH-A4 isozymes from human and mouse have been determined. In human cancer, an increase is noted in LDH activity and unusual isozymes LDH-K, LDH-Z and an altered configuration of LDH-A are reported. An important recent finding by Dr. Li is that the cancer-associated LDH-K isozyme induced by Kirsten sarcoma virus is a modified form of human LDH-A4 complexed with ras P21 protein. He has shown that bovine LDH-A4 is phosphorylated in vitro by src tyrosine kinase.

Recently the LDH-A4 protein was found to bind single strand DNA while LDH-B4 isozymes show only weak ssDNA binding. The low-salt eluting ssDNA binding protein from mouse myeloma was shown to have LDH enzymatic activity and to cross react immunologically with LDH-A4 isozymes.

The mouse LDH-A functional gene and four different pseudogenes have been isolated and compared by the Li group. The organization of the gene shows six introns in the translated region and one in the 5' untranslated region. Initiation sites of transcription and translation have been identified, as have some putative regulatory sequences. A comparison of the functional gene with a pseudogene sequence shows about 87% homology over the 1.7 kb length. There were about twice as many transitions (114) as transversions (65) followed by

deletions/insertions numbering 36. Only four out of 25 CpG dinucleotides present in the cDNA sequence remained unchanged.

Recently the molecular cloning of LDH-C has been accomplished by Dr. Li's group. Future plans include the isolation of LDH-B clones as well, the complete molecular characterization of the organization and expression, and the evolutionary relationships that exist between isozymes in several mammalian species.

The Li group, in collaboration with Drs. C. L. Lee and T. M. Chu of Roswell Memorial Park Institute has also been studying two prostatic acid phosphatase isozymes (PAP-I and PAP-II). The proteins have been purified and partially characterized. A glycoprotein (GP), immunochemically and functionally related to PAP has also been characterized. It is clear that PAP-I and PAP-II are different isozymes and that GP is a distinct glycoprotein that shares some common enzymatic and antigenic characteristics with PAP. The complete primary structures and the antigenic determinants of these proteins as well as some human acid phosphatase isozymes purified from lung, kidney, bladder, spleen, pancreas and liver will be determined and compared in future work.

The Johnson group is investigating spontaneous and induced mutations in mice. Germinal mutations are detected at specific loci by examining electrophoretic characteristics of about 30 different gene products. A large number of mutations induced by ethylnitrosourea, (ENU), x-rays and ethylene oxide have been detected along with several mutations of spontaneous origin. Animals derived from the electrophoretic studies have also been subjected to a series of measurements on skeletal structures. Using quantitative measurements and multivariate statistical techniques, comparisons were made between the observed morphological variation and the induced mutation frequencies measured by the electrophoretic screening tests. The progeny of ENU treated parents appeared to have less skeletal variation than the control animals; an interesting observation considering this is in contrast to results obtained by other investigators, whose data have been widely used as a basis for genetic risk assessment.

Dr. Johnson has also been involved in the analysis of some of the mutants recovered in the electrophoretic screen. One of these, apparently of spontaneous origin, produces a β-thalassemia. Analysis shows it to be due to a deletion.

The Malling group is studying *in vivo* mutagenesis at the molecular level in mammals. A major hurdle in detection of genetic damage directly in DNA is that genes for the most part are single copy per genome. Dr. Malling has been developing systems designed to use well characterized DNA sequences that are amplified in mammalian cells, such as mitochondrial DNA, or that can be isolated, cloned and analyzed *in vitro*. The virus ϕX174 is being tested for use in the latter approach. Mouse *L cells* were transformed with ϕX174 containing the mutations *am3* and *cs70*. ϕX174 DNA sequence is completely known and reverse mutations of *am3* and *cs70* can be conveniently scored. Techniques for recovery and analysis of the ϕX174 DNA are now being worked out and plans are being made to produce transgenic mice carrying ϕX in the nuclear genome so that mutations in different somatic tissues at various stages of gametogenesis can be recovered, studied and compared.

Mitochondrial DNA isolated from cultured cells or from mammalian tissues can also be monitored for sequence changes since its structure is well characterized. Plans include screening cloned mtDNA for restriction site changes following treatment with mutagens.

The Langley group in collaboration with Dr. Shiu Huang have been characterizing spontaneous mutations in the hypoxanthine-guanine phosphoribosyl transferase gene (HGPRT) of humans. Primary cell cultures established from neonatal foreskins were screened for HGPRT mutations by selection in 6-TG medium. DNA purified from each of the mutants was digested with various restriction endonucleases and, following fractionation and blotting, was probed with labeled HGPRT DNA. Eighteen mutant clones have thus far been analyzed and seventeen show no detectable changes in restriction enzyme patterns relative to wild type. One mutant showed modified sites for three restriction enzymes and had no detectable HPRT activity. Nine of the seventeen mutants that have normal restriction patterns have been analyzed for HPRT activity and all show some level of enzyme activity.

A survey of genetic variation in natural populations of Drosophila is being carried out by Drs. Kreitman, Simmons and Langley. Two loci, Alcohol dehydrogenase (Adh) and white (w) are being examined at the nucleotide level in samples of about 100 lines from each of several populations of D. melanogaster and a single population of its sibling species, D. simulans. The technique involved here is the use of restriction endonucleases that have four-nucleotide specificies. This allows identification of essentially all haplotypes for the genes being surveyed. Dr. Kreitman has accepted a faculty position at Princeton University and will continue this work there. He will continue to collaborate with Dr. Simmons who is studying geographic patterns of polymorphism of Adh using the techniques described above. Isogenic lines of Drosophila from three east coast populations span the region containing a well studied cline in Adh allozyme frequencies. The objective is to test hypotheses on the origin and maintenance of the allozyme cline.

Mutagenesis: Members of this section evaluate the molecular and genetic mechanisms of mutational processes in model systems ranging from bacteriophage to mammalian cells in culture. Of particular interest is defining the general and specific pathways by which mutations may arise. These studies provide important information concerning the genetic interaction of environmental mutagens and have implications in the study of carcinogenesis and the etiology of human genetic disease.

The Drake group continues the analysis of error-prone repair (EPR) systems utilizing bacteriophage T4. The WXY EPR system of T4 appears to be similar to the SOS system of E. coli with regard to damage processing and mutagenic response. However, little is known of the molecular mechanisms involved in either of these important pathways. The elegance of T4 as a genetic system allows specific responses to mutagenic DNA damage to be defined within a limited number of genes that may be easily manipulated for further characterization. A number of genes, e.g., 32, 46, 47, 49, 58, 59, and hm, encoded by T4 are thought to act in an accessory role in the mutagenic processing of DNA damage. Studies are currently underway to evaluate the contribution of these accessory genes to known mutagenic pathways. In specifically analyzing the WXY EPR response, temperature-sensitive (ts) mutations of uvsX and uvsY have been isolated, characterized,

and initial results suggest the involvement of a recombinational intermediate either at or near the site of the EPR response. These data imply a more complex mechanism for EPR than the traditionally held view of a simple EPR-mediated reduction of polymerase fidelity. The success of studies utilizing ts mutants of X and Y has motivated the isolation of a series of ts mutations in the W gene. In addition, the uvsX gene has been cloned, and sequenced, and is being inserted into an expression vector for isolation of the protein. In related studies, a clone of the uvsW gene has been obtained and is currently being characterized at the molecular level. Finally, specific mutations in gene 32 (single-stranded-DNA binding protein) and gene 41 (resolvase) have been used to study a newly proposed DNA repair pathway. Genetic studies clearly indicate that this pathway is separate from the WXY EPR system. Moreover, this pathway appears to operate during DNA replication and may involve the uncoupling of leading and lagging-strand DNA synthesis.

The Sugino group studies eukaryotic DNA replication, repair and recombination utilizing yeast as a model system. Using a yeast 2μ plasmid plus another yeast plasmid with an autonomously replicating sequence (ARS), it has been possible to fractionate a crude yeast extract and identify various components that are involved in replication in vitro. A number of proteins have been identified that together can replicate these plasmids in vitro. They include two recently isolated proteins, ATPase III (which also has a DNA-helicase activity) and DNA topoisomerase II. In addition to these proteins, three previously identified single-stranded-DNA binding proteins (ySSBs) and three different RNase Hs have been purified to near homogeneity and antibodies have been produced. The in vitro studies have provided detailed information concerning the effects of several of these accessory proteins in yeast Pol I-mediated DNA replication. These studies have been simplified by the development of large-scale purification techniques for the isolation of the Pol I "core" enzyme. The in vitro results indicate that the three different ySSBs appear not to effect the processivity or the accuracy of yeast Pol I replication but rather influence replication by preventing nonproductive template binding of the Pol I replication complex. A system has been developed to further access the role of ySSBs in replication. The ATPase III protein (ostensibly under the regulatory control of RAD 3) has been shown to increase the processivity of yeast Pol 3- to 5-fold in a catalytic fashion, implying a direct interaction between yeast Pol I and ATPase III. RNase H has also been assigned a role as a yeast Pol I subunit or accessory protein.

Genetic studies have produced more than 100 new temperature-sensitive DNA replication mutants useful for the analysis of identified proteins and cloned genes involved in replication, recombination and repair. The genes for three different ySSBs and two RNase Hs have been cloned and sequenced. Gene disruption experiments provide a method for the analysis of the in vivo role of these proteins. Such studies indicate that at least one protein (20 kd SSB) is essential for viability. An interesting aspect of the function of this protein is that deletions involving the C-terminus retain the essential functions of the protein, i.e., viable cells can be recovered; however, the recovered cells are now UV-sensitive. This result argues that the 20 kd protein may play a role in both replication and DNA repair. A modification of the well defined in vitro replication system described above has been developed to the isolate and characterize proteins involved in yeast recombination. Although the RAD 52 gene is cloned, the gene product (essential in recombination and repair) has been shown to be

extremely unstable and rapidly degraded in vivo and, therefore, has eluded isolation in several labs. Antibodies directed against synthetic oligopeptides derived from the known protein sequence of RAD 52 have been developed and will be used for the identification and purification of the RAD 52 gene product. In related studies, the RAD 18-1 allele, which is associated with a mutator phenotype and with radiation sensitivity, has been cloned and partially characterized. This allele may code for an altered subunit of Pol I. Therefore, isolation of the Pol I holoenzyme from rad18 and comparisons with the holoenzyme isolated from wild-type cells is underway.

The Schaaper group continues to explore mechanisms of mutation induction in E. coli both in vivo and in vitro. The mutational target for the in vivo studies is the lacI gene. This program has been significantly augmented by the development of techniques for the rapid analysis of lacI$^-$ mutations by recombinational transfer of the mutant lacI sequences to a recipient single-stranded-DNA phage vector for DNA sequence analysis. This system has been useful in the analysis of both spontaneous and UV-induced mutagenesis. More specifically, the spontaneous spectrum of mutations derived in an E. coli strain that is wild-type for DNA repair has been produced and compared with the spontaneous mutational spectra observed in the E. coli mutH, mutL, and mutS mutator strains. These data have provided clues as to role of the mismatch repair system in response to replication errors. The UV spectrum indicates that both the cyclobutane dimer and the pyr(6-4)pyo lesions may be mutagenic under SOS-induced conditions. In addition, the mutational spectra allow the distinction of specific mutations and/or mutational sites as being diagnostic of normal replication errors vs SOS-dependent processes.

The in vitro studies utilize a replication system in which the accuracy of DNA damage processing in the presence of cell extracts or other modifiers, (e.g., dNTP pool alterations) is measured in the sensitive lacZ α-complementation assay. A single-stranded phage carrying the lacZ α region is replication in vitro and mutations induced in the lacZ target region are scored following DNA transformation of E. coli. The replication mix can be supplemented to access the role of specific factors in the induction of mutation during replication of either an undamaged or a damaged template. Initial results indicate that the accuracy of replication in a defined reaction mix approaches the accuracy of in vivo replication. Analyses of the effects of perturbations of the nucleotide pools during the reaction, as well as assessment of the effects of the addition of crude extracts from SOS-induced cells, are in progress. Notably, this system has demonstrated reduced accuracy with extracts derived from mutD5 strains which are deficient in the 3' - 5' exonuclease proofreading function. These data are consistent with the in vivo mutator phenotype of mutD5 strains.

The Kunkel group has refined the phage M13mp2 lacZ α-complementation assay for use in studies of the fidelity of replication in vitro and in analyses of mutagenesis by defined DNA lesions within the lacZ α target. Using this system, an experimental measure has been obtained of the in vitro accuracy of replication for yeast DNA Pol I as well as for the three major animal cell polymerases (α, β and γ). An interesting result of the yeast Pol I studies is that the highly purified form of the enzyme generates many deletions in the in vitro assay. These results have provided insights into the mechanisms of deletion errors during replication. Studies are currently underway to evaluate the effect of yeast accessory proteins on both the quantitative and qualitative nature of

Yeast Pol I replication errors, (e.g., mutation frequency, site specificity, incidence of transitions, transversions and frameshift-mutations). Mutational specificities were observed with the animal polymerases as well. Using frameshift reversion assays, a detailed study of the mechanisms of induction of frameshift mutations using animal cell polymerases has provided a correlation between processivity and replication fidelity. In addition, other studies have provided *in vitro* support for both the Streisinger and the dislocation models of frameshift mutagenesis. Finally, non-templated DNA synthesis by TdT (terminal deoxynucleotidyl transferase) has long been thought to serve in the generation of immunoglobulin diversity. The production of complex mutations by TdT using the M13mp2 *lacZ* α system has provided data bearing on the *in vivo* role of TdT.

The role of specific premutagenic DNA lesions has also been explored using the M13mp2 *lacZ* α system. The earlier application of this system to the study of the abasic site as a premutagenic lesion has now been followed by a detailed analysis of cytosine deamination. The deamination of cytosine produces uracil and, therefore, could lead to GC → AT transitions. Rate constants, activation energies and the effects of local DNA sequence on the frequency of deamination at specific sites all have been studied. Of particular interest are the possible local effects of DNA sequence. Such studies have been simplified by the development of a method for site-directed mutagenesis that yields mutations within a target region with extremely high (40-60%) efficiency. Thus, the DNA sequence can be readily modified, providing insight into local sequence effects. A separate project, in collaboration with the laboratory of A. Sancar (UNC, Chapel Hill, NC), examines the substrate recognition for the *E. coli* ABC excision nuclease. These studies indicate that undamaged mismatched bases involving one, three, or four extrahelical bases are not recognized by the ABC enzyme complex unless they are chemically modified. Furthermore, extrahelical bases located near UV photoproducts do not inhibit incision by the ABC enzyme complex but may affect the incision pattern.

The Tindall group studies molecular mechanisms of mutation in mammalian cells using a chromosomally integrated *gpt* gene as a mutational target. This laboratory has focused on a well defined Chinese hamster ovary (CHO) cell line with a single copy of the *gpt* gene stably integrated into the CHO genome. The *gpt* gene is the bacterial equivalent of the mammalian *hgprt* gene. Use of an SV40 promoter to express the *gpt* gene in mammalian cells provides a useful target for molecular studies allowing easy manipulation of the 456 base-pair *gpt* structural gene. In contrast, the CHO *hgprt* structural gene contains 654 base pairs but, with introns, is distributed over 35-40 kb of the CHO genome. The cell line with the single *gpt* integration, designated AS52, has been extensively characterized with regard to spontaneous and induced mutation frequencies at the *gpt* and *hgprt* loci. An interesting aspect of these studies is that the frequency of induced mutations at *gpt* or *hgprt* is quite similar when standard point mutagens are used as the inducing agent, (e.g. UV, EMS, ICR-191). However, if X-rays or a variety of radio-mimetic/clastogenic agents are used, the mutation frequency at *gpt* is substantially greater than at *hgprt*. These data support the hypothesis that large deletions at the *hgprt* locus may extend into adjacent sequences required for viability and, therefore, lead to dominant lethal events. The site of integration of the *gpt* locus, however, is proposed to permit viable progeny following large deletions, thus resulting in a higher observed mutant frequency. Experiments are in progress to determine the chromosomal location and molecular organization of *gpt* in the AS52 cell line. In addition, approximately 250

independently isolated gpt spontaneous and induced (UV, EMS, ICR-191) mutants have been partially characterized for sequence analysis of point mutations induced in the AS52 cell line. A collaborator in these studies is L. F. Stankowski (Pharmacon Research International, Inc., Waverly, PA).

In collaboration with P. de Jong of the Sugino group, defective retroviral shuttle vectors have been developed for the study and rapid recovery of chromosomally integrated target genes. In these studies, the gpt gene has been selected as a target sequence and the vector includes a linked neo gene as well as SV40, ColE1 and M13 origins of replication for the convenient shuttling of mutant sequences from between mammalian and E. coli cells. The gpt and neo genes are expressed in mammalian cells using the Mo-MLV LTR. The vector also includes a supF allele in the LTRs for the easy recovery of deletions or rearrangements of the gpt sequences by direct cloning into λ libraries. Passing the retroviral DNA through an amphotropic packaging line allows the production of defective retroviral particles that will infect a variety of cell lines, usually resulting in a functional single-copy integration of the vector sequences. Thus, one can study mutation at the gpt locus in a variety of cell lines. Initial results indicate that the vector is functionally expressing both the gpt and neo genes. Characterizations of gpt integrations of human cell lines that have proven useful in mammalian cell mutagenesis studies are currently underway.

P. de Jong also studies the molecular basis of mutation in mammalian cells. Two approaches have been developed for such studies. First, the chromosomal adenine phosphoribosyl transferase (aprt) gene has been utilized as a target gene for mutagenesis studies in Chinese hamster ovary (CHO) cells. This project has relied upon collaboration with the laboratory of B. W. Glickman (York University, Toronto). With the development of λ cloning vectors that allow the rapid isolation of mutant aprt sequences, DNA sequence spectra have been generated for spontaneous as well as for UV- and gamma-ray-induced mutations in CHO cells. Initial results indicate that spontaneous and induced mutations in mammalian cells show both agent-specific and site-specific responses. These results parallel observations of specificities in DNA sequence mutational spectra in microbial and phage systems. There appears to be a strong bias among the spontaneous mutants for GC → CG tranversion (3/4 sequence mutants) as opposed to the GC → AT transitions normally associated with UV mutagenesis in lower organisms. Forty gamma-ray-induced mutational alterations are point mutations or small deletions. In seven mutants sequenced, transitions, transversions, and frameshift mutations have all been observed, with no apparent hot-spots. While the data are intriguing, the number of sequenced mutants is quite small and more sequences are required to fully evaluate the specificity of UV- or gamma ray-induced mutations in CHO cells.

A second approach to the study of chromosomally induced mutations in mammalian cells has made use of defective retroviral shuttle vectors. The vector expresses the aprt and neo genes utilizing an LTR. Also included are the SV40, ColE1, and M13 origins of replication, as well as a supF allele positioned in both LTRs. Thus, mutant sequences can be shuttled between mammalian cells and E. coli while the supF gene allows the isolation of deleted or rearranged vector sequences. Amphotropic packaging cell lines produce defective virus that will infect a wide range of host cells. Integration of a single copy of the virus into the chromosomal DNA thus allows the use of the aprt as a target gene

for mutagenesis studies and the linked neo gene provides a marker for the easy recovery of mutant aprt sequences. Preliminary results indicate that the aprt, neo vector is functional in several mammalian cell lines. However, most aprt mutations involve deletions of rearrangements of the aprt sequences. Work is currently underway to identify a stable pro-viral insert. A parallel effort in collaboration with K. R. Tindall involves the construction of a defective retrovirus expressing the gpt and neo genes. Dr. de Jong will assume a position elsewhere in the Fall of 1986. The analysis of aprt chromosomal mutations will be continued by the Glickman group (York University). The retroviral vector project will be continued by K. R. Tindall (NIEHS), B. W. Glickman (York University) and P. de Jong (elsewhere).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60099-07 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Organization-regulation of mammalian lactate dehydrogenase genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Ikuya Sakai	Visiting Fellow	LG, NIEHS
	Kayoko M. Fukasawa	Visiting Fellow	LG, NIEHS
	Fu-zon Chung	Visiting Fellow	LG, NIEHS
	Esther W. Hou	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
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SECTION
Eukaryotic Gene Structure Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

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

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

Mouse LDH-A functional gene and four different pseudogenes have been isolated. The complete sequence of 12,856 nucleotides of mouse genomic clone M15 containing LDH-A functional gene has been determined. The initiation sites of transcription and translation, and the putative regulatory sequences including the hexanucleotides CCGCCC and the cAMP-responsive sequence AAATCTTGCTCAA of mouse LDH-A gene have been identified. Seventeen human genomic clones containing the LDH-A functional gene and several pseudogenes have been isolated, and the exon-intron organization of human LDH-A functional gene of 12 Kb has been determined. The protein-coding sequence of the LDH-A genes from both mouse and human is interrupted by six introns. An additional intron was found in the 5' untranslated region, while there was no intron present in the entire 3' untranslated sequence. The relationships between the exon-intron organization of the LDH-A gene and the structural-functional domains of LDH-A protein are illustrated. The nucleotide sequences of LDH-A processed pseudogenes present in four different mouse genomic clone and two human genomic clones have been determined. A sequence comparison between LDH-A cDNA and processed pseudogenes revealed the types and rates of nucleotide changes, presumably without selection pressure, and these results have significant implications on spontaneous mutations and molecular evolution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60146-04 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mutagenic Consequences of Defined Lesions in DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Senior Staff Fellow	LG NIEHS
Others:	L. Frederico	Guest Worker	LG NIEHS
	K. Bebenek	Visiting Fellow	LG NIEHS

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D. Thomas, Postdoctoral Fellow, Dept. of Biochemistry, UNC
A. Sancar, Assistant Professor, Dept. of Biochemistry, UNC

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Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.2	1.2

CHECK APPROPRIATE BOX(ES)

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

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

The mutagenic consequences of introducing defined lesions into DNA are being examined in a mutational system capable of detecting a wide spectrum of mutational events, both at and some distance away from the actual site of damage. Our efforts are focused on two lesions known to present major challenges to the stability of genetic information in animal cells, depurination and deamination. Both have been found to be highly mutagenic for base substitution errors, the former for transversions at purine bases, the latter for transitions at cytosine residues. Newly developed and highly sensitive genetic assays allow detection of spontaneous deamination mutagenesis using physiologically relevant conditions. Extension of this work to measurements of deamination rates within mispaired and extrahelical bases is currently in progress. This is the first step in examining deamination due to cross strand protonation induced by DNA adducts in double stranded DNA. In a related study, the ability of the E. coli uvr ABC excinuclease to incise both normal and damaged DNA containing mispairs and extrahelical bases is being examined. These studies are intended to probe the structure and processing of both premutagenic adducts and the actual mispaired and misaligned intermediates which lead to base substitution, frameshift and deletion errors.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60147-03 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Molecular Mechanisms of SOS-Mutagenesis in Escherichia Coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. Schaaper	Visiting Associate	LG NIEHS
	R. Dunn	Biologist	LG NIEHS
	R. D. Wallace	Student Employee	LG NIEHS

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	0.75	0.35

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this project is to study the molecular events that lead to mutation in the bacterium E. coli after induction of the SOS-response. The error-prone type of DNA replication that is presumed to be responsible for SOS-mutagenesis will be studied in an in vitro replication system. The accuracy with which crude extracts of E. coli cells copy normal or damaged single-stranded bacteriophage M13 DNA will be used as an indicator for in vitro SOS-expression. Characterization of the components involved is important for the study of SOS-mutagenesis and for the question of the regulation of mutation rates in general. The crude-extract replication system was developed to the point that reliable estimates can be made about the accuracy of phage DNA replication in vitro. The accuracy is extremely high and resembles the values expected for in vivo replication. The system is currently used to further define the parameters that determine this accuracy and for a comparison of these parameters in normal and SOS-induced extracts.

GPO 914-910

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 60148-03 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Error-Prone Repair in Bacteriophage T4

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head	LG NIEHS
Others:	L. K. Derr	Biologist	LG NIEHS
	A. G. Morton	Biological Aid	LG NIEHS
	D. C. Nguyen	Chemist	LG NIEHS
	K. Fukasawa	Visiting Scientist	LG NIEHS

COOPERATING UNITS *(if any)*

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.4	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The central objective of this project is the analysis of error-prone repair in bacteriophage T4, with related explorations into other mechanisms of mutagenesis and into a novel mechanism for avoiding ultraviolet-induced killing. Most chemical and all radiation mutagenesis in T4 occurs via error-prone repair and depends on the functions of the genes uvsW, uvsX and uvsY, most or all of the genes required for DNA replication, and perhaps several other genes as well. We have recently characterized temperature-sensitive alleles of the X and Y genes, alleles that differentially affect mutagenesis and inactivation. We are now cloning these mutant genes in order to obtain their proteins in amounts sufficient to determine which kinetic parameters correlate with mutagenesis and which with survival. Temperature-sensitive alleles of uvsW have also been obtained and will be characterized as to survival and mutation after UV irradiation; at the same time, a clone of the uvsW gene is being trimmed down to minimum size for further studies of the structure and function of this gene. We plan to explore which other genes may be required for error-prone repair in T4, with particular attention to genes 46, 47, 49, 58 and 59, and to continue with mapping experiments to localize a mutation, hm, which promotes ultraviolet mutagenesis. Experiments will be conducted to determine whether error-prone repair and genetic recombination are correlated, both processes being controlled by genes of the WXY pathway. Finally, our work on a recently discovered new mode of survival after UV irradiation will be prepared for publication; this system involves the genes encoding the ssDNA-binding and helicase proteins.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61005-07 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Biosynthesis and function of RNA Polymerase II in Drosophila melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Voelker	Research Geneticist	LG, NIEHS
Others:	L. L. Searles	Senior Staff Fellow	LG, NIEHS

COOPERATING UNITS *(if any)*

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

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

This study was initiated to genetically analyze the biosynthesis and function of the components of the RNA polymerase II transcription complex in Drosophila melanogaster. RNA polymerase II is a heteromultimer consisting of approximately ten different subunits, each of which is presumably specified by a different locus. The number of associated transcription factors (which are not structurally a part of RNA polymerase II) is unknown, but evidence for their existence has been found in other systems. The genetic locus that confers α-amanitin-resistance to RNA polymerase II has been identified. That locus has now been cloned as recombinant DNA molecules and was found to encode the 215,000 dalton subunit. The genetic control of the biosynthesis of that subunit is being analyzed at the molecular level by analyzing a number of revertants of the P-element induced mutant that was used to clone the DNA sequences of the region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61019-06 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Collaborative Protein Sequencing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Farida S. Sharief	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

Department of Diagnostic Immunology Research and Biochemistry, Roswell Park Memorial Institute, Buffalo, New York

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Two prostatic acid phosphatase isozymes (PAP-I & PAP-II) and a glycoprotein (GP) which is immunochemically and biologically related to PAP, have been purified and partially characterized from human seminal plasma. Amino acid compositions, peptide maps and carbohydrate contents of PAP-I, PAP-II and GP have been obtained. Amino-terminal sequences of PAP-I and GP have also been determined. These chemical data as well as immunological results demonstrate that PAP-I and PAP-II are different human PAP isozymes and that the GP represents a distinct glycoprotein which shares some common enzymatic and antigenic characteristics with PAP. The antigenic structure of Human PAP has been analyzed by using three partial tryptic peptide fragments and the entire PAP molecule comprised a minimum of four distinguishable, non-overlapping antigenic determinants. Several additional human acid phosphatases have also been purified from normal lungs, spleens, kidneys and bladders, and their structural and functional properties were compared with those of human malignant PAP.

The primary structure information of protein is very important in elucidating the fundamental biological function. The collaborative research of protein sequencing provides accurate information that can be used for cloning and identification of eukaryotic genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61021-05 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Molecular Analysis of the cut Locus of D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joseph W. Jack	Senior Staff Fellow	LG, NIEHS
Other:	Willie Gibson	Research Chemist	LG, NIEHS

COOPERATING UNITS *(if any)*

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

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

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

We are interested in knowing how cells of a single organism can differentiate to form specific tissue types. We have chosen to address one aspect of the question by learning how one gene, the cut locus of Drosophila melanogaster, is expressed differently in different tissues of the fly.

We have now cloned DNA sequences representing the entire gene, which encompasses 200 kb or more of DNA. A large number of mutants have been analyzed. We find that the deletion of sequences in the leftmost part of the gene cause phenotypic effects primarily in the legs, while deletion of or insertions into sequences slightly to the right cause effects primarily in the wings. Mutations in a 70 kb to the right affect the wings, head, and thorax and cause lethality. A fourth group of mutations lacks cut locus function in all tissues. These mutations map at the rightmost end of the gene.

The availability of tissue specific mutants of a gene afford the opportunity to experiment to find out how the gene normally operates in tissue specific ways. We are currently studying the transcriptional activity of the cut locus to find out how the tissue specificity of the mutant phenotypes relates to the transcriptional activity of the gene.

We now know that many of the cut mutants are insertions of retrovirus-like sequences into the cut locus DNA, and we are interested in understanding the affect of these sequences on gene activity. Some of these mutations are suppressible and will be useful in determining how a mutation caused by a retrovirus-like sequence can be suppressed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61022-05 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The Population Genetics of Transposable Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Langley	Research Geneticist	LG, NIEHS
Others:	Elizabeth A. Montgomery	Bio. Lab. Tech.	LG, NIEHS
	James Presson	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

Drs. N. Kaplan and R. Hudson, Biometry and Risk Assessment Program
Dr. Brian Charlesworth, Department of Biology, University of Chicago
Dr. Wolfgang Stephan, Department of Genetics, University of Edinburgh

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NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The role of natural selection against the deleterious effects of individual transposable element insertions was investigated by comparing the numbers of transposable elements on the X chromosomes versus autosomes of Drosophila. There is little evidence for selection against copia-like elements on the X chromosomes. This suggests other possible mechanisms (e.g. chromosome rearrangement) are likely to be important in removing transposable elements from natural populations.

A second study investigated the theory of the evolution of copy number regulation of a transposable element in an outbreeding population. The prediction of this study is that dominant effects, such as chromosome rearrangements, are likely to be the events that ultimately make the evolution of copy number regulation likely. Finally, the theory of the evolution of reduced recombination in centromeric and telomeric regions was developed and the associated accumulation of tandemly repeated DNA was investigated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61023-04 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Drosophila Germ Cell Determination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. Boswell	Staff Fellow	LG, NIEHS
Others:	Marcia Meltzer	Microbiologist	LG, NIEHS

COOPERATING UNITS *(if any)*

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0.0

CHECK APPROPRIATE BOX(ES)

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

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

It is a fundamental concept in developmental biology that the fate of embryonic cells is regulated by morphogenetic determinants localized in the ooplasm. In Drosophila, heterotopic transplantation experiments have conclusively demonstrated that cytoplasmic factors localized to the posterior pole plasm of the oocyte and embryo are requisite for the formation of pole cells, the primordial germ cells. However, the molecular nature of these cytoplasmic factors, the mechanism of localization within the ooplasm, and their mode of action in development are unknown. The genetic and developmental analysis of maternal effect mutants that affect pole cell formation in Drosophila melanogaster are intended to allow one to elucidate the mechanism of determination and how the determined state is maintained throughout development.

A detailed genetic and developmental analysis of one such grandchildless mutant, tudor, (tud) has been undertaken. The properties of mutations of the recessive maternal effect gene tud indicate that the gene product of the tudor locus is required for the proper determination of germ cells in Drosophila melanogaster. Specifically, the germ plasm of six different alleles of tud has been analyzed at the ultrastructural level, and it is found that different alleles contain different amounts of assembled polar granule material (a cytoplasmic organelle classically thought to be the germ cell determinants). The ability or inability to form germ cells correlates directly to the amount of assembled polar granule material observed in the germ plasm. For example, one allele produces polar granules approximately 1/3 the size of wild type polar granules and this allele produces fertile progeny. On the other hand, alleles that produce no apparent assembled polar granule material in the germ plasm produce no fertile progeny. Therefore, mutations at the tudor locus, which disrupt the normal assembly of the germ plasm, result in the failure to localize the germ plasm determinants to the posterior pole.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61024-04 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Genetic and Molecular Analysis of Suppressor-of-Sable Function in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Voelker	Research Geneticist	LG, NIEHS
Others:	K. Hiraizumi	Staff Fellow	LG, NIEHS
	S. P. Bainbridge	Visiting Fellow	LG, NIEHS
	J. F. Sterling	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	2.4	1.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Recent findings in Drosophila have shown that 1) a significant proportion of spontaneous mutations are caused by insertions of mobile genetic elements, and 2) certain genetic suppressor systems are mediated through insertions of specific mobile elements. We are investigating the molecular mechanism of one such suppressor system: recessive mutations at the suppressor-of-sable [su(s)] locus suppress recessive mutations at the vermilion (v) locus that are caused by insertions of the mobile elements 412 and B104 (Project Number Z01 ES 61029-02 LG).

DNA sequences of su(s) have been cloned and are being characterized. The insertions of foreign DNA that are associated with 14 su(s) mutant alleles have been localized to a region of DNA that encodes the 5' end of the message. The DNA sequences that give rise to the ~5 kb poly A^+ su(s) message consist of at least 5 exons interspersed over 8 kb of genomic DNA. Coding sequences from the two largest exons have been ligated into an expression vector to produce fusion proteins against which antibodies can be produced. Antibodies against the su(s) portion of the fusion protein will be recovered and used as probes to identify the location and function of the su(s) protein product within the organism. Wild type su(s) sequences will be introduced into su(s) mutant flies by P element transformation to determine the effect of the su(s) protein product on the biology of mobile element 412.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61029-04 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cloning and Characterization of the vermilion Locus of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lillie L. Searles	Senior Staff Fellow	LG, NIEHS
Others:	Robert A. Voelker	Research Geneticist	LG, NIEHS
	Mary L. Tate	Bio. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

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SECTION
Eukaryotic Gene Structure

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project are use vermilion (v), a gene required for eye pigment synthesis in Drosophila, as a model system (1) to investigate the phenomenon of suppression of transposable element insertion mutations and (2) to investigate the molecular mechanisms which regulate the expression of the gene during development.

The vermilion gene has been cloned and the structure of the gene has been determined in some detail. Mutations that disrupt v gene expression are clustered within approximately 2 kilobases of DNA. A 1.4 kb transcript, homologous to this same region, is present in v^+ RNA and altered in either size or level of accumulation by various v mutations. The spontaneous v mutations that are suppressed by the suppressor of sable [su(s)] are apparently identical insertions of 412, a retrovirus-like transposable element.

Transcription mapping and DNA sequencing experiments have shown that the v transcript consists of 5 or more exons. The positions of several mutations relative to introns and exons have been determined by sequencing. The suppressible 412 insertion mutations appear to be located within an intron near the 5' end of the gene. A revertant of one of the mutations is a secondary insertion of 2.6 kb of DNA into one end of the 412 element. The insertion of 412 has been shown to prevent accumulation of the v transcript, and su(s) partially restores the level of v transcription.

Comparison of the developmental profiles of vermilion transcription and enzyme activity indicates that the expression of the gene product is regulated both at the level of transcription and post-transcriptionally.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61030-03 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular analysis of the Om mutator in Drosophila ananassae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. H. Langley	Research Geneticist	LG, NIEHS
Others:	Elizabeth A. Montgomery	Bio. Lab. Tech.	LG, NIEHS
	James Presson	Bio. Lab. Tech.	LG, NIEHS

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

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

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

Hinton (1984) described an unusual mutator in D. ananassae which he speculated was a transposable element, tom, with (previously) novel properties: the element could only be detected phenotypically by its effect on eye morphology at 21 non-pleiotropic, non-dosage compensated, non-random loci. Because of the recovery of a spontaneous singed mutation, sn^{9g}, in an ocullar morphology (Om) mutant line derivative, Om(1D)9g, it was possible to investigate his speculation. D. melanogaster singed, sn, DNA probes were used to isolate and recover a D. ananassae singed gene. A comparison of the sn^{9g} and wild type singed restriction map implicated a 6.5 kb insert as the element responsible for the mutator effect. A total of 186 recombinant lines from four X-linked Om loci were examined. 80 were Om and 106 were non-Om; in all instances an in situ hybridization signal, when probed with sn^{9g} insert, was found at appropriate locations on the polytene chromosomes. Linkage was complete and showed that the sn^{9g} insert was homologous to sequences localized at the sites of Om mutants.

Preliminary analysis of several isolated clones of the tom element indicate a fairly conserved structure typical of copia-like elements. DNA sequence analysis of the tom at sn^{9g} and Om(1D)9g showed direct repeats at the termini that is also characteristic of copia-like elements. Ongoing southern blot studies of several Om(1D) mutations indicate that some are due to insertions of a single tom, others appear to be due to multiple copies and yet others show no obvious alterations in the cloned region surrounding the tom insertion site in Om(1D)9g. Further investigations of the tom elements and the Om mutations should provide insight into the mechanisms underlying this very unusual mutational process.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61032-03 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure-function of mammalian lactate dehydrogenase isozymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	Farida S. Sharief	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

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Eukaryotic Gene Structure Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
I.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

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

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

The complete primary structure of LDH-A4 isozymes from human and mouse has been determined by sequence analyses of both proteins and cDNA clones. Recently, the cancer-associated LDH-K isozyme induced by Kirsten sarcoma virus has been shown to be a modified form of human LDH-A4 protein complexed with ras P21 protein. Bovine LDH-A4 isozyme was shown to be phosphorylated in vitro by src tyrosine kinase. The low-salt eluting ssDNA binding protein of 36,000 daltons from mouse myeloma was shown to exhibit LDH enzymatic activity and cross-reacted immunologically with LDH-A4 isozymes from mouse, bovine and human. The LDH-A4 isozymes purified from human and bovine were also shown to bind to ssDNA on Western-blot and filter-binding assay. Human and bovine LDH-B4 isozymes showed only weak ssDNA-binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61034-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Survey of Genetic Variation in Natural Populations of Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Kreitman	Staff Fellow	LG, NIEHS
Others:	W. Quattlebaum	Bio. Lab. Tech.	LG, NIEHS
	C.H. Langley	Research Geneticist	LG, NIEHS
	Gail Simmons	Staff Fellow	LG, NIEHS
	Cynthia Newlin	Biological Aid	LG, NIEHS
	Barbara Lange	Graduate Student	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Montserrat Aguadá
University of Barcelona
Barcelona, Spain

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.0	1.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The evolutionary histories of two genetic loci in Drosophila species are being examined by studying genetic variation at the DNA level in natural populations. A methodology has been developed to identify classes of haplotypes in large samples (100 lines or greater). The method involves probing filters containing an image of genomic DNA cut with four-cutter restriction enzymes and run under denaturing conditions on DNA sequencing-type gels. Approximately 20% of all nucleotide polymorphisms and all length polymorphisms can be identified within the probed region. This approach is being applied to samples of approximately 100 lines from each of several populations of D. melanogaster and a single population of its sibling species, D. simulans. Two loci are under investigation - Alcohol dehydrogenase (Adh) and white (w).

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61035-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The Molecular Characterization of Spontaneous HGPRT Mutations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.H. Langley	Research Geneticist	LG, NIEHS
Others:	Elizabeth A. Montgomery	Bio. Lab. Tech.	LG, NIEHS
	Shiu L. Huang	Guest Worker	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Shiu L. Huang
Environment Health Research and Testing Inc.
Research Triangle Park, N. C.

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The types of gene structural changes causing deficiency of hypoxanthine guanine phosphoribosyl transferase (HGPRT) activity in spontaneous mutations is being examined in cultured human fibroblasts. The deficiency of this enzyme activity causes a human disease (Lesch-Nyhan Syndrome). The restriction enzyme cleavage patterns of HGPRT gene sequences in mutant lines are being analyzed. The work is presently focused on obtaining a large number of independent spontaneous mutants that existed in new born baby's foreskins. Forty independent mutants have been isolated from different normal newborns. The mutant cells were grown to large numbers. Portions of cultured cells were frozen in liquid nitrogen for cytogenetic and enzymology studies at a future time and portions of cultured cells were frozen for DNA extraction. Southern blot analysis is now in progress to assess the possible involvement of DNA rearrangements in spontaneous mutation.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61037-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanism of DNA Replication in Eucaryotes: Yeast as a Model System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sugino	Visiting Scientist	LG NIEHS
Others:	R. K. Hamatake	Staff Fellow	LG NIEHS
	S. L. Eberly	Staff Fellow	LG NIEHS
	P. S. Alexander	Biologist	LG NIEHS
	A. B. Clark	Biologist	LG NIEHS
	R. Desai	Q	LG NIEHS
	T. Sugino	Guest Worker	LG NIEHS

COOPERATING UNITS *(if any)*

Lucy M. S. Chang, Professor and Chairperson, Dept. of Biochemistry, The Uniformed Service University of Health Sciences, Bethesda, MD 20705.
Dr. Errol C. Friedberg, Professor, Dept. of Pathology, Stanford Univ., School of Medicine, Stanford, CA 94305.

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.0	2.5

CHECK APPROPRIATE BOX(ES)

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

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

An in vitro DNA replication system of yeast 2-μm and ARS1 plasmid DNAs has been used as a model to investigate the mechanism of DNA replication in eucaryotes. To identify various enzymes and protein factors required for DNA replication, the system has been further fractionated and reconstituted by using separated fractions. From these experiments, two additional proteins have been identified and purified. These are newly identified single-stranded DNA-dependent ATPase (ATPase III) and DNA topoisomerase II and have been extensively studied in this year. The ATPase III has additional enzymatic activity which unwinds double-stranded DNA utilizing energy of ATP hydrolysis (helicase activity) Furthermore, RNase Hs and single-stranded DNA binding proteins which might participate in DNA replication have been purified to homogeneity by following their enzymatic activities. To prove that these proteins are required for yeast DNA replication, antibodies have been raised against each and their genes have been identified and cloned from lambda gt11 expression yeast genomic library using the antibodies. Their nucleotide sequences have been determined and the genes have been mapped on the chromosomes. Finally, the essentiality of each gene has been tested by gene disruption and one of single-stranded DNA binding proteins (20k dalton) is found to be required for yeast cell viability. In order to permit identification and isolation of other DNA replication proteins, new temperature-sensitive DNA replication mutants of yeast have been isolated, characterized genetically, some of these mutant genes have been cloned and their nucleotide sequences have been determined. Currently, to ease the purification of their gene products overproduction of the gene products is being carried out.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 61039-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanism of DNA Recombination and Repair in Yeast Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sugino	Visiting Scientist	LG NIEHS
Others:	T. Sugino	Guest Worker	LG NIEHS
	C. C. Dykstra	Guest Worker	LG NIEHS

COOPERATING UNITS *(if any)*

Dr. F. Coleman-Wilson, Assistant Professor, Dept. of Microbiology, Univ. of North Carolina at Asheville, NC, Asheville, NC

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	0.25	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Proteins binding to single-stranded DNA are expected to participate in DNA recombination and repair as well as DNA replication. Thus, three different single-stranded-DNA-binding proteins (SSB) have been purified from the yeast Saccharomyces cerevisiae and antibodies have been raised against them. Using the antibodies as probes, their genes have been identified and cloned from a λgt11 yeast DNA library. Deletions of these genes were then constructed, the wild-type genes were replaced by the disrupted genes, and the resulting phenotypes were studied. 20kd SSB gene is essential for cell viability. However, C-terminal region of the 20kd SSB is still functional, but the cell is UV-sensitive. This strongly indicates that 20kd SSB is required for DNA repair and recombination as well as DNA replication. The RAD52 gene product is required for DNA recombination and repair in yeast. The gene has been cloned and its nucleotide sequence determined by other groups. However, this important gene product has not yet identified and purified. By aid of a computer we identified several possible antigenic regions in the RAD52 gene. The oligopeptides covering the antigenic regions were chemically synthesized and conjugated to BSA and antibodies were raised against the conjugates. In addition, several fusion plasmids of the RAD52 gene and either the λpL promoter or the yeast α-mating type pheromon leader sequence or the yeast ADH promotor will be constructed in order to overproduce RAD52 protein in E. coli and yeast. Finally, one of the excision repair genes of yeast (RAD18), which also has been expected to be a subunit of yeast DNA polymerase I, has been cloned and characterized.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61040-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Genetic and Biochemical Analysis of Yeast DNA Polymerase I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. K. Hamatake	Staff Fellow	LGM, NIEHS
	A. Sugino	Visiting Scientist	LGM, NIEHS
Others:	A. B. Clark	Biologist	LGM, NIEHS

COOPERATING UNITS *(if any)*

Lucy M. S. Chang, Professor and Chairperson, Dept. of Biochemistry, The Uniformed Service, Univ. of Health Sciences, Bethesda, MD 20705

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Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.75	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A detailed analysis of the yeast replicative DNA polymerase, DNA pol I, is being undertaken at the molecular and genetic level. The objectives of this project are to map and clone the gene for DNA pol I, to identify subunits and accessory proteins that influence DNA pol I activity and, ultimately, to identify the proteins that regulate its activity on native DNA templates and to determine the mechanism of their interactions.

Using purified DNA pol I, we have identified several proteins that stimulate its synthetic activity. These include three different RNase H proteins, three different single-stranded DNA binding proteins (ySSBs) and a DNA-dependent ATPase (ATPase III) that possesses a helicase activity. In this year, we have studied the mechanism of stimulation by each protein in detail. Three different ySSBs primarly prevent non-productive binding form of yeast DNA polymerase I on DNA templates, as the proteins increase neither processivity nor accuracy of DNA polymerase I reaction. On the other hand, ATPase III increases the processivity at least 2-3 fold. Furthermore, a catalitic amount of ATPase III is required for maximal stimulation of DNA polymerase I reaction strongly suggests that ATPase III interacts with DNA polymerase I.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65021-14 LG

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Investigation of Germinal Mutation Induction in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. M. Johnson	Research Geneticist	LG, NIEHS
Others:	M. L. Snell	Bio. Lab. Technician	LG, NIEHS
	D. P. Lovell	Statistician	BIBRA
	S. E. Lewis	Senior Geneticist	RTI

COOPERATING UNITS *(if any)*

Research Triangle Institute, Life Sciences Group, Research Triangle Park, N.C.; British Industrial Biological Research Association, Carshalton, Surrey, England

LAB/BRANCH

Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this project is to detect natural and induced mutations in mice for the purpose of providing understanding of the specific molecular events involved in germinal mutation and the effects of these events on the life, form and function of the mammalian organism. Results are relevant to human exposures to mutagens and the potential for increased risk of genetic disease that may accompany mutagen exposure. The problem is approached by detecting mutations at specific biochemical loci with electrophoretic methods, by conducting characterization studies on the mutant genes and gene products, and by examining the animals for expressed physical abnormalities correlated with mutation rate increases and with specific induced-mutant genotypes. The methods have led to successful identification of many new mutations induced by ethylnitrosourea, x-rays and ethylene oxide. A number of naturally occurring mutations have been identified as well. Results have generally shown little or no increased incidence of harmful gene expression attributable to induced mutations. The results raise questions as to the appropriateness of depending on the mutagenic properties of a substance for environmental risk assessment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65033-03 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. V. Malling	Research Geneticist	LG, NIEHS
	J. G. Burkhart	Research Chemist	LG, NIEHS

COOPERATING UNITS *(if any)*
C. A. Hutchinson, III & M. H. Edgell, UNC, Chapel Hill, N. C.
S. C. Hardies, Univ. of Texas, San Antonio, Texas
E. J. Eisen, NCSU, Raleigh, N. C.

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Laboratory of Genetics

SECTION
Eukaryotic Gene Structure

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2	0.3

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

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

The objective of this research is to study mutagenesis in mammals at the DNA level using both nuclear and mitochondrial DNA. Studies are to be based upon variation among individual copies of a particular sequence rather than variation between averages for the same sequence. Special emphasis is to be placed on the differential sensitivity to mutagenesis of gametogenic stages and in comparison with the response in the various somatic tissues. A major problem for detection of genetic damage directly in mammalian DNA is that most genes occur in one, or few copies. Our approach will utilize well characterized DNA sequences. The basic requirements for direct analysis of specific DNA sequences are that the sequences: (1) are already amplified in the cell, (2) can be isolated from the mammalian genome, and (3) can be amplified in vitro. Two genetic entities met these requirements. The first is the use of mitochondrial (mt) DNA. Cloned mouse mtDNA has been used for restriction analysis of sperm mtDNA isolated from a single mouse; after additional technical improvements, the mtDNA from treated mice will be examined for mutations. The second is the use of viral DNA transformed into mammalian DNA. Double stranded DNA from ϕX174 am3, cs70 has been transformed into mouse L-cells. The DNA is incorporated into several places in tandem arrangements. Using restriction enzymes and ligase it has been possible to transfect spheroplasts with ϕXDNA from the transformed mammalian cells. Conditions for purification of ϕX from the mammalian genome have been developed and conditions for measuring reverse mutations of am3 and cs70 have been established. Attempts are being made to create a mouse strain with ϕXDNA in the genome for the study of mutation induction in any part of the animal tissue.

PHS 6040 (Rev. 1/84) GPO 914-918

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65034-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The Specificity of Spontaneous and Induced Mutation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. Schaaper	Visiting Associate	LG NIEHS
	R. L. Dunn	Biologist	LG NIEHS
	R. D. Wallace	Student Helper	LG NIEHS

COOPERATING UNITS *(if any)*

Dr. R. A. Fuchs
IBMC
Strasbourg, France

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	0.25	0.85

CHECK APPROPRIATE BOX(ES)

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

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

The complexity of mutational mechanisms operating in the bacterium E. coli is studied by DNA sequence analysis of large groups of lacI⁻ mutants produced under selected conditions. LacI⁻ mutations are selected on an F'prolac and then transferred by genetic (homologous) recombination onto a single-stranded phage vector for DNA sequencing. An analysis of the resultant mutational spectra has yielded clues with regard to the mechanisms of both spontaneous and SOS-mediated mutagenesis. Spontaneous mutation is a highly varied process in which multiple classes of mutations are produced. DNA replication errors on the other hand are of mainly two classes, base substitutions and single-base frame-shifts. Mismatch repair and the occurrence of mutations from sources otner than DNA replication are responsible for this discrepany. The mechanisms of SOS-induced mutation were investigated by studying the specificity of ultraviolet-light-induced mutation. It was shown that wild-type and excision-repair-deficient strains of E. coli exhibit a very similar specificity which has implications for the mutagenic pathways in these strains which have been thought to be distinct. The data could furthermore be used to draw conclusions regarding the nature of the premutagenic lesions in DNA after UV-irradiation.

GPO 914-911

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65035-02 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Specificity of Mutagenesis in Mammalian Genes Using a Natural Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. J. de Jong Visiting Fellow LG NIEHS

COOPERATING UNITS *(if any)*

Dr. B. W. Glickman
Biology Department, York University
Downsview, Ontario, Canada

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Environmental mutagens induce mutations in prokaryotes and presumably also in eukaryotes in a non-random fashion with respect to the DNA sequence. An understanding of the non-random character and the underlying mechanism of induced mutagenesis, will eventually lead to better risks estimates for mutagens present in the environment. To investigate the mutational specificity in mammalian cells at the molecular level, the adeninephosphoribosyltransferase (aprt) gene in a cell-line hemizygous for aprt was chosen as a model. This gene was elected in view of its small size (less than 2.5 kb, including all regulatory sequences and four introns) and the well-established selective growth conditions for aprt-deficient cells. Special lambda vectors have been developed to allow the efficient and repetitive isolation of mutant alleles for DNA sequence analysis. A large number of mutant alleles have now been processed through these cloning protocols : 58 resulting from spontaneous mutations, 40 induced by gamma ray and 44 induced by UV. For a considerable fraction of the cloned genes, the mutational changes have been elucidated. Spontaneous and UV-induced mutations are, indeed, spread across the gene in a non-random way. Three specific transition hot-spots are observed among the spontaneous mutants and a transversion hot-spot is suggested by data collected for UV-induced mutants.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65036-02 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Organization and Regulation in D. melanogaster

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Judd	Chief	LG, NIEHS
Others:	Patricia S. Davis	Chemist	LG, NIEHS
	Shu-Mei Huang	Biologist	LG, NIEHS
	Deborah A. Adams	P Appointment	LG, NIEHS
	Katherine M. Peterson	Q Appointment	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION
Eukaryotic Gene Structure Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.25	1.75

CHECK APPROPRIATE BOX(ES)

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

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

Selected genes in Drosophila melanogaster are being studied in an effort to understand their organization and regulation through the analysis of mutations that upset regulatory functions. A locus of particular interest is white, which controls one of the steps in the pigmentation of the eyes, Malpighian tubules and testis sheath in Drosophila. The molecular analysis of mutants shows that a large proportion of spontaneous changes result from the insertion/deletion of transposable elements. We are studying mutations that cause mosaic expression of the white locus. The original mutation of this series resulted from the insertion of the transposon BEL into the large intron of white. This mutant, w^{zm}, produces a mottle-eye phenotype only when combined with the mutation z^1, otherwise it has a wild-type phenotype. w^{zm} is mildly unstable and has produced a series of other alleles including a transposition of an X chromosome segment containing white into the third chromosome. In the new position, the white locus produces a wild-type phenotype except when the transposition is homozygous and the z^1 mutation is present. That genotype produces a mosaic eye-color that is nonautonomous and nonclonal in expression, marking a dramatic change from the autonomous, clonal patterns seen in the non-transposed w^{zm} and its derivatives. The molecular analyses of these mutant alleles are being done to determine the basis for the modified expression of white and its interaction with z^1. We have also resolved the basis for four unexpected regularly-occurring asymmetrical recombination products recovered from w^{bf}/w^a heterozygotes. The transposon roo (also known as B104) is found within the white locus and ~30 kb proximal to white in the w^{bf} chromosome. roo had also invaded copia at the w^a site in the w^a chromosome. The three copies of roo pair and undergo exchange in two separate configurations, resulting in two sets of reciprocal duplication and deficiency products. This defines another important way that transposons can reshape eukaryotic genomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65037-02 LG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transposon - mediated chromosomes instabilities in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. H. Judd	Chief	LG, NIEHS
	J. W. Jack	Senior Staff Fellow	LG, NIEHS
	Shu-Mei Huang	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*
Dr. Johng K. Lim, Professor of Biology
University of Wisconsin, Eau Claire

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Laboratory of Genetics

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Eukaryotic Gene Structure Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The retrovirus-like transposon gypsy has been shown to account for high rates of mutation in some strains of Drosophila melanogaster. This transposon is usually found in low copy number (3-4) per genome and is extremely stable in most strains. The Uc strain shows a wide range of copy number in sublines (3-60+) and when this strain is outcrossed to stable strains, some matings result in high rates of mutation associated with mobilization and insertion of gypsy into chromosomal sites previously free of gypsy. From such dysgenic crosses only the F_1 female and her offspring, both male and female, show the high mutation rates. F_1 males are mutationally stable and gypsy is not mobilized. Reciprocal crosses give similar results, suggesting that cytoplasmic factors are not involved in the mobilization process. Examination of polytene chromosomes from F_2 and F_3 offspring of dysgenic crosses show that gypsy is amplified in copy number and mobilized to insert at new chromosomal locations in somatic cells. This is consistent with the observation that most mutations transmitted through the germ cells appear in clusters.

We are studying the molecular structure of gypsy to determine whether incomplete or defective copies can be mobilized when a complete copy is also present in the cell. We also are making crosses of various types to determine the genetic factors that are important in mobilization of the transposon.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65038-01 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Analysis of Molecular Mechanisms of Mutagenesis with Defined Components

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. A. Kunkel	Senior Staff Fellow	LG NIEHS
Others:	A. Sugino	Visiting Scientist	LG NIEHS
	J. Roberts	Staff Fellow	LG NIEHS
	K. Bebenek	Visiting Fellow	LG NIEHS
	A. Soni	Biologist	LG NIEHS
	M. Smith	Biologist	LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.2	1.9

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are investigating the fidelity mechanisms used to control the level of mutations produced during the replication and maintenance of genetic information in cells. For this purpose assays have been developed to determine the frequency and specificity of mutations produced by eukaryotic DNA replication and repair proteins during a single round of *in vitro* DNA synthesis with a biologically active DNA molecule. None of the highly purified DNA polymerases themselves nor the somewhat more complex multisubunit enzymes examined to date can account for the high fidelity required *in vivo*, suggesting that additional fidelity components are still to be identified. The specificity of frameshift, deletion and base substitution errors produced by purified DNA polymerases has suggested several mechanisms for the formation of these errors. Two of the models formulated from these data have been experimentally verified using highly sensitive reversion assays involving template DNA sequences engineered by site-directed mutagenesis. In one instance, direct *in vitro* verification of the Streisinger strand slippage model (which explains frameshift errors in runs of a common base) has been obtained. In a second instance, single-base misinsertions by DNA polymerase at specific target sequences have been shown to ultimately result not in base substitution, but rather in frameshift errors, providing a mechanistic link between these two very different classes of mutations. These and other studies are being expanded to include additional protein components, and where possible, to *in vivo* circumstances, in order to provide detailed information on the protein-nucleic acid interactions important in determining accuracy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65039-01 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Molecular Analysis of Mutation in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. R. Tindall	Staff Fellow	LG NIEHS
Others:	D. L. Halderman	Biol. Lab. Tech.	LG NIEHS

COOPERATING UNITS *(if any)*

Leon F. Stankowski, Pharmakon Research International, Inc., Waveryl, PA 18471

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Molecular analyses of mutation in mammalian cells have been hindered by the complexity of the mammalian genome and the lack of rapid methods for the sequence analysis of induced mutations. Most mammalian loci useful for studying mutagenesis in mammalian cells (hgprt, tk, oua^r, dpt^r, etc.), are not readily amenable to molecular analyses due to the large size of the gene in its normal genomic state, e.g., 35-40 kb in the cast of hgprt, or due to the lack of cloned sequences and/or sequence information concerning the nature of mutations that will lead to the resistant phenotype. There are three notable exceptions, however, which appear promising in their ability to analyze mutations at the molecular level in a genomic target gene. Two of these are the aprt and tk loci in mammalian cells. These loci have been cloned are sequenced and are sufficiently small to allow the application of rapid cloning techniques for analysis of induced mutations. A third system, allowing a somewhat greater range of applications, utilizes a single copy of the bacterial gpt gene (456 basepairs) linked to an antibiotic resistance gene, Ampicillin (Ap^r) or Neomycin (neo^r), integrated into the mammalian genome. Using the SV40 early promoter or a retroviral LTR, the gpt gene expresses the purine salvage pathway enzyme, HGPRT (the bacterial equivalent of HGPRT). In this system, standard techniques used for the isolation of mutations at hgprt can be applied to the isolation of mutations at the integrated gpt sequences.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65040-01 LG

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Analysis of mutagenic specificity using a retrovirus shuttle vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. J. de Jong Visiting Fellow LG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Previous work has focussed on the analysis of mutagenic specificity at the natural adenine phosphoribosyltransferase (APRT) gene in Chinese Hamster ovary cells. These studies included the isolation of over 50 mutant genes and their subsequent DNA sequence determination. Although the determination of mutagenic specificity in mammalian cells appears feasible now, it still remains very laborious, requiring the construction of genomic libraries for each mutant gene. To facilitate the characterization of chromosomal mutations, an aprt cDNA gene (540 bp coding region) has been constructed and has been stably integrated in the genome of several mammalian cell lines using an infectious retrovirus shuttle vector. Cell lines carrying a single aprt provirus are useful for isolating mutations in the cDNA gene. A number of other sequences are present in the shuttle vector in order to allow rapid recovery of mutant sequences and subsequent DNA sequence analysis. These additional sequences include (1) the replication origin from Simian virus 40 (SV40 ori), which permits extensive in vivo amplification of the gene upon fusion of the mutant cells to cells with constitutive expression of the SV40 T-antigen; (2) the pBR322 plasmid replication ori, to propagate the amplified sequence in E. coli; (3) the tn5 neo gene, to select the recovered shuttle vector in E. coli; and (4) the bacteriophage M13 replication ori and packaging sequences, in order to produce transducing M13 particles carrying a single-stranded mutant gene, thus allowing rapid DNA sequencing. The aprt shuttle vector has been used to correct the APRT$^-$ phenotype of several mammalian cells, including human fibrosarcoma cells, human lymphoblasts, murine L cells.

GPO 914-918

LABORATORY OF MOLECULAR BIOPHYSICS
SUMMARY STATEMENT

Research efforts in the Laboratory of Molecular Biophysics are directed towards understanding at the molecular level the mechanisms of toxicity of environmental chemicals and the developmnt of techniques to detect, analyze and quantitate extremely low levels of toxic agents in biological tissues. The studies draw mainly on the research disciplines of physics, physical chemistry, organic chemistry, pharmacology, biochemistry and cell biology. Special emphasis is placed on the following (1) the development, improvement and utilization of spectroscopic methods (nuclear magnetic resonance, electron spin resonance, fluorescence spectroscopy) to characterize and measure the molecular interactions that occur between environmental agents and target biological systems; (2) the conduct of physical organic and biorganic studies of environmental agents, biological materials and their metabolic conversion products with emphasis on elucidation of chemical mechanisms in biological damage; (3) the development, improvement and utilization of analytical methodology (mass spectrometry, chromatography) for specified agents present in trace amounts; (4) the provision of collaborative and service functions (mass spectrometry, nuclear magnetic resonance) for other NIEHS laboratory and research programs. The Laboratory is organized into five Workgroups: Molecular Biophysics, Mass Spectrometry, Nuclear Magnetic Resonance, Prostaglandin Biochemistry, and Metabolism.

MOLECULAR BIOPHYSICS

The Molecular Biophysics program is concerned with understanding, at the molecular level, the interaction of environmental agents with target biological systems, including nucleic acids, proteins and membranes. For these studies a number of highly sophisticated spectroscopic techniques (electron spin resonance, fluorescence and absorption spectroscopy, circular dichroism and stopped flow spectrometry) are employed. Particular emphasis is placed on the detection and identification of reactive free radicals (including active oxygen species) generated from environmental chemical agents by metabolic and other processes. There is now convincing evidence that free radicals are involved in a number of pathologic conditions including chemically-induced carcinogenesis, pulmonary fibrosis, methemoglobinemia, hemolytic anemia and cutaneous photosensitization.

Before free radicals can be clearly implicated in any toxic effect, the free radical species must be demonstrated to exist under appropriate biological conditions. Studies to detect, identify and quantitate free radicals generated during the metabolism of endogenous and exogenous compounds have continued. Sulfur-centered (thiyl) radicals have been detected when either cysteine or reduced glutathione was incubated with horseradish peroxidase. These studies have been extended to thiol-containing drugs and a variety of peroxidases, including prostaglandin hydroperoxidase, lactoperoxidase, and glutathione peroxidase. Free radical metabolites are formed during the oxidation of inorganic anions, such as sulfite, azide and even cyanide, by a variety of peroxidases and proteins with peroxidase activity such as catalase and methemoglobin. Free

radical metabolites, formed by both the one-electron reduction and oxidation of toxic chemicals, have been successfully detected in a variety of cells. An investigation of carbon tetrachloride-derived free radical metabolites in the perfused liver and *in vivo* has begun.

Light is known to interact with chemical agents in tissues, such as the skin or eyes, to produce photosensitization. The chemical agent may be endogenous (protoporphyrin in erythropoietic protoporphyria), a drug (sulfonamides, declomycin, chlorpromazine), a topical agent (4-aminobenzoic acid and its esters in sunscreens; halogenated salicylanilides in soaps) or an environmental agent (polycyclic aromatic hydrocarbons in coal tar; amyl esters of 2-aminobenzoic acid in printer's ink). The photosensitivity response may be one of two types, phototoxic or photoallergic. While the initial step in all forms of photosensitivity must be the absorption of light by the chemical or its metabolites, the precise mechanisms of photoxicity and photoallergy are unknown. Evidence has been sought for the involvement of free radicals and active oxygen species in photosensitization. UV irradiation (330 nm) of CPZ in aqueous solution resulted in the homolytic cleavage of the carbon-chlorine bond to yield an aryl radical which extracted a hydrogen atom from suitable donors. CPZ photoionized when irradiated at 280 nm (but not at 330 nm) to give the CPZ cation radical. CPZ generated singlet oxygen (luminescence at 1270 nm) when photo-irradiated in benzene, hexane, and cyclohexane (strong), and methanol, and ethanol (weak) but not in aqueous solutions. CPZ sulfoxide, a major CPZ metabolite in man, generated $^{\bullet}OH$ and the CPZ cation radical upon irradiation with near UV light. Halogenated salicylanilides eg. 3,3',4',5-tetrachlorosalicylanilide (TCSA) and 3,4',5-tribromosalicylanilide (TBSA) generated aryl radicals during photoirradiation. Irradiation of TBSA with glutathione generated the corresponding thiyl radical, while under the same conditions TCSA abstracted hydrogen atom from the glycyl α-carbon atom of the dipeptide Gly-Ala. The skin photoallergy of TBSA or TCSA may be due to reactions involving protein-derived radical intermediates. The chlorinated phenols bithionol and fentichlor also dehalogenated upon irradiation and in addition underwent photohydrolysis to yield semiquinone radicals. The aryl radical formed by photodeiodination of the anti-arrhythmic drug amiodarone readily abstracted a hydrogen atom from linoleic acid. Reaction of the resultant linoleyl radical with oxygen would initiate lipid peroxidation and provide an explanation for lipofuscin skin deposits in patients receiving this drug.

MASS SPECTROMETRY

The work of the Mass Spectrometry Group involves the application of advanced mass spectrometric methods to solve analytical problems and the development of new techniques in anticipation of future analytical requirements.

Recent work in method development has focused on two areas: progress in liquid chromatography-mass spectrometry, and utilization of tandem mass spectrometry. Liquid chromatography-mass spectrometry studies have provided an improved understanding of the ionization processes involved. Thermospray ionization of neutral compounds, for example, has been shown to be predominantly a gas-phase process. The use of a tandem double-focusing mass spectrometer has provided

important structural data in the study of peptides. Similar techniques applied to analysis of acyl carnitines in clinical samples have permitted the differentiation of isomeric species.

A new method for structure determination based upon endothermic ion/molecule reactions of accelerated ions with a reactive collision gas has been tested successfully: isomeric $C_2H_5O^+$ ions react differently with NH_3 at 1-10 eV kinetic energy, and the thresholds of endothermic processes agree for the most part with thermochemical predictions. Fast atom bombardment has been shown to favor species concentrated near solution surfaces, and has been applied to the analysis of silicate solution surfaces. New applications of sector-instrument collisional activation have been made, including the development of kinetic energy release data for identification of isomers at the 1 ng level and the development of theory and experiment to show that fragment ion intensities relate to thermochemistry of fragmentation even when the parent ion is not thermalized. Collisional activation studies were shown to require transmission of the entire primary beam to be usable for structure determination; further, the relation of fragment ion intensities to unattenuated main beams is required in quantitative analysis of isomers.

Approximately 400 samples have been analyzed as part of the service and collaborative support program. The techniques employed spanned the full range of mass spectrometric methods and instrumentation available to the group. Collaborative projects included applications of combined liquid chromatography-mass spectrometry, contributions to drug metabolism studies and the use of tandem mass spectrometry in structure elucidation.

NUCLEAR MAGNETIC RESONANCE

The objective of the Nuclear Magnetic Resonance (NMR) program is to elucidate of the mechanisms by which chemicals and heavy metals present in the environment cause cell injury. Specific studies carried out within the framework of this objective fall into two categories: in vivo metabolic analysis by NMR spectroscopy focused on parameters thought to be involved in the mediation of cell injury; in vitro NMR studies of the interaction of various chemicals or heavy metals with proposed or demonstrated biochemical targets.

As a consequence of the postulated role of an increase in intracellular calcium levels in the mediation of cell injury, effort has been focused on methods used to determine this parameter. Due to the relatively poor sensitivity of direct observation by ^{43}Ca NMR, an indirect method was recently developed by Feeney and coworkers utilizing a fluorinated calcium chelate - fluoroBAPTA. This chelate can be loaded into a variety of cells using a strategy initially developed for fluorescent dyes in which the chelate is administered as a neutral membrane permeable ester which then loads as a consequence of the action of cell esterases. Fluorine-19 NMR studies then allow detection of resonances from both the free and calcium complexed chelate, and hence a determination of the cellular calcium levels. Since one of the most significant limitations of fluorescent calcium sensitive dyes has been an inability to deal with cells with a large fluorescent background such as arises from hemoglobin, initial investigations using the NMR sensitive chelate have focused on erythrocytes. In addition to studies with

normal erythrocytes, studies on cells derived from patients with sickle cell anemia have been carried out, and the effects of anoxia (which leads to sickling) on cell calcium levels have been determined, for the first time. The group has also carried out NMR studies of cytosolic calcium levels in the perfused rat heart, with initial efforts focused on the determination of basal levels, on the effects of cardiac arrest, and on the effects of varying hypoxic periods.

The application of the fluorine-19 NMR calcium determination method to different cell types has emphasized the need for additional chelators with: (1) varying calcium dissociation constants and (2) increased sensitivity. Consequently, a synthesis effort has been in progress to prepare such fluorinated chelators. A series of chelators with dissociation constants up to ten times lower than those originally available were designed and tested and have found particular use in determinations of the cytosolic calcium levels in erythrocytes which have relatively low basal calcium (30 nM). Additional work is in progress on the development of more sensitive chelators containing trifluoromethyl groups.

As a consequence of the central role of the liver in the metabolism of xenobiotics, NMR studies of hepatic metabolism represent an important component of the research effort. Studies are directed either at the direct observation of biotransformations of chemicals which are present at sufficient levels to permit detection, or at the observation of changes in various metabolic parameters in response to the administration of different chemical or heavy metals. In the first category, we have carried out a series of studies of the metabolism of various fluorinated anesthetics, particularly halothane. Interest in halothane metabolism reflects the occasional toxic effects associated with its use. *In vivo* studies demonstrate that the primary hepatic metabolite is trifluoracetate, and that the rate of production of this metabolite is dramatically increased by induction of the hapatic P-450 system with barbiturates. Studies falling into the second category include the effects of L-ethionine on hepatic ATP levels. Interest in this problem derives in part from the desire to study the role of lowered cellular ATP levels in the production of irreversible cell injury.

In addition to these *in vivo* NMR studies, several *in vitro* studies have been carried out as well. One series of studies is aimed at elucidating the nature of the interaction between clinically important anti-folate drugs used in the treatment of neoplastic disease, and the target enzyme dihydrofolate reductase. These studies have involved the use of the specifically ^{13}C labeled inhibitors methotrexate and trimethoprim, in combination with ^{13}C NMR analysis of the inhibitor-enzyme complex. During the past year, these studies have been extended to include comparisons of the drug interactions with wild type dihydrofolate reductase derived from *E. coli*, and with mutants in which the active site aspartic acid-27 residue is replaced by either an asparagine or a serine residue. The results indicate dramatic differences in the protonation of the inhibitors in complexes with these mutated forms of the enzyme. NMR has also been used to characterize the solution structure of an age related crosslink which forms in bovine and human collagen. These studies, carried out in collaboration with Drs. G. Mechanic and M. Yamauchi at the University of North Carolina, represent the first analysis of a crosslink involving three amino acids, the concentration of which is age related.

PROSTAGLANDIN BIOCHEMISTRY

The Prostaglandin Group investigates the metabolism of arachidonic acid to prostaglandins (PG), hydroxy fatty acids and leukotrienes and studies their role in a number of important physiological and pathophysiological events. The group also studies the metabolism of chemicals by prostaglandin synthase (co-oxidation) in order to determine the role of this metabolic pathway in the development of chemically-induced carcinogensis.

Investigations into the role of prostaglandin hydroperoxidase and peroxyl radicals (cooxidation) in the metabolism of carcinogens and other toxicants to electrophilic or toxic metabolites have continued. Two classes of carcingens are being studied, polycyclic aromatic hydrocarbons and aromatic amines. Prototypes of these classes are benzo(a)pyrene and 2-aminofluorene (2-AF) respectively. The mechanisms involved in metabolism have been elucidated. Metabolism by prostaglandin hydroperoxidase is via a one-electron oxidation, producing free radical intermediates. Stable metabolites, which are reaction products of the free radicals, have been isolated and characterized. These studies yielded several suitable biochemical markers for cooxidation that are being used both in intact cells and in vivo to determine the importance of cooxidation. The formation of unique DNA adducts serves as a useful biochemical marker for 2-AF metabolism while for (±) benzo(a)pyrene 7,8-diol, selective stereochemical oxidation of the (+) isomer is a useful marker. Our ultimate goal in this work is to determine whether cooxidation is an alternate or additional pathway for activating toxicants and carcinogens in extra-hepatic tissues.

Work has also continued on the oxidation of arachidonic acid by prostaglandin synthase, lipoxygenases and possibly cytochrome P-450, and the involvement of this metabolism in the regulation or modulation of biological processes. Arachidonic acid metabolism has been studied in isolated cells, tissues or explants, where both the arachidonic acid oxidation and the biological event can be measured. Since the profile of arachidonic acid metabolism varies among different cells and tissues, characterization of the metabolites continues to be an important part of the studies. We have investigated arachidonic acid metabolism in canine tracheal epithelial cells and shown that the formation of PGD_2 correlates with Cl^- secretion. Factors that control or regulate arachidonic acid metabolism have also been examined. Prostaglandin hydroperoxidase and peroxides play a role in regulation of PG biosynthesis. Other studies on arachidonic acid metabolism in cells were done primarily for future cooxidation studies. A major effort now, and in the near future, is an investigation of the possible role of arachidonic acid metabolism in mitogenesis.

METABOLISM

The Metabolism program is concerned with the development of methodologies to detect and identify metabolites of environmental chemicals in biological systems. Research is also carried out on the mode of interaction of environmental agents with biological systems at the molecular level with particular emphasis on metabolic factors.

The undesirable biological effects of phthalate esters (the most ubiquitous of all environmental pollutants), which include acute testicular atrophy resulting in male sterility, hepatocarcinogenesis in rats and mice, and proliferation of

peroxisomes, are mediated through their metabolites. Some twenty seven metabolites of the most widespread phthalate [di-(2-ethylhexyl)phthalate] have been identified in several mammalian species, a metabolic pathway for their formation has been postulated, and details of the pathway elucidated through in vitro studies. As the biological activities of the different metabolites are elucidated, it becomes clear that species differences in metabolism can potentially result in the resistance of non-rodent species to the undesirable biological effects. This renders extrapolation of toxicity tests from rodents to man highly unreliable.

The emphasis this year has been on the biochemical effects of tumor promoters. This presently exploratory research has as its objectives (a) the determination as to whether selected tumor promoters have lipid peroxidation as part of their mechanisms of action, (b) the development of an approach to measuring lipid peroxidation in vivo that permits quantitative interpretation, and (c) an evaluation of the role of the hepatocyte plasma membrane in tumor promotion. The tumor promoters being used as probes include 2,3,7,8-TCDD, selected PCB isomers, and phthalate esters, all of which reportedly promote liver carcinoma, but which differ drastically in physicochemical properties and metabolism. A new approach to studying lipid peroxidation has been developed which involves preloading the liver lipids with uniformly-C-14 labeled linoleic acid, then exposing the animals to the promoters. This technique has the unique advantage that everything from the earliest to the latest events in the peroxidation sequence can be studied using essentially the same protocol. Future studies will focus on the plasma membrane include effects of tumor promoters on protein kinase C-mediated phosphorylation of specific membrane proteins (and how that correlates with intracellular calcium levels), effects on the turnover of components of the polyphosphoinositide cycle, and effects on the ratio of cyclic to non-cyclic inositol polyphosphates. Thus far effects of all the tumor promoters on protein kinase C activity using purified enzyme have been seen.

Other research has focused on the description of mechanisms at various biochemical and molecular levels including development of structure-activity correlations as a predictive tool in toxicology. A theoretical model for the dioxin (Ah) receptor interaction with polychlorinated biphenyls (PCBs) and dibenzofurans based on molecular parameters and molecular mechanics has been developed and extended to interpret associated enzyme induction potencies. PCB conformational structures have been based on X-ray crystallographic measurements and energy minimization calculations. A similar stacking interaction experimental model was developed for thyroid hormone interactions with donor aromatic compounds, providing results in substantial agreement with their relative binding potencies to the triiodothyronine nuclear receptor. The important elements in this possible experimental binding model for the nuclear receptor are essentially the same as those proposed for the Ah receptor interaction with its ligands. In related work, a theoretical model for PCB (and related compound) interaction with human prealbumin was developed which depended on lateral chlorine substitution.

The role of thyroid hormone binding proteins in mediating the toxic effects of certain halogenated aromatic hydrocarbons of environmental importance is being studied. Current interest is in the binding proteins specific for thyroxine (T_4). Prealbumin (TBPA) is a major thyroxine binding protein in blood which has been proposed as a model for the thyroxine nuclear receptor in tissue.

Molecular interactions of TBPA with the dioxins, furans and polychlorinated biphenyls (PCBs) have been studied with use of computer graphics and predictions made regarding relative binding affinities for such structures. These modeling predictions were tested by experimentally measuring the binding affinities of soluble derivatives of those structures, and the results are in good agreement with prediction. The binding model can account for the requirements for lateral halogens and for a linear and symmetrical molecular shape in toxicity. Similar results were obtained in studies investigating the inhibitory potency of outer (phenolic) ring deiodination of reverse triiodothyronine ($r\text{-}T_3$ 5'-deiodinase) in microsomal fractions of rat liver *in vitro* for which TBPA served as an active site model. The thyroxine nuclear protein solubilized from rat liver tissue also showed competitive binding interactions with similar structural specificity but with the additional requirement of a preference for structures which were planar and highly polarizable. A soluble dioxin approximate isostere shows a remarkably high affinity for the nuclear receptor. Thus the nuclear receptor affinity has the expected sensitivity for possible involvement in toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10004-07 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

NMR Studies of the Mechanisms of Cell Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. London	Research Physicist	LMB	NIEHS
OTHER:	Louis Levy	Research Chemist	LMB	NIEHS
	Elizabeth Murphy	Senior Staff Fellow	LMB	NIEHS
	C. Tyler Burt	Expert	LMB	NIEHS
	Barry Selinsky	Staff Fellow		
	Ronnie R. Rippy	Electrical Engineer	LMB	NIEHS
	Scott Gabel	Biologial Laboratory Technician	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of the biophysical nuclear magnetic resonance (NMR) program is the elucidation of the mechanisms by which chemicals and heavy metals present in the environment cause cell injury. The development and application of NMR methodology in order to achieve this objective may be considered to fall into two broad categories: 1) In vivo metabolic analysis using NMR spectroscopy. Such studies probe directly the metabolism of various xenobiotics when sufficient concentrations are present to permit detection. Additionally, studies of the effects of these agents on metabolic parameters thought to play an important role in the mediation of cell injury are carried out. In addition to measurements of intracellular pH and levels of high energy phosphate compounds, current emphasis is on the measurement of free intracellular calcium levels and on the development of fluorinated NMR active spin traps for the in vivo detection of intracellular free radicals. Cellular calcium levels are measured using a fluorinated calcium chelate in combination with 19F NMR detection. Measurements have been carried out in a variety of cell and perfused organ systems, with current emphasis on red blood cells in which fluorescent calcium sensitive dyes cannot readily be used, and in the perfused heart. Efforts are in progress to develop more specific and sensitive probes for calcium and other cellular cations. 2) In vitro studies of the interaction of various chemicals with known or proposed biological targets. Recent studies have involved the use of ^{13}C labeled antifolate drugs, particularly [2-^{13}C] methotrexate, with the enzyme dihydrofolate reductase. Studies of the biosynthesis of tabtoxin, an inhibitor of glutamine synthetase produced by Pseudomouos syringal, have also been carried out.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 20015-03 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Estimation of Pollutant Concentrations in Groundwaters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Warren T. Piver	Chemical Engineer	LMB NIEHS
Other:	F. Thomas Lindstrom	Associate Professor Dept. Mathematics	Orgeon State University Corvallis, OR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Biochemical/Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been discontinued.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30003-15 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of Analytical Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS
Other:	Joseph Evans	"Q"	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Traditional methods for the study/monitoring of lipid peroxidation are difficult if not impossible to interpret quantitatively. The few methods applicable to *in vivo* studies are especially inadequate in this regard. Use of pairs or sets of measurements on the same samples was shown to be more quantitatively interpretable than any single assay. New methods based on pre-loading with radio-labeled lipids are in development and offer some clear advantages in that early and late stages of lipid peroxidation can be studied by equivalent techniques. The mechanisms of lipid peroxidation can be studied by identifying the isomeric peroxidation products. A method for the quantitative determination of sterol hydroperoxide isomers has been developed and is being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30015-12 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Studies in Gaseous Ion Chemistry.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Christian Guenat	Visiting Associate	LMB
OTHER:	Earl White	Chemist	LMB
	Mike Kinter	Chemist	LMB

COOPERATING UNITS *(if any)*

Dr. M.M. Bursey, UNC Chapel Hill, N.C.; Dr. J.C. Tabet, Ecole Polytechnique, Palaiseau, France

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.20	0.20	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Detailed studies in gas phase ion chemistry, using a triple quadrupole mass spectrometer, have continued with a view to the development of novel analytical procedures. Selected reaction monitoring analyses with a triple quadrupole using ion/molecule reactions rather than collisionally-activated decompositions have been found to be more sensitive and more selective in two model systems.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30020-15 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Transport and Metabolism of Phthalate Esters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS
OTHER:	Richard Philpot	Research Chemist	LP	NIEHS
	Rudolpho Gasser	Visiting Fellow	LP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Bio-organic Chemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This report has been combined with Z01 ES 50082-03 LMB.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30064-09 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of Analytical Methodology for Environmental Health Sciences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carol Parker	Chemist	LMB
	Christian Guenat	Visiting Associate	LMB
OTHER:	Richard Cole	Chemist	LMB
	Richard Smith	Visiting Fellow	LMB

COOPERATING UNITS *(if any)*

Dr. D.S. Millington, Duke University Medical Center, Durham, NC, Dr. M.M. Bursey, UNC, Chapel Hill, NC and Dr. S.J. Gaskell, Tenovus Inst., Cardiff, Wales

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.1	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Recent studies on the mechanism of ionization during thermospray liquid chromatography-mass spectrometry have extended earlier work and substantiated the conclusion that, for most analytes, ion formation is predominatly a gas phase process akin to chemical ionization. The finding has important implications for the prediction of suitable experimental conditions for new analytes.

Tandem mass spectrometry of acylcarnitines and steroid glucuronides has indicated appropriate conditions for the analyses, with a particular view to the differentiation of isomers. These studies have additionally improved understanding of the processes of ion production following fast atom bombardment and of collisionally-activated decomposition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-08 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanisms of Chemically Induced Photosensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Colin F. Chignell	Chief, LMB	LMB	NIEHS
OTHER:	Robert D. Hall	Staff Fellow	LMB	NIEHS
	Anson S.W. Li	Staff Specialist	CSC	NIEHS

COOPERATING UNITS *(if any)*

Enrico Gratton, Dept. of Physics, University of Illinois, Champaign, Urbana, IL., Ann G. Motten, Department of Chemistry, Duke University, Durham, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.7	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Light is known to interact with endogenous or exogenous chemical agents in the skin or eyes, to produce photosensitization (phototoxicity or photoallergy). The objective of this study is to determine whether light-induced free radicals or active oxygen species play a role in photosensitization. Chlorpromazine (CPZ) is an antipsychotic drug that causes both phototoxic and photoallergic reactions. UV irradiation (330 nm) of CPZ in aqueous solution resulted in the homolytic cleavage of the carbon-chlorine bond to yield an aryl radical which extracted a hydrogen atom from suitable donors. CPZ photoionized when irradiated at 280 nm (but not at 330 nm) to give the CPZ cation radical. CPZ generated singlet oxygen (luminescence at 1270 nm) when photo-irradiated in benzene, hexane, and cyclohexane (strong), and methanol, and ethanol (weak) but not in aqueous solutions. CPZ sulfoxide, a major CPZ metabolite in man, generated $^{\bullet}OH$ and the CPZ cation radical upon irradiation with near UV light. Halogenated salicylanilides eg. 3,3',4',5-tetrachlorosalicylanilide (TCSA) and 3,4'5-tribromosalicylanilide (TBSA) generated aryl radicals during photo-irradiation. Irradiation of TBSA with glutathione generated the corresponding thiyl radical, while under the same conditions TCSA abstracted hydrogen atom from the glycyl α-carbon atom of the dipeptide Gly-Ala. The skin photoallergy of TBSA or TCSA may be due to reactions involving protein-derived radical intermediates. The chlorinated phenols bithionol and fentichlor also dehalogenated upon irradiation and in addition underwent photohydrolysis to yield semiquinone radicals. The aryl radical formed by photodeiodination of the anti-arrhythmic drug amiodarone readily abstracted a hydrogen atom from linoleic acid. Reaction of the resultant linoleyl radical with oxygen would initiate lipid peroxidation and provide an explanation for lipofuscin skin deposits in patients receiving this drug.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50077-04 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Free Radical Intermediates of Antiparasitic Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert Docampo	Visiting Scientist	LMB	NIEHS
OTHER:	Silvia N.J. Moreno	Visiting Fellow	LMB	NIEHS
	Ronald P. Mason	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50078-04 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Radical Anion Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Kim Morehouse	Staff Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Clinical Pharmacology, VA Hospital, Minneapolis, MN
Department of Pharmacology, UNC, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

This project has been combined with Z01 ES 50086-01 LMB.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50079-04 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Free Radical Metabolite Formation by Peroxidases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Klaus Stolze	Visiting Fellow	LMB	NIEHS
	Carolyn Mottley	IPA	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This report as been combined with Z01 ES 50086-01 LMB.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-04 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Environmental Health Applications of Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Carol E. Parker	Chemist	LMB
OTHER:	Christian Guenat	Visiting Associate	LMB
	John Dino	Chemist	LMB
	Richard Smith	Chemist	LMB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	0.9	0.20

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Approximately 400 samples have been analyzed as part of the service and collaborative support program. The techniques employed spanned the full range of mass spectrometric methods and instrumentation available to the mass spectrometry group. Collaborative projects included applications of combined liquid chromatography-mass spectrometry, contributions to drug metabolism studies and the use of tandem mass spectrometry in structure elucidation.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50082-03 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Studies on Tumor Promoters and Antipromoters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS
Other:	Ram Rudra Shukla	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	1.8	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Those tumor promoters that are common environmental pollutants and to which humans have a high probability of exposure include the polychlorinated biphenyls (PCBs), phthalic acid esters (PAEs), and chlorinated dibenzo-p-dioxins (TCDD). Well-studied promoters such as the phorbol esters have a wide variety of biochemical effects. By looking for biochemical effects common to the above list of highly diverse compounds, it is hoped that effects relevant to hepatic tumor promotion can be identified. Current studies, which are in a purely exploratory phase, are concentrating on such membrane-related effects as lipid peroxidation and activation of protein kinase C. Lipid peroxidation studies are also directed toward the development of more quantitatively interpretable, in vivo monitoring procedures.

Z01 ES 30020-15 incorporated in this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50083-02 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Specific Binding of Halogenated Aromatic Hydrocarbons to Thyroxine Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James D. McKinney	Supervisory Research Chemist	LMB	NIEHS
OTHER:	Kun Chae	Chemist	LMB	NIEHS
	Urs Rickenbacher	Visiting Fellow	LMB	NIEHS
	Ricky Fannin	Chemist	LMB	NIEHS
	Sandy Jordan	Biologist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Biochemical/Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this work is to study the role of thyroid hormone binding proteins in mediating the toxic effects of certain halogenated aromatic hydrocarbons of environmental importance. Current interest is in the binding proteins specific for thyroxine (T_4). Prealbumin (TBPA) is a major thyroxine binding protein in blood which has been proposed as a model for the thyroxine nuclear receptor in tissue. Molecular interactions of TBPA with the dioxins, furans and polychlorinated biphenyls (PCBs) have been studied with use of computer graphics and predictions made regarding relative binding affinities for such structures. These modeling predictions were tested by experimentally measuring the binding affinities of soluble derivatives of those structures, and the results are in good agreement with prediction. The binding model can account for the requirements for lateral halogens and for a linear and symmetrical molecular shape in toxicity. Similar results were obtained in studies investigating the inhibitory potency of outer (phenolic) ring deiodination of reverse triiodothyronine ($r\text{-}T_3$ 5'-deiodinase) in microsomal fractions of rat liver in vitro for which TBPA served as an active site model. The thyroxine nuclear protein solubilized from rat liver tissue also showed competitive binding interactions with similar structural specificity but with the additional requirement of a preference for structures which were planar and highly polarizable. A soluble dioxin approximate isostere shows a remarkably high affinity for the nuclear receptor. Thus the nuclear receptor affinity has the expected sensitivity for possible involvement in toxicity. Dose-dependent regulation (increase) of the T_4 nuclear receptor number by dioxin was demonstrated and suggest a possible mechanism for potent and persistent expression of thyroid hormone activity which could result in toxicity.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50084-02 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Modeling Approaches to the Study of Molecular Mechanisms of Toxic Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James D. McKinney	Supervisory Research Chemist	LMB	NIEHS
OTHER:	Thomas Darden	Computer Scientist	BRAP	NIEHS
	Andy Maynard	Computational Chemist	LMB	NIEHS
	Lee Pederson	Theoretical Chemist	LMB	NIEHS
	Herbert Posner	Biochemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Department of Chemistry, University of North Carolina, Chapel Hill, NC
Department of Chemistry, University of California, San Diego, CA
Laboratory of Reproductive and Developmental Toxicology

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular/Theoretical Modeling

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This program develops and maintains an interconnected system of computer programs which would allow a chemist or biologist to conveniently ask theoretical questions about molecules of interest. Using structure-activity, molecular and theoretical modeling approaches, we attempt to explain how structure determines toxicity and propose testable mechanistic hypothesis for toxic action. A theoretical model for the dioxin (Ah) receptor interaction with polychlorinated biphenyls (PCBs) and dibenzofurans based on molecular parameters and molecular mechanics has been developed and extended to interpret associated enzyme induction potencies. PCB conformational structures have been based on X-ray crystallographic measurements and energy minimization calculations. A similar stacking interaction experimental model was developed for thyroid hormone interactions with donor aromatic compounds, providing results in substantial agreement with their relative binding potencies to the triiodothyronine nuclear receptor. The important elements in this possible experimental binding model for the nuclear receptor are essentially the same as those proposed for the Ah receptor interaction with its ligands. In related work, a theoretical model for PCB (and related compound) interaction with human prealbumin was developed which depended on lateral chlorine substitution. Conformationally restricted PCBs were shown to be effective binding ligands for the estrogen receptor. In defining important structural properties for activity, molecular mechanics (MM2p), modified neglect of diatomic overlap (MNDO) and ab initio (STO-3G) calculations were performed on a class of similar molecules which bind the estrogen receptor. Related ab initio studies on nitroarenes established a relationship between their electron affinities and mutagenic activities.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50085-02 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Halogenated Aromatic Hydrocarbons as Thyroxine Agonists (or Antagonists)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James D. McKinney	Superivsory Research Chemist	LMB	NIEHS
OTHER:	Ellen Cheung	Visiting Fellow	LMB	NIEHS
	Ricky Fannin	Chemist	LMB	NIEHS
	Sandy Jordan	Biologist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Biochemical Risk Assessment Branch, BRAP, NIEHS
Toxicology Research and Testing Program, NTP, NIEHS
Duke Medical Center, Department of Radiology

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Biochemical/Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

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

TCDD and thyroxine have common molecular reactivity properties which enable them to present a planar face and lateral halogens in interactions with proteins. These molecular properties are consistent with the structure-toxicity relationship of TCDD and related compounds. Polybrominated naphthalenes with four or more bromine atoms and diiodobenzens were shown to bind specifically and with high affinity to the Ah (dioxin) receptor in rat liver cytosol. The binding results further showed little dependency on bromine substitution pattern and the toxic potency of the compound. The diiodobenzenes served as models for the accessible planar faces of thyroid hormone (possible endogenous ligands for the Ah receptor). Other studies demonstrated that dioxin toxicity is modulated by thyroid hormones and that T_3/T_4 combinations can mimic certain toxic effects. These results are compatible with our two receptor mechanism proposal in which the Ah receptor can modulate toxicity by controlling access to a second nuclear receptor, possibly a thyroid hormone receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50086-01 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Free Radical Metabolites of Toxic Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
OTHER:	Kim M. Morehouse	Staff Fellow	LMB	NIEHS
	Ramakrishna D.N. Rao	Visiting Associate	LMB	NIEHS
	Klaus Stolze	Visiting Fellow	LMB	NIEHS
	William D. Flitter	Visiting Fellow	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Ronald G. Thurman, Department of Pharmacology, UNC, Chapel Hill, NC, Dr. Thomas E. Eling, LMB, Dr. Jack Bend, LP

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this study is to determine the role played by free radicals in the reductive and oxidative metabolism of xenobiotics. The anaerobic incubation of almost all nitroaromatic xenobiotics, e.g., nitrobenzene, by the microsomal, mitochondrial, or cytosolic fractions of rat liver in the presence of either NADH or NADPH, leads to a multiple-line electron spin resonance (ESR) spectrum characteristic of the nitro anion free radical. Halogen-substituted nitro compounds are radiosensitizers and are among the most toxic nitro compounds. Loss of halide by the nitroaromatic anion forms a very reactive carbon-centered free radical, detected by spin trapping, which reacts with cellular macromolecules. The irreversible binding of these nitro compounds to DNA, protein, etc. may be inhibited by spin traps. Free radical formation by hepatic microsomal cytochrome P-450 reduction of gentian violet, SO_2, CCl_4 and O_2 has also been investigated. Investigations of the prostaglandin hydroperoxidase and a model enzyme system, horseradish peroxidase, have demonstrated the enzymatic formation of free radical metabolites by oxidation. ESR, on a millisecond time scale, has revealed the formation of a transient phenoxyl radical in the reaction of acetaminophen with horseradish peroxidase/H_2O_2 and bovine lactoperoxidase/H_2O_2. The short-lived radical is clearly distinguished from the persistent paramagnetic melanin polymers that are generated by prolonged incubation of acetaminophen in the presence of oxidizing enzymes. Sulfur-centered free radicals have been detected when cysteine was incubated with horseradish peroxidase and H_2O_2. Reduced glutathione (GSH) was also oxidized to a sulfur-centered radical (GS·) by horseradish peroxidase and H_2O_2. Since cysteine and glutathione play an important role in the structure and function of sulfhydryl-containing proteins, these oxidation reactions may modulate the biological function of these compounds.

Project Nos, Z01 ES 50078-04 LMB & Z01 ES 50079-04 LMB incorporated in this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80008-12 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Biosynthesis of Prostaglandins, Hydroxy-Fatty Acids and Leukotrienes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
OTHER:	Roberta Danilowicz	Biologist	LMB	NIEHS
	Roger Nolan	Visiting Fellow	LMB	NIEHS
	Jorg Schrieber	Visiting Fellow	LMB	NIEHS
	Ronald P. Mason	Research Chemist	LMB	NIEHS
	Beth Kagen	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

R. Boucher	Associate Professor	Dept. Medicine	UNC
Michael Luster	Research Microbiologist	TRTP	NIEHS
Ann Tucker	IPA	TRTP	NIEHS

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.1	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Investigations concerning the oxidation of arachidonic acid by prostaglandin synthase, lipoxygenases and possibly cytochrome P-450, and the relationship of this metabolism to regulation or modulation of biological processes. Arachidonic acid metabolism is studied in isolated cells, tissues or explants, where both the arachidonic acid oxidation and the biological event can be measured. Since the profile of arachidonic acid metabolism varies among different cells and tissues, characterization of the metabolites is an important part of the studies. We have investigated arachidonic acid metabolism in canine tracheal epithelial cells and showed that the formation of PGD_2 correlates with Cl^- secretion. Factors that control or regulate arachidonic acid metabolism are also studied. Prostaglandin hydroperoxidase and peroxides play a role in regulation of PG biosynthesis. Other studies on arachidonic acid metabolism in cells were done primarily for future cooxidation studies. A major effort now, and in the near future, is an investigation of the possible role of arachidonic acid metabolism in mitogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-10 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cooxidation of Xenobiotics by the Prostaglandin Synthetase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Thomas Eling	Research Chemist	LMB	NIEHS
Other:	Ronald Mason	Research Chemist	LMB	NIEHS
	Thomas Petry	Staff Fellow	LMB	NIEHS
	Robert Krauss	Biologist	LMB	NIEHS
	John Curtis	Chemist	LMB	NIEHS
	Beth Kagen	Chemist	LMB	NIEHS
	Jorg Schrieber	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Jack Bend, Laboratory of Pharmacology; and Dr. L. Marnett, Wayne State University; Dr. William Caspary, TRTP, Dr. Fred Kadlubar, NCTR.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.1	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The long range goal of this project is to study the oxidation of chemicals to toxic metabolites by prostaglandin synthetase (PHS) and to demonstrate the significance of this system in chemically induced toxicity or carcinogenesis. We have shown that PHS converts both polycyclic hydrocarbons and aromatic amines to mutagens as measured by bacterial tester systems. Other in vitro studies have demonstrated the formation of electrophilic metabolites that react with macromolecules. Benzo(a)pyrene-7,8-diol is metabolized to an anti-diol epoxide by PHS. We have compared PHS and NADPH-dependent metabolism in mouse skin epidermal cells. The aromatic amine carcinogen 2-aminofluorene (2-AF) is metabolized to free radical intermediates by PHS. The stable end products are azo-, nitrofluorene and 2-aminodifluorenylamine. We have studied the formation of phenolic 2-AF adducts and obtained evidence that 2-AF is oxidized to several free radicals or free radical derived products (nitrenium ion). These radicals may not only be responsible for covalent binding to DNA but also may indeed be the proximate carcinogenic and mutagenic agents. We have also studied the formation of 2-AF DNA adducts catalyzed by PHS. Several unique 2-AF-DNA adducts were detected. We have also shown that 2-napthylamine is oxidized to unique metabolites by PHS and demonstrated a free radical mechanism for the formation of styrene-GSH adducts. Our studies indicate that PHS activates chemicals to ultimate carcinogenic metabolites which may be of importance in the initiation of tumors in extrahepatic tissue. Thus PHS is an enzyme system that, like cytochrome P-450, is important in the metabolism of xenobiotics.

PHS 6040 (Rev. 1/84)

GPO 914-918

LABORATORY OF PHARMACOLOGY

Summary Statement

The Laboratory of Pharmacology carries out research to elucidate the relationships between the transformation and translocation of chemicals and toxicity in various target organs and cells of the body. A multidisciplinary approach is used in these investigations. This laboratory provides a central focus at NIEHS for using pharmacological and pharmacokinetic concepts to characterize, in detail, the biochemical and chemical mechanisms by which environmental contaminants which must be metabolized exert biological effects. It plans and conducts studies 1) to determine the metabolic basis for selective/specific damage to certain organs and cell types which is characteristic of some toxins, 2) to elucidate the mechanisms whereby hormones or chemicals with hormonal activity imprint tissues for the expression of specific sex-dependent cytochrome P-450s, and 3) to determine the role of membrane structure and function in excretion and toxicity of pollutants. The Laboratory of Pharmacology also serves as a focal point within NIEHS, NIH and DHHS for marine and freshwater biomedical research. In this context we are especially interested in possible direct impact on human health by contaminants present in the aquatic environment (including drinking water) and accumulated by aquatic animals. Presently, the Laboratory of Pharmacology contains the Molecular and Comparative Pharmacology and Cell Pharmacology sections.

A. Molecular and Comparative Pharmacology Section (Head: Dr. J.R. Bend):

The overall activity of this group can be described as an integrated, multifaceted effort concerned with understanding the role of chemical metabolism, transport and excretion in the mediation of toxicity such as overt tissue damage, or more subtle effects such as carcinogenesis, mutagenesis and teratogenesis.

For many chemicals, the processes of metabolism are means of both activation and inactivation and the relative activities of these pathways/steps, as well as their location in different cells, and parts of cells, are most critical to the particular outcome of exposure to any given chemical. That these processes of metabolic activation and inactivation are themselves often controlled by genetics, as well as being affected by age, sex, disease and environment, further complicate the understanding of their role in the modulation of the biological activity of any chemical in any tissue or animal species or individual of that species at any specific time of exposure.

Major emphasis is currently focused on toxication-detoxication systems at the isozyme level, transport and excretory mechanisms, and membrane toxicity.

Another major purpose of this section is to serve as a national focus for an aquatic pharmacology/toxicology program -- to promote awareness of and use of certain aquatic species and experimental systems for studies, the results of

which will give us a better understanding of human disease and contributions of pollution to such disease.

The collaborative efforts of this group demonstrate both its desire to share expertise where possible as well as to make use of the many opportunities for introducing more powerful and new approaches in this research area of chemical metabolism as related to toxicity.

Recent Accomplishments:

1. Dr. Bend's laboratory:

   a. The majority of rabbit pulmonary cytosolic glutathione S-transferase activity has been isolated as an apparently cationic homodimeric protein of subunit molecular weight 27.5 Kdaltons. This isozyme is absent from (or present at very low concentrations in) liver.

   b. In collaboration with Drs. Eling and Mason, Laboratory of Molecular Biophysics, NIEHS, we demonstrated a radical mechanism for the formation of styrene-glutathione adducts. The first step in this reaction sequence is the formation of the glutathione thiyl radical by the peroxidase.

   c. At an intravenous dose of 10 μmol/kg, N-α-methylbenzyl-1-aminobenzotrazole, a suicide inhibitor of P-450 that we synthesized and characterized, was shown to be a pulmonary specific (vs. liver), isozyme 2 specific suicide inhibitor of rabbit cytochrome P-450 *in vivo*.

2. Dr. Negishi's laboratory:

   a. Two loci (Rip and Rsh) were defined which regulate the female-specific expression of "I"-P-450, 16α (a cytochrome P-450 isozyme which catalyzes testosterone 16α-hydroxylation) in mouse liver microsomes and of P-450, 15α (a P-450 isozyme which catalyzes testosterone 15α-hydroxylation) in mouse renal microsomes, respectively. The Rip locus was localized to mouse chromosome 7.

   b. Growth hormone, when administered in a pulsatile manner, was found to be a masculinizing factor which acts by expressing the male-specific isozyme of testosterone 16α-hydroxylase ("C"-P-450, 16α) and by repressing female-specific hydroxylases ("I"-P-450, 16α and P-450, 15α).

   c. Four different cDNAs of the "I"-P-450, 16α family were isolated and DNA sequence data showed that three of these cDNAs represent mRNA derived from a single gene.

   d. "C"-P-450, 16α cDNA (1481bp) was sequenced and the amino acid sequence deduced. This P-450 isozyme has only 20% sequence homology with other known forms of P-450.

3. Dr. Philpot's laboratory:

   a. A number of cDNA probes of rabbit P-450 isozymes 2 and 5 have been isolated and the sequences of the coding regions of two cDNAs of isozyme 2 were found to be identical. The derived amino acid sequence differs from the published amino acid sequence in only 6 of 491 positions. cDNA for these forms of P-450 prepared from rabbit lung are now being sequenced. Large increases in mRNA of isozymes 2 and 5 are observed in liver following treatment of rabbits with phenobarbital; no such changes occur in lung.

   b. Apparent genetic polymorphism was found for rabbit lung and kidney flavin-containing monooxygenases. Three different combinations involving three different forms of the enzyme have been observed in each tissue.

   c. Homologues of rabbit P-450 isozyme 5 were detected in the lungs of mice, rats, guinea pigs, hamsters and monkeys. The metabolism of the carcinogen 2-aminofluorene by pulmonary microsomes of all species tested was inhibited by antibodies to rabbit isozyme 5.

4. Dr. Pritchard's laboratory:

   a. A sodium independent carrier was identified in renal brush border membranes (BBM) that, in conjunction with the basolateral membrane anion exchanger previously described, may mediate secretory sulfate flux. The BBM carrier is a symmetrical, electroneutral anion exchanger.

   b. Organic anion and, to a lesser extent, organic cation transporters are stimulated by in vitro treatment of fish renal tubules with spermine and spermidine, polyamine growth factors.

   c. The crustacean urinary bladder was shown to share multiple features with mammalian proximal tubule. These include sodium-coupled solute reabsorption, secretion of organic anions and cations, and the electrical properties of a leaky epithelium. Since this tissue is a simple, flat sheet epithelium of single cell type, it has excellent potential for an experimental model of the proximal tubule.

   d. Cryomicrodissection and reference phase analysis of intracellular solute activities and compartmentalization have been used to analyze individual nuclei from amphibian oocytes. This system provides the ability to analyze nuclear function and its modulation by ions, hormones and exogenous chemicals.

B. Cell Pharmacology Section (Head: Dr. J.W. Putney, Jr.)

This section was formerly headed by Dr. James R. Fouts. Dr. Fouts transferred from the Laboratory of Pharmacology to the Office of the Director, NIEHS, this year. Dr. James W. Putney, Jr., Department of Pharmacology, Medical College of

Virginia, has been recruited to fill this position, and he will arrive at NIEHS late in FY'86.

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.

Examples of collaborative programs outside of NIEHS for the senior scientists are: Dr. Bend with Dr. Bengt Mannervik of the University of Stockholm, and with Dr. Mike Meredith, Vanderbilt University; Dr. Philpot with Dr. Eric Johnson of Scripps Clinic and Research Foundation, Drs. Paul Thomas and Wayne Levin, Hoffmann-LaRoche, and Dr. Lucy Waskell, Veterans Administration, San Francisco; Dr. Pritchard with Drs. Paul Linser and Margaret James, University of Florida, Gainsville and Dr. Gaylen Neufeld, Emporia State University; and Dr. Negishi with Dr. J. E. Shively, City of Hope, California.

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.

D. Personnel:

Dr. James W. Putney, Jr. joined the Laboratory of Pharmacology as Head of the Cell Pharmacology Section this year. Dr. James R. Fouts, a former Scientific Director and Chief, Laboratory of Pharmacology, who had been an integral part of this laboratory for 16 years, and its Chief for most of this period, transferred to Dr. Rall's office to assist the NIEHS Director with some of his scientific administration duties. Members of Dr. Fouts' research program also transferred to other NIEHS programs. Ms. Theodora Devereux, a long-time member of LP moved to the Biochemical Applications Laboratory of the Biometry and Risk Assessment Program, Ms. Janet Diliberto transferred to the Toxicological Research and Testing Program, and Mrs. Blair Hoyle transferred to the National Park Science, Department of the Interior. New arrivals included Dr. Kaname Kawajiri, a Visiting Scientist with Dr. Negishi, Dr. Peter Smith, a Visiting Fellow with Dr. Pritchard, and Ms. Merja Lakso, a Guest Worker with Dr. Negishi. Other individuals leaving the Laboratory of Pharmacology this year were Dr. James Mathews, a Staff Fellow, Dr. Julie Horton, a Visiting Fellow and Dr. Beresford Stock, a Visiting Scientist (all with Dr. Bend).

E. Other Activities:

Dr. J.R. Bend: Adjunct Professor, Interdepartmental Toxicology Program, Department of Entomology, North Carolina State University, Raleigh; Adjunct Professor, Curriculum in Toxicology, School of Medicine, University of North

Carolina; Member Editorial Advisory board for Drug Metabolism and Disposition and Board of Editors, Environmental Health Perspectives; Associate Editor, Reviews in Biochemical Toxicology; served on graduate student committees at North Carolina State University and University of North Carolina; Member, Committee for Toxicokinetics Section, WHO-sponsored International Program of Chemical Safety; Invited presentation given at a Symposium on the Lung, Norwegian Society of Pharmacology and Toxicology; Invited speaker at a FASEB-sponsored conference on "Lung Pharmacology and Pathophysiology"; and Research Seminar at the School of Medicine, University of Western Ontario, London, Canada.

Dr. M. Negishi: Adjunct Associate Professor, School of Veterinary Medicine, North Carolina State University; Invited speaker at a Symposium on "Genetics and Biology of Cytochrome P-450", Sendai, Japan.

Dr. R.M. Philpot: Adjunct Professor, Department of Entomology, North Carolina State University, Raleigh; Member, Toxicology Advisory Committee, North Carolina State University; Associate Managing Editor (U.S.A.) Chemico-Biological Interactions; Associate Editor Reviews in Biochemical Toxicology; Member, Editorial Board Molecular Pharmacology.

Dr. J.B. Pritchard: Appointed to Editorial Board of American Journal of Physiology; Organized an international conference on "Mechanisms of Pollutant Action in Aquatic Organisms" and edited monograph based on conference proceedings; Invited to present the W.B. Kinter Memorial Lecture at Mt. Desert Island Biological Laboratory; Presented research seminars at Duke University and SUNY, Buffalo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70132-06 LP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.M. Schiller	Research Chemist	LP	NIEHS
Others:	M.W. King	NIH-Postdoctoral Fellow	NRSA	NIEHS
	D.E. Chapman	Toxicologist	UNC	NIEHS

COOPERATING UNITS *(if any)*
Curriculum of Toxicology, University of North Carolina, Chapel Hill, N.C.

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Cell Pharmacology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

Dr. Schiller is no longer with the NIEHS. The project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80001-14 LP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microsomal Mixed-Function Oxidase System: Specificity and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.M. Philpot	Research Chemist	LP NIEHS
Others:	B.A. Domin	Staff Fellow	LP NIEHS
	R.R. Vanderslice	Graduate Student	LP NIEHS
	G. Carver	Biologist	LP NIEHS
	R. Tynes	Guest Worker	LP NIEHS
	R. Gasser	Visiting Fellow	LP NIEHS

COOPERATING UNITS *(if any)*
Department of Pharmacology, Scripps Clinic and Research Foundation, LaJolla, CA; Department of Biochemistry, University of Michigan, Ann Arbor, MI.

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular and Comparative Pharmacology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

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

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

Rabbit cytochrome P-450 isozymes 2 and 5 are present in lung and liver. In the liver, but not the lung, the concentrations of these isozymes are increased by treatment of rabbits with phenobarbital. Homologues of isozymes 2 and 5 have been detected in lungs of mice, rats, hamsters, guinea pigs and monkeys. Although homologues of isozyme 2 are also present in livers of these species, hepatic homologues of isozyme 5 are not detected in any species except the hamster. Treatment of hamsters with phenobarbital does not increase the concentration of the isozyme 5 homologue in liver. In microsomal preparations from the Clara cell, alveolar type II cell, and alveolar macrophage fractions isolated from rabbit lung, the relative concentrations (% total P-450) of cytochrome P-450 isozyme 2 and 5 are similar to those determined with the microsomal fraction from intact lung. The absolute concentrations of these isozymes are somewhat (about 2-fold) higher in the Clara cell preparation than in preparations from type II cells or intact lung and substantially higher than the concentrations found with the macrophage. In contrast, the highest concentrations of isozyme 6 are found in microsomes prepared from intact lung. Analysis of cDNA probes for rabbit liver and lung isozyme 2 indicates that microheterogenous (greater than 95% homology) forms of this isozyme exist. Results of restriction mapping show a difference in the probes at a position corresponding to amino acid 174. The derived sequence for the hepatic isozyme is not identical to the sequence that has been published for the protein. This difference may be due to the animal populations used in the separate studies or to microheterogeniety in liver.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80007-15 LP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Conjugation and Oxidation Pathways for Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Bend	Chief	LP	NIEHS
Others:	C. Serabjit-Singh	Research Chemist	LP	NIEHS
	D. Brier	Chemist	LP	NIEHS
	G. Parker	Chemist	LP	NIEHS
	J. Horton	Visiting Fellow	LP	NIEHS
	T. Eling	Research Chemist	LMB	NIEHS
	R. Mason	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Arrhenius Laboratory of Biochemistry, Stockholm University; Laboratory of Molecular Biophysics, NIEHS; Department of Biochemistry, University of Dundee, Scotland; Department of Biochemistry, Vanderbilt University.

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	1.9	1.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Epoxides are frequently formed as metabolites of unsaturated hydrocarbons by cytochrome P-450-dependent monooxygenase activity. Many arene and alkene oxides are known to react covalently with macromolecules and to transform cells in vitro, and some of these are ultimate carcinogens, mutagens and/or cytotoxins. We are studying various aspects of the enzymatic formation and metabolism of epoxides and of glycols and phenols which are products of subsequent epoxide biotransformation, in relationship to cell-selective and organ-selective toxicity of compounds metabolized to epoxides by both hepatic and extrahepatic tissues. Particular attention is given to the respiratory tract because this is a common site for pollutant-mediated damage. We are currently investigating the rabbit pulmonary glutathione S-transferases using a combination of biochemical (purification), immunochemical (polyclonal and monoclonal antibodies) and chemical (stereoselectivity with polycyclic arene oxides and alkene oxides as substrates) techniques; the biosynthesis and status of the tripeptide glutathione, which is important in detoxication of electrophilic metabolites, in Clara cells, alveolar type II cells, and alveolar macrophages isolated from rabbit lung; and the biochemical and immunochemical properties of pulmonary and renal UDP-glucuronosyltransferases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80031-10 LP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Role of Altered Membrane Function in Xenobiotic Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.B. Pritchard	Research Physiologist	LP NIEHS
Others:	D.S. Miller	Expert	LP NIEHS
	P.M. Smith	Visiting Fellow	LP NIEHS

COOPERATING UNITS *(if any)*

University of Florida, C.V. Whitney Laboratory; Duke University; University of North Carolina

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	2.6	2.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The ability to transport solutes across epithelial membranes is vital for the function of many organs, e.g., secretion and reabsorption by the kidney. In turn, epithelial transport depends upon the coordinated function of individual transport systems located at opposite poles of the cells in the apical (BBM) and basolateral (BLM) membranes. Many of these membrane processes, particularly for anions, are not yet understood. Furthermore, because of their complex organization, functional importance, and exposed location, epithelial membranes are particularly susceptible to toxic effects of foreign chemicals. Our major recent emphasis has been on increasing our understanding of vectorial solute transport in polar epithelia, including the properties of specific carrier systems, the driving forces energizing transport, the regulation of transport events, and the coupling between events at opposite poles of the cells. Isolated BBM and BLM vesicles are used to examine cell membrane events. Intact epithelial preparations, including teleost renal tubules and crustacean urinary bladder, allow assessment of electrical and transport properties and permit study of regulatory mechanisms. Cryomicrodissection of intact amphibian oocytes permits direct analysis of solute activities in a living cell. Results in mammals, lower vertebrates and crustaceans demonstrated the intricate interrelations between solute transport, ion gradients and metabolic energy transduction. Transport may, therefore, be disrupted by xenobiotics at multiple sites in these complex chains of events. However, certain features of these events are common to the transport of several solutes. Thus, the same mechanism may account for impaired transport of multiple solutes, e.g., collapse of proton gradients by pentachlorophenol or of the sodium gradient by ouabain lead to reduced transport of numerous solutes whose secondary active transport is energized by these gradients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80038-03 LP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Suicide Inhibitors of Cytochrome P-450: Isozyme and Tissue/Cell Selectivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.R. Bend	Chief	LP NIEHS
Others:	J. Mathews	Staff Fellow	LP NIEHS
	D. Brier	Chemist	LP NIEHS
	G. Parker	Chemist	LP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Molecular and Comparative Pharmacology

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.6	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Microsomal monooxygenase systems contain multiple isozymes of cytochrome P-450 which contribute differentially to the oxidative metabolism of endogenous and exogenous substrates; isozyme differences in Km, Vmax, regioselectivity and stereoselectivity are common. Hence, modulation of the relative amounts of various P-450 isozymes can have pronounced effects on metabolism of endogenous and exogenous chemicals. For this reason we are studying isozyme selectivity and tissue/cell selectivity of suicide inhibitors of cytochrome P-450. The suicide inhibition by 1-aminobenzotriazole (ABT) and some of its novel N-alkylated derivatives, which we synthesized and characterized, is being studied in rabbit lung and liver. Although ABT is a potent suicide inhibitor, it shows little P-450 isozyme selectivity. N-α-methylbenzyl-ABT, on the other hand, is much more potent and highly selective. At certain doses in vivo it destroys only isozyme 2-catalyzed benzphetamine N-demethylase activity in lung, having no effect on isozymes 5 and 6 in lung and no effect on any of the parameters tested in liver.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80039-03 LP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

Xenobiotic Transformation in Isolated Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James R. Fouts	Research Pharmacologist	LP	NIEHS
	Theodora Devereux	Research Biologist	LP	NIEHS
Others:	Janet Diliberto	Biological Lab. Technician	LP	NIEHS
	Blair Hoyle	Biological Lab. Technician	LP	NIEHS
	Thomas Eling	Research Chemist	LMB	NIEHS
	Richard Philpot	Research Chemist	LP	NIEHS
	Barbara Domin	Staff Fellow	LP	NIEHS

COOPERATING UNITS *(if any)*
Biometry and Risk Assessment Program (BRAP); Histology, NIEHS; Department of Pulmonary Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina (human tissues).

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Cell Pharmacology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Major cell types of the lung and upper airways of rabbits and rats are being isolated and used in studies of xenobiotic and endogenous substrate metabolism. Cell types being studied are Clara cells, alveolar type II cells, alveolar macrophages, ciliated cells and other tracheal cells. Some comparisons are being made with hepatocytes. Studies of metabolism and Western blotting analysis on the effects of the cell isolation procedures on the isolated cell populations are being made using antibodies to selected cytochrome P-450 isozymes and to P-450 reductase. Pulmonary cells from humans and dogs are also being prepared and analyzed for P-450 isozymes and xenobiotic metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80040-03 LP

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Developmental Pharmacogenetics of Liver Microsomal Testosterone Hydroxylases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Negishi	Visiting Scientist	LP	NIEHS
Others:	M. Noshiro	Visiting Associate	LP	NIEHS
	B. Burkhart	Biologist	LP	NIEHS
	T. Ichikawa	Visiting Fellow	LP	NIEHS
	K. Kawajari	Visiting Scientist	LP	NIEHS
	J. Squires	Visiting Fellow	LP	NIEHS
	M. Lakso	Guest Researcher	LP	NIEHS
	G. Wong	"Q"	LP	NIEHS

COOPERATING UNITS *(if any)*: City of Hope, CA; Laboratory of Genetics, NIEHS; Department of Pediatrics, Duke University; Department of Pharmacology, University of Minnesota.

LAB/BRANCH: Laboratory of Pharmacology

SECTION: Molecular and Comparative Pharmacology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	5.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is aimed at understanding the molecular mechanism(s) of sex-dependent gene regulation of steroid hydroxylases in liver and renal microsomes from mice. Genetic regulation: We defined two loci (Rip and Rsh) which regulate female-specific expression of "I"-P-450, 16α (an isozyme of testosterone 16α-hydroxylase) in liver microsomes and expression of P-450, 15α (an isozyme of testosterone 15α-hydroxylase) in renal microsomes, respectively. With inbred strain (9XA), the Rip locus was located on mouse chromosome 7. The two loci are expected to be trans-acting genetic elements whose gene products are not yet identified. Hormonal regulation: Growth hormone (GH) is a masculinizing factor which acts by expressing the male-specific isozyme of testosterone 16α-hydroxylase ("C"-P-450, 16α) and repressing female-specific hydroxylases ("I"-P-450, 16α and P-450, 15α) in male liver. Estrogen is another repressor of "I"-P-450, 16α in male liver and estrogen-dependent repression is under control of either the Rip locus or a locus closely linked to the Rip locus. Expression of P-450, 15α is growth hormone- and androgen-dependent in mouse kidney. It was also found that the induction of "I"-P-450, 16α by phenobarbital in 129/J mice is due to a derepression, presumably through an interaction with pathway of the hormonal repression of this gene. Cloning and characterization of cDNAs and genomic DNAs: Four different cDNAs for the "I"-P-450, 16α family were isolated and DNA sequence data revealed that three of these cDNAs represent "I"-P-450, 16α mRNAs derived from a single gene by an alternative splicing and utilization of poly A sites. One other cDNA shared 90% DNA sequence homology with "I"-P-450, 16α. 1481bp of "C"-P-450, 16α cDNA were sequenced and the amino acid sequence was deduced from it. "C"-P-450, 16α had only 20% sequence homology with other known P-450s. The "C"-P-450, 16α gene family is on mouse chromosome 15. Two closely related P-450, 15α cDNAs were sequenced (98% homology) and two tandomly repeated P-450, 15α genes were found on mouse chromosome 7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80041-02 LP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Detection and Quantitation of Cytochrome P-450 Isozymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.M. Philpot	Research Chemist	LP	NIEHS
Others:	B.A. Domin	Staff Fellow	LP	NIEHS
	P. Bent	Biological Lab. Technician	LP	NIEHS

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Department of Biochemistry and Drug Metabolism, Hoffmann-LaRoche, Nutley, NJ

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Molecular and Comparative Pharmacology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project has been combined with Z01 ES 80001-14 LP.

THE LABORATORY OF PULMONARY PATHOBIOLOGY

SUMMARY STATEMENT

The Laboratory of Pulmonary Pathobiology (LPP) studies basic aspects of pulmonary cell biology and biochemistry as well as mechanisms-of toxic cell injury leading to the development of pulmonary diseases. By investigating normal cell functions as well as their disruption by toxic agents we hope to elucidate pathogenetic mechanisms of disease. The scope of research approaches employed spans from whole animal experiments to cell culture and molecular studies. The Laboratory's research efforts revolve around three major themes: regulation of cell differentiation, mechanisms of neoplastic transformation and mechanisms of particle and fiber toxicity.

In the following I will highlight a few select research projects in which significant advances have been made during the past year.

Mechanisms of Cell Differentiation of Airway Epithelium. The epithelium of the airways has a high degree of plasticity. Normally it is composed of two types of differentiated cells namely mucous and ciliated cells. However this state of differentiation can rapidly change when the epithelium is injured by physical or chemical insults or when the host is deprived of vitamin A. Under these circumstances the epithelium changes from a mucociliary to a squamous phenotype, showing many signs typical of keratinocyte differentiation.

Studies were conducted to identify markers of keratinocytic differentiation of airway epithelial cells, to identify key factors regulating the expression of this phenotype and to elucidate mechanisms involved in it's expression. Three biochemical markers were found to be useful for measuring keratinocyte differentiation in these cells: epidermal transglutaminase, cholesterol sulfate and two keratins with the molecular weights of 48kD and 56kD. Primary epithelial cell cultures, established from the tracheas of rabbits in serum and vitamin A free medium were used. The expression of the keratinocyte phenotype was found to be markedly influenced by several factors: confluency, transforming growth factorβ (TGFβ) and retinoids. During logarithmic growth the cultured tracheal cells were undifferentiated. Confluency triggered a program of terminal keratinocyte differentiation, clearly manifested by dramatic changes in morphology and expression of three biochemical markers (see above). The same program of differentiation was triggered in rapidly growing cultures by addition of pM quantities of TGFβ. In both situations differentiation was accompanied by cessation of cell replication and a marked loss of clonogenic cells (presumed to be stem cells) from the cultures, suggesting that cessation of cell replication and keratinocyte differentiation are closely coupled events. However if the culture media contained small amounts of retinoid ($\leq 10^{-9}$M), keratinocyte differentiation was blocked even though the cells were undergoing terminal cell division. Thus two distinct events can be descerned in the expression of the squamous phenotype of airway epithelial cells: Terminal cell division and keratinocytic differentiation; the latter is not an obligatory consequence of the former but terminal cell division may be a necessary prerequisite for keratinocyte differentiation. Molecular studies have shown that with the onset of keratinocyte differentiation two mRNAs of 1.0 and 1.25kb length are expressed at high levels, which are pre-

sent only in low abundance in undifferentiated, proliferating cells or in confluent retinoic-acid treated cells, supporting the notion of a two-step process in keratinoctye differentiation.

Modulation of the Transformed Phenotype by Retinoids. Vitamin A and some of its derivatives, so called retinoids, have been shown by a number of investigations to inhibit tumor formation in vivo and neoplastic transformation in cell culture; however other studies have shown the opposite effect, namely enhancement of neoplastic transformation and tumor promotion. This is perhaps not surprising if one takes into account the known effects of retinoids on cell proliferation and differentiation. Depending on the cell type and the state of commitment, retinoids can either stimulate cell replication or induce (terminal) differentiation. It seems likely, that the retinoid effects on proliferation and differentiation are closely linked to the modulation of the transformation process.

Studies were conducted to examine the effects of retinoids on the multistep process of neoplastic transformation of rat tracheal epithelial cells. In this in vitro transformation model the development of immortal and neoplastic cellular phenotypes are preceded by the so called enhanced growth variant (EGV); the clonal evolution of this preneoplastic phenotype was recently described in considerable detail. Studies showed that retinoic acid (RA) inhibited the evolution of this early preneoplastic transformant in a dose dependent manner. Even when RA treatment was delayed for 3 weeks after the cultures were exposed to a transforming dose of MNNG, a marked reduction of transformation frequency was detected at 5 weeks (at which time the transformed colonies are scored). Importantly, inhibition of transformation was found to be irreversible. Based on a number of studies it was concluded, that the inhibition of transformation was probably due to the antiproliferative effect RA was found to have on cells isolated from early transformed clones, as well as on normal rat tracheal cells. The most important finding was that, most of the EG variant clones lost their sensitivity to RA, as they progressed to the "immortal" stage, which is the second stage of neoplastic transformation of rat tracheal cells. Thus the transition from the first to the second transformation stage of RTE cells is marked by the loss of sensitivity to retinoids, a normal growth regulatory factor for this cell type.

In other studies the effect of RA on the expression of the transformed phenotype of v-src and v-Ha-ras transfected Syrian hamster embryo (SHE) cells was examined. RA suppressed the transformed phenotype of v-src transfected cells, as measured by anchorge independence of growth, but enhanced the transformed phenotype of v-Ha-ras transfected cells. Interestingly the synthesis of the onc gene products pp60^{src} and p21^{ras}, was not measurably altered, nor was the tyrosine kinase activity of the src gene product significantly affected. This suggests that the RA effect on the transformed phenotype of cells transfected by these two oncogenes probably does not involve regulation of these two oncogenes but is directed at other molecular targets important for expression of the transformed phenotype in these cells. Possible target mechanisms currently being investigated include TGFβ synthesis and processing in v-Ha-ras transfected SHE cells.

Cellular and Molecular Mechanisms of Neoplastic Transformation. The development of cancer proceeds in multiple stages during which cells progressively acquire

many phenotypic changes culminating in the neoplastic/ malignant phenotype, the hallmark of which is unregulated, invasive and metastatic growth. The Syrian hamster embryo (SHE) cell transformation system has been used as a paradigm to investigate sequential steps in neoplastic transformation. These cells, following exposure to chemical carcinogens, undergo several discrete changes, such as morphological transformation and immortalization before acquiring anchorage-independent and neoplastic growth characteristics. Thus they provide a useful tool to examine mechanisms involved in early as well as late stages of neoplastic transformation.

Investigations from various laboratories have indicated that inappropriate expression or mutation of certain genes, so called proto-oncogenes, can play a role in some steps of neoplastic transformation. Studies were undertaken with several viral oncogenes, to determine whether they can neoplastically transform normal SHE cells and whether a single step or multiple steps are involved in the transformation process. These studies showed that neither v-myc nor v-Ha-ras, by themselves are able to transform normal SHE cells. However, when cells were co-transfected with these two viral genes, neoplastic transformation resulted. Chromosomal analyses of the tumors produced by injecting such cells into nude mice revealed a most interesting finding: all of the tumors showed monosomy 15. This suggested that at least 3 steps were involved in their transformation; two steps brought about by the cooperation of the two oncogenes and the third step by the loss of chromosome 15. Presumably chromosom 15 carries a gene inhibiting expression of the transformed state. This interpretation was strongly supported by subsequent experiments in which cytogenetic studies were performed on cell-cell hybrids between the ras-myc tumor cells and normal cells. Such hybrids were not tumorigenic inspite of the fact that they expressed ras and myc oncogene products. When these nontumorigenic cell hybrids were passaged, tumorigenic segregants appeared which had lost chromosome 15. Together these experiments suggest that loss of chromosome 15 results in the loss of a tumor as suppressing gene. Thus it appears that both, activation of transforming genes as well as loss or inactivation of suppressor genes, are important events in multistep neoplastic transformation.

Mechanisms of Particle and Fiber Toxicity. Inorganic particles such as abestos and silica, when inhaled, cause severe obstructive pulmonary disease. In spite of decades of research, the cellular and biochemical mechanisms leading to fibrotic lung disease are only poorly understood.

Previous studies indicated that the first noticeable cellular change following brief asbestos exposure of rats was the accumulation of macrophages at alveolar duct bifurcations, which was shown to be the primary site of fiber deposition. A major research effort was then devoted to identify the macrophage chemotactic factor generated as the fibers land on the alveolar duct surface. It was found that the fibers activated a complement dependent chemotactic factor in alveolar fluids. Partial chemical characterization of the chemotactic activity suggests that it is complement 5a. Genetically complement deficient mice and chemically complement deprived rats can not generate the factor, have a depressed macrophage response upon inhalation of asbestos and, most importantly, show markedly reduced pulmonary fibrosis. Thus it was concluded that the first step in the pathogenesis of pulmonary fibrosis following asbestos exposure is activation of C5 complement to macrophage chemotactic C5a.

The next important objective was to identify the role of macrophages in particle induced fibrogenesis. The hypothesis under investigation is, that such macrophages secrete factors which stimulate the growth of various cell types in

alveolar duct regions, leading to fibrosis and obstruction of small airways. Autoradiographic studies with ^{3}H-thymidine in asbestos exposed animals indeed showed that within a few hours after accumulation of macrophages in alveolar duct regions, an increased number of epithelial cells and interstitial fibroblasts entered DNA synthesis. To determine whether the macrophages are responsible for stimulating cell proliferation in interstitial fibroblasts, an *in vitro* system was developed to measure mitogenic stimulation of rat lung fibroblasts (RLF) by macrophage products. It was shown, that pulmonary macrophages exposed *in vitro* to asbestos fibers, secrete a factor or factors which stimulate the growth of RLF. Partial physical and chemical characterization of the factor indicates that it has many similiarities with platelet derived growth factor (PDGF). Whether the growth factor secreted by particle stimulated macrophages is identical with PDGF remains to be determined.

Another research effort is concerned with the pulmonary toxicity of silica particles and the effects of silica on the pulmonary surfactant system. Recent studies have layed the ground work for examining the intracellular and extracellular surfactant phospholipid pools quantitatively and to determine the relative pool sizes under various normal (development and growth) and abnormal conditions. It was shown, that within 28 days after exposure, the intracellular surfactant pool increased 123-fold over control values in the lungs of rats; at the same time the extracellular surfactant pool increased 22-fold. Morphometric and cell isolation studies showed that the number of type II alveolar cells (the producers of surfactant phospholipids) increased by about 75% above normal. Perhaps more importantly almost 40% of the type II cells were increased in size, and, based on this size change, could be separated from the normal type II alveolar cells by centrifugal elutriation. These hypertrophic type II cells were increased by 50% in volume. The number of lamellar bodies per cell and the size of the lamellar bodies were on the average increased by > 60% compared to normal. Taken together these data suggest that the silica particles cause a marked type II cell hyperplasia and a marked increase in type II cell biosynthetic activity. In addition they cause a severe imbalance between intra and extracellular surfactant phospholipid pools. Future studies will attempt to determine the metabolic alterations in isolated hypertrophic type II cells, in order to elucidate the mechanisms of silica toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-25001-09 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. C. Barrett	Research Chemist	LPP, NIEHS
Others:	M. Oshimura	Expert	LPP, NIEHS
	N. Tanaka	Visiting Fellow	LPP, NIEHS
	P. Lamb	Biological Lab Tech	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Environmental Carcinogenesis Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

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

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

Most chemical carcinogens induce DNA damage and are mutagenic at specific genetic loci; however, certain carcinogens (including the human carcinogens diethylstilbestrol, asbestos, arsenicals and benzene) usually do not induce gene mutations. We have examined the activity of these chemicals, proposed as carcinogenic but not mutagenic, to induce morphological transformation, gene mutations and chromosome mutations in Syrian hamster embryo cells in culture. We have reported previously that diethylstilbestrol (DES) induces transformation in the absence of mutations at specific genetic loci. Furthermore, we have proposed that the mechanism of action of DES is related to its ability to induce numerical chromosome changes, i.e., aneuploidy. We have now shown that DES has colcemid-like activity in that it disrupts microtubule organization. Inhibition of polymerization of spindle microtubules is therefore a possible mechanism for DES-induced aneuploidy and possibly cell transformation according to our hypothesis. This hypothesis is further supported by our observations that colcemid and vincristine sulfate, two well-known inducers of aneuploidy, are also inducers of morphological transformation. The mechanism of another important human carcinogen, asbestos, was also examined. The ability of asbestos and other mineral fibers to induce cell transformation was observed to depend on fiber dimension similar to the results found in vivo in studies on mesothelioma induction. We have proposed that asbestos induces cell transformation due to its ability to induce chromosomal changes in the treated cells. We recently found that asbestos fibers induce anaphase abnormalities indicating that a direct physical interaction of the fibers with chromosomes occurs during mitosis. In the asbestos-induced cell lines a nonrandom chromosome change, trisomy of chromosome 11, was found. These results further support our hypothesis that the mechanism of asbestos-induced transformation is due to a chromosomal mutation. Thus, our results suggest an important role for carcinogen-induced aneuploidy in carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25020-04 LPP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Regulation of the Pulmonary Surfactant System and its Modification by Toxic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	L. B. Gilmore	Biologist	LPP, NIEHS
	B. A. Miller	Graduate Student	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

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Biochemical Pathology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Pulmonary surfactant is a complex mixture of lipids and proteins that prevents collapse of the alveoli and distal airways at low lung volumes. Numerous chemical and particulate toxicants, when inhaled, affect the surfactant system. Silica dust administered by either intratracheal injection or by inhalation, causes massive increases in the surfactant content of the lungs. The mechanisms through which silica stimulates the surfactant system are not known. The pulmonary surfactant system consists of two major anatomically distinct pools, an intracellular pool contained within the alveolar Type II cells and an extracellular pool that overlies the alveolar epithelium. These pools appear to be under some kind of common regulation because the relationship between them appears to be highly stable. Intratracheal injection of silica dust increased the levels of surfactant in both compartments but not to the same degree, indicating that the ratio between the two pools could be changed by toxic materials. These data suggest the existence of a size relationship between the intra- and extra-cellular pools of surfactant, a relationship that implies a common regulatory mechanism that can be disturbed by pulmonary injury. The effects of silica on the surfactant system appears to be mediated through the alveolar Type II cells. These cells increased 2-fold in response to silica and, in addition, many of them increased in size. We have isolated these hypertrophic Type II cells and preliminary evidence indicates that the increased levels of phospholipid in the lungs of silica-treated rats may be associated primarily with the appearance of these highly active cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-25021-03 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Differentiation and Differentiative Functions of Airway Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Jetten	Senior Staff Fellow	LPP, NIEHS
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	J. Rearick	Staff Fellow	LPP, NIEHS
	M. Deas	Chemist	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Cell Biology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Rabbit tracheal epithelial cells grown on fibronectin-albumin-vitrogen coated dishes in serum-free and vitamin A-free medium undergo terminal squamous differentiation under a variety of conditions. This differentiation occurs in several stages. First cells undergo terminal cell division; cells accumulate in GO/G1 phase of the cell cycle and loose the ability to form colonies. This terminal cell division occurs at high density or is induced at low density when epidermal growth factor is omitted from the medium or when TGFβ is added. This is followed by the expression of a squamous phenotype that is characterized by the induction of a squamous morphology, increase in transglutaminase and cholesterol sulfate levels, increase in the expression of a 48 and 56 kd keratin and the formation of cross-linked envelopes. The increase in cholesterol sulfate levels appears to be related to an enhancement in sulfotransferase activity. High calcium concentration promotes the expression of the squamous phenotype. Retinoic acid has no effect on the commitment to terminal cell division but inhibits the expression of the squamous phenotype; retinoic acid inhibits the increase in transglutaminase and cholesterol sulfate levels the alterations in keratin expression and the formation of cross-linked envelopes. To study the regulation of differentiation at the molecular level, we established a cDNA library using poly $(A)^+$ RNA from squamous differentiated cells and isolated recombinant cDNA clones that hybridize with mRNA's isolated from squamous differentiated cells but not from undifferentiated and retinoic acid-treated cells. This indicates that these clones represent gene products that function as markers for differentiation. We are in the process to analyze at what level these gene products are regulated and how retinoids control them.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-25022-03 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of the Molecular Mechanisms of Action of Retinoids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. M. Jetten	Senior Staff Fellow	LPP, NIEHS
Others: J. Shirley	Biological Lab Technician	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Cell Biology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this study was to determine whether the expression of specific oncogenes can influence the response of cells to retinoids. To test this idea we examined the effect of retinoic acid on several neoplastic cell lines that were clonally derived from immortal, non-tumorigenic Syrian hamster embryo DES-4 cells after transfection with v-src or v-Ha-ras DNA. We have shown that retinoic acid inhibits the expression of the transformed phenotype in src-453 cells which express the v-src oncogene. This is indicated by the restoration of contact inhibition and reduction in anchorage-independent growth. In these cells retinoic acid also causes a reduction in the levels of ODC activity. In contrast, retinoic acid stimulates the expression of the transformed phenotype and increases the levels of ODC activity in ras-C2 cells which express v-Ha-ras. These results show that the expression of specific oncogenes can influence the response of cells in vitro to retinoids. Retinoids do not affect the synthesis of the oncogene products pp60^{src} and p21ras. For the pp60^{src} we have shown that retinoic acid does not significantly affect the protein kinase activity and therefore does not appear to act at the level of the kinase. The v-Ha-ras transformed cells produce TGFβ, which inhibits anchorage-independent growth of these cells. Retinoids appear to reduce the level of active TGFβ secreted into the medium indicating that the increase in anchorage-independent growth by retinoids is related to reduced levels of TGFβ. TGFβ appears to act synergistically with the src-oncogene by enhancing the expression of the transformed phenotype; retinoic acid also inhibits this increase in anchorage-independent growth. We are examining now the levels of TGFβ-synthesis in these cells and try to identify specific protein(s) that might be affected by TGFβ and retinoic acid and would be involved in the action of these agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25023-03 LPP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Molecular Mechanisms of Neoplastic Progression in Airway Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Nettesheim	Chief	LPP, NIEHS
Others:	D.J. Fitzgerald	Visiting Fellow	LPP, NIEHS
	H. Kitamura	Visiting Associate	LPP, NIEHS
	C. Walker	Staff Fellow	LPP, NIEHS
	T.E. Gray	Biologist	LPP, NIEHS

COOPERATING UNITS *(if any)*

Environmental Carcinogenesis Group
(J.C. Barrett and T. Gilmer)

LAB/BRANCH

Laboratory of Pulmonary Pathobiology

SECTION

Epithelial Carcinogenesis Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of our studies is to elucidate cellular and biochemical mechanisms involved in "Neoplastic Development", using rat tracheal epithelial (RTE) cells transformed in culture by chemical carcinogens. In studies designed to define the growth and differentiation abnormalities of early transformants we found that the so called Enhanced Growth (EG) variant, has a markedly increased self renewal capacity, but nevertheless gives rise to a large number of terminally differentiating progeny. The cell composition of the EG variant clones changes dramatically with time. At 5 weeks after exposure, only ~ 1% of the cells have clonogenic potential, while at 12 weeks such clones contain 10-30% colony forming cells. These changes in transformed stem cell pool sizes are accompanied by changes in responsiveness to physiologic regulators of growth (and differentiation) such as retinoids. Early transformants were growth inhibited by 10^{-9} M concentrations of retinoic acid (RA), however with time their RA sensitivity decreased >100-fold.

Another aspect of our studies is concerned with elucidation of molecular mechanisms of RTE cell transformation. A number of neoplastically transformed RTE cell lines were analyzed for expression of oncogenes. The oncogenes N-*myc*, *abl*, *fes*, *erbB* and *myb* were not expressed and the genes *myc*, *fos*, *raf* and K-*ras* were expressed at levels similar to normal RTE cells. H-*ras* expression was increased in 3 of the tumor lines. Most interesting was the elevated expression (5-19-fold) of a c-*fms* related message in several of the cell lines. The message size detected was 9.5 kb. In comparison the *fms* message size in normal rat alveolar macrophages was ~4 kb. No evidence for gene amplification or rearrangement was found. Future studies will be aimed at idenitifying the ligand for this putative, *fms*-related growth factor receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25024-03 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Early Pulmonary Lesions Induced by Inhaled Inorganic Particles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. R. Brody	Research Biologist	LPP, NIEHS
Others:	L. H. Overby	Chemist	LPP, NIEHS
	V. Roggli	Guest Worker	LPP, NIEHS
	M. Bauman	NRSA Fellow	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
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SECTION
Pulmonary Pathology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

We have established animal models to elucidate the basic cellular mechanisms associated with interstitial fibrosis caused by inhalation of asbestos and silica. The initial deposition sites of the particles and the nature of the earliest lesions consequent to deposition have been demonstrated. Autoradiography and ultrastructural morphometry have been used to show the cellular alterations which occur at alveolar duct bifurcations (i.e., sites of particle deposition) at varying times after a brief exposure (1 or 5 hrs) to chrysotile asbestos. Within 24 hrs after a 5-hr exposure, there was a highly significant increase in macrophage accumulation at the bifurcation of alveolar ducts. Now we have shown for the first time that concurrent with macrophage accumulation increased numbers of terminal bronchiolar epithelial cells, proximal alveolar duct epithelial cells and alveolar duct bifurcation epithelial cells incorporated tritiated thiymidine (^{3}HTdr) into nuclei. In addition, there were significant increases in the number and volume of epithelial and interstitial cells in all three anatomic compartments. We have attempted to establish the biochemical and cellular mechanisms through which these initial pulmonary lesions are induced. We postulate that the pulmonary macrophages which accumulate in association with inhaled asbestos produce growth factors that are mitogenic for interstitial fibroblasts and possibly for epithelial cells. We have demonstrated *in vitro* that rat pulmonary macrophages secrete a growth factor for early passage rat lung fibroblasts when stimulated with a variety of particles. This macrophage-derived growth factor has several biochemical characteristics similar to platelet-derived growth factor and is chemotactic for other pulmonary macrophages. Ongoing studies are designed to establish whether or not this factor is secreted by macrophages *in vivo* and if it plays a role in the pathogenesis of pulmonary fibrosis.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-25025-03 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Complement-Dependent Chemotactic Factors for Macrophages

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold R. Brody	Research Biologist	LPFT, NIEHS
Others:	D. B. Warheit	Postdoctoral Fellow	LPFT, NIEHS
	L. H. Overby	Chemist	LPFT, NIEHS
	G. George	Visiting Fellow	LPFT, NIEHS
	S. Kouzan	Visiting Fellow	LPFT, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

Pulmonary macrophages migrate to the sites where inhaled chrysotile fibers initially are deposited (i.e., surfaces of alveolar duct bifurcations) and form a major component of an early asbestos-induced interstitial lesion in rats. To establish the basic cellular mechanisms of asbestos-induced lung disease, it is essential to determine the chemical mediators which attract macrophages to these sites of fiber deposition. Fluids lavaged from the lungs of exposed rats contain substantial chemotactic activity for macrophages compared to fluids from sham-exposed animals. This chemotactic activity is derived from complement activated by inhaled asbestos on alveolar surfaces. This contention is supported by observing: 1) Production in vitro of chemotactic activity by asbestos in serum or in lung lavageates was blocked by complement inhibitors, and 2) Fractionation, by molecular sieve chromatography, of serum proteins and concentrated proteins lavaged from the lungs of asbestos-exposed rats showed chemotactic activity in the 14-18,000 MW range. This fractionation profile is identical to C5a, the chemotactic product of complement activation. Rats treated with cobra venom factor (CVF) to deplete circulating complement as well as complement-deficient mice demonstrated significantly depressed macrophage accumulation at sites of asbestos deposition. Most recently time course studies have shown that the complement-dependent chemotactic factor is rapidly activated during a 3-hr exposure to asbestos, peak activity is maintained through 48 hrs post-exposure, and the chemotactic activity is no longer detectable by 8 days after exposure. In addition, once the C5a is activated, no detectable C5 is present in the lungs until 2 wks post-exposure. Now we have learned that complement-deficient asbestos-exposed mice fail to develop significant interstitial lung disease, suggesting that the complement-dependent macrophage response may play an essential role in mediating disease progression.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES-25027-03 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Identification and Characterization of Materials Secreted by Pulmonary Clara Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. E. R. Hook	Research Chemist	LPP, NIEHS
Others:	S. E. Patton	Postdoctoral Fellow	LPP, NIEHS
	R. P. Gupta	Visiting Fellow	LPP, NIEHS
	L. B. Gilmore	Biologist	LPP, NIEHS

COOPERATING UNITS *(if any)*
Cell Biology Group (A. Jetten)
Epithelial Carcinogenesis Group (P. Nettesheim)

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Biochemical Pathology Group

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

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

The functions of the bronchiolar Clara cell are not known although numerous morphological investigations in many species have led to the hypothesis that the cell is secretory. The objectives of this research are to eludicate the secretory nature of the Clara cell, identify and characterize those secretions and determine their extracellular functions. Using a model system, developed in this laboratory, involving isolated and purified Clara cells, we have identified a low molecular weight protein (12 Kd) as the major protein secreted by those cells. Using antisera prepared against this low molecular weight protein we have demonstrated that it is also present in the pulmonary extracellular lining accounting for approximately 10% of the total proteins secreted by the pulmonary epithelium. We have isolated and purified this low molecular weight protein from the extracellular lining, analyzed it for its amino acid composition and general electrophoretic properties. The protein appears to consist of one major and five minor isoforms. The protein has weak inhibitory activity against human PMN elastase and papain. These studies identify the low molecular weight protein as a major secretory product of the Clara cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-25028-03 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Basis For Cellular Changes in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.C. Barrett	Research Chemist	LPP, NIEHS
Others:	T. Gilmer	Senior Staff Fellow	LPP, NIEHS
	M. Koi	Visiting Fellow	LPP, NIEHS
	M. Oshimura	Expert	LPP, NIEHS
	O. Sugawara	Visiting Fellow	LPP, NIEHS
	L. Annab	Biological Lab. Tech.	LPP, NIEHS
	P. Lamb	Biological Lab. Tech.	LPP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Environmental Carcinogenesis Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.5	1.2

CHECK APPROPRIATE BOX(ES)

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

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

Neoplastic development of Syrian hamster embryo (SHE) cells is a multistep process. However, the number of steps and the genes or changes involved are unknown. SHE cells transfected with v-Ha-ras DNA plus v-myc DNA formed tumors with short latency periods. To determine whether activation of ras plus myc was sufficient for tumorigenicity, we performed cytogenetic analyses of tumors formed following transfection. Tumors (ras/myc-T) induced by v-Ha-ras plus v-myc oncogenes were monoclonal and had a nonrandom chromosome change, monosomy of chromosome 15. Thus, an additional change, loss of chromosome 15, is required or advantageous for tumorigenicity induced by v-Ha-ras plus v-myc oncogenes. To determine if normal cellular factors or genes can regulate the phenotypic expression of tumorigenicity and/or oncogenes, cell-cell hybrids between neoplastic and nontumorigenic hamster cells were isolated. Hybrids between ras/myc tumor cells and SHE cells were nontumorigenic and failed to grow in agar. These suppressed hybrids still expressed the ras and myc oncogenes. After several passages, variants arose in the hybrid cells which re-expressed tumorigenicity and anchorage-independence. Karyotypic analysis of the suppressed and re-expressed hybrids showed a non-random loss of chromosome 15 associated with re-expression of tumorigenicity. Hybrids between SHE cells and chemically transformed hamster cell lines were also suppressed for tumorigenicity. Carcinogen treatment of SHE cells induced immortal cells as an early step in a multistep, neoplastic transformation. At early passages immortal cells suppressed tumorigenicity of fully transformed cells. At later passages the cells lost the ability to suppress tumorigenicity in cell hybrids but were still not completely transformed. Cells which had lost the tumor suppression function were readily converted to tumorigenicity by a transforming oncogene (eg ras) whereas cells which retained this function were resistant to neoplastic transformation. These results suggest that chemically induced neoplastic progression of Syrian hamster embryo cells involves at least three steps: (1) induction of immortality; (2) activation of a transforming oncogene; and (3) loss of a tumor suppression function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-25029-02 LPP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Neoplastic Transformation by Viral and Cellular Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Tona Gilmer Senior Staff Fellow LPP, NIEHS

Others:

COOPERATING UNITS *(if any)*
Cell Biology Group, LPP
(A. Jetten)

LAB/BRANCH
Laboratory of Pulmonary Pathobiology

SECTION
Environmental Carcinogenesis Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

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

The product of the oncogene v-src, a phosphoprotein (pp60src) with tyrosine-specific kinase activity, is responsible for transformation of cells by Rous sarcoma virus (RSV). An immortal cell line (10W) isolated after treatment of Syrian hamster embryo (SHE) cells with asbestos, was cotransfected with pSV2-neo DNA and RSV DNA which encodes the v-src oncogene. Neo^R 10W RSV cell clones contained multiple copies of the RSV genome, but failed to express v-src RNA or pp60src. Most of these clones were not morphologically transformed and failed to grow in soft agar. Three neo^R RSV clones were tumorigenic with latency periods of three weeks. Tumor-derived cell lines from these clones expressed high levels of v-src RNA and protein. This increased expression was associated with an approximately ten-fold amplification in RSV DNA sequences and the appearance of double minute chromosomes. Our results suggest that gene amplification alters the expression of the v-src gene in these cells. The mechanism of v-src transformation was compared to the mechanism of another viral oncogene, v-Ha-ras. Cell lines containing either the v-src gene or the v-Ha-ras gene were analyzed following treatment with retinoic acid. The transformed phenotype was inhibited in cells transformed with the v-src oncogene, whereas retinoic acid stimulated parameters of transformation in v-Ha-ras transformed cells. The different effect of retinoic acid on the cells was not due to alterations in the synthesis of the oncogene products. Future studies will use retinoic acid to identify critical cellular targets for the v-src and the v-Ha-ras proteins.

LABORATORY OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY
Summary Statement

Research in the Laboratory of Reproductive and Developmental Toxicology seeks to understand basic mechanisms underlying normal and abnormal development and reproduction. The studies draw mainly on the research disciplines of endocrinology, reproductive physiology, developmental biology, pharmacology, neurochemistry, and cell biology. The research in development concentrates on craniofacial, genital tract, and germ cell differentiation. Reproductive studies are conducted largely to understand neurotransmitter/neuropeptide regulation and function, germ cell biology, and the functional cell biology of estrogens. The toxicological mechanisms associated with perturbations of processes leading to craniofacial teratogenesis, transplacental hormonal carcinogenesis, neurotoxin-induced neuroendocrine dysfunction, and failure of germ cell function are investigated. While research problems are developed in depth, the diversity of scientific disciplines establishes the Laboratory as a unique resource within the NIH. As a component laboratory of the NIH, scientists are cognizant of the human health implications of their basic research which contributes to public health efforts and clinical investigations.

OVERVIEW OF CURRENT RESEARCH EFFORTS

The Laboratory of Reproductive and Developmental Toxicology is organized in four Sections. The research emphases in the Sections are described below.

Developmental Endocrinology and Pharmacology Section

Research in the Developmental Endocrinology and Pharmacology Section focuses on three major topics: (1) Pharmacology of estrogens, including structure-activity relationships (SAR) and target organ specific metabolism. Metabolic studies are directed towards understanding the modification of hormonal activity of different estrogenic chemicals and the induction of long-term defects in cell differentiation, including neoplasia, associated with metabolism of estrogens. SAR studies strive to elucidate the structural basis of estrogenicity. (2) Cell biology of estrogens, including studies of estrogen regulation of protein secretion and cell proliferation. Research is designed to understand the role of growth factors in the mitogenicity of estrogens. Major efforts evaluate receptor biology, protein synthesis control mechanisms, and hormone responsive gene structure and function. These studies utilize *in vitro* models for hormone action, including primary cultures of mouse uterine and seminal vesicle epithelial cells. (3) Developmental biology of estrogens, including studies to elucidate the mechanisms for estrogen-induction of differentiation defects in the genital tract, and the expression of these alterations.

Results obtained in the last year have further characterized the structure-activity relationships of a series of stilbene estrogens with regard to estrogen receptor (ER) binding and biological responses. Some of these compounds show a variety of differences compared to the intracellular responses of diethylstilbestrol (DES), including lack of receptor synthesis, poor nuclear translocation, excessive retention of the receptor complex in the nucleus and the inability to stimulate certain tissue responses such as DNA synthesis, mitosis, and induction of specific enzymes and proteins. Studies on the metabolism of estrogens have previously focused on stilbene estrogens such as DES. In comparative studies, it was found that among steroidal estrogens the catechol forms exhibit reactivity similar to DES. Very recently, the formation of catechol estrogens was demonstrated in target tissue microsomes derived from the uterus.

In continuing studies on the estrogen-inducible mouse uterine 70 kDa protein, the cDNA to the message for this protein has been isolated and sequenced. It was found to be a member of the transferrin gene family, but distinct from serum transferin. This protein should provide a useful marker for mouse uterine ontogeny and function. New aspects of mouse uterine growth induced by estrogen and growth factors has utilized studies with epidermal growth factor (EGF). Thus, modeling studies in mouse kidney have developed methodology to localize and characterize the precursor form (140 kDa) of EGF bound to cell membranes. These techniques are being applied to the uterus. Characterization of the receptor in mouse uterus has indicated multiple forms which are proteolytic fragments and found in nuclear and cytoplasmic tissue fractions. The protease action results in a receptor form which has lost its ability to bind DNA. In the absence of phenol red in the media, proliferative and differentiative (secretory) responses induced by estrogen could be documented in organ cultures of the mouse uterus. Control of proliferation in uterine epithelial cells grown in serum-free media was further studied and found to involve prostaglandin E_1 or E_2, as well as epidermal growth factor (EGF) and insulin.

Studies have continued to determine the molecular and cellular targets of estrogenic chemicals and establish the mechanisms by which interactions of estrogens with developing genital tract target cells result in permanently altered differentiation, including dysmorphology and neoplasia. In the period covered by the report, the developmentally estrogenized mouse model was used effectively to suggest an etiology for paraovarian cysts in similarly exposed women.

Experimental Teratogenesis Section

The Experimental Teratogenesis Section conducts basic research to understand at the morphological, cellular and biochemical levels various aspects of normal and abnormal embryonic development, especially relating to craniofacial development. Retinoids are craniofacial teratogens in the human, and our results using mouse whole embryo culture demonstrate that they exert a direct effect on the embryo. Cranial neural crest cells are extremely important in development of craniofacial tissues; our studies using neural crest-derived facial mesenchyme cells in primary culture indicate that this may be the primary target cell for retinoid-induced

craniofacial anomalies. Future studies are aimed at determining the effect that retinoids have on extracellular matrix production by these cells and the role that retinoid receptors play in normal and abnormal facial development.

Growth and differentiation of secondary palatal epithelial cells in culture are dependent upon epidermal growth factor (EGF) and a fibronectin-rich extracellular matrix (ECM) substrate. A serum-free culture system is utilized to examine the hormonal infuences on normal and abnormal epithelial development in the palate. A major focus relates to the manner by which EGF and α-transforming growth factor (α-TGF) influences epithelial development. Evidence suggests that α-TGF is an important embryonic growth factor, and studies are in progress at the molecular level to determine when and in what tissues α-TGF and its receptor are expressed during embryonic development.

Extracellular matrix (ECM) components play a critical role in palatal development especially hyaluronate, Types I, III, IV and V collagen, fibronectin and laminin. Studies are in progress using cryostat sections and cultured cells from the secondary palate to localize these macromolecules under conditions of normal and abnormal development. Monoclonal antibodies are being produced to palatal epithelial and mesenchymal cells and will be utilized as probes for normal and abnormal development in vivo and in culture.

The dioxin, TCDD, is a potent cleft palate inducer in the mouse; results indicate that this is due to a receptor-dependent inhibition of palatal epithelial cell differentiation. On the other hand, glucocorticoids, such as dexamethasone, induce cleft palate by a receptor-dependent inhibition of palatal mesenchymal cell growth. Studies are in progress in vivo as well as in cell and organ culture to ascertain specific biochemical events which are altered by TCDD or glucocorticoids in palatal epithelial or mesenchymal cells which ultimately result in cleft palate formation. This human embryonic palatal mesenchyme (HEPM) cell line is being used to determine mechanisms of teratogenicity as well as a teratogen screening assay. Chemically-induced growth inhibition of HEPM cells, along with a liver-derived metabolic activating system, has been developed as a short-term screening assay for potential human teratogens.

Gamete Biology Section

Research in the Gamete Biology Section emphasizes identification of molecules that are specific to male germ cells or associated somatic cells and the development of systems to study these cells and molecules in vitro. Two of the major limitations in the study of gamete biology are the lack of cell-specific biochemical markers and of adequate in vitro systems. Monoclonal antibodies have been produced to isolated cells and molecules and are being used to localize and characterize cell-specific and stage-specific antigens. These highly selective probes are being used to study the distribution, synthesis, regulation, and roles of molecules of interest in reproductive and developmental processes.

Antibodies to cell-specific surface and cytoskeletal antigens of mouse spermatogenic cells are available and efforts are continuing to produce antibodies to stage-specific surface antigens and to cell-specific heat shock proteins of germ cells. Monoclonal antibodies have been prepared against rat Leydig cells which also react with mouse Leydig cells. These antibodies are being used to study development of Leydig cells, morphogenesis of the testis, and the influence of germ cells on steroid production by Leydig cells. Antibodies to spermatozoa are being used to examine modification of the sperm surface during epididymal maturation, which occurs as sperm gain the ability to fertilize, and to study the distribution, synthesis, and role of sperm-specific molecules. A factor produced by Sertoli cells stabilizes the association antigens and the sperm plasma membrane, and studies are underway to further characterize the factor. Other antibodies have been produced against purified antigens, including a sulfated glycolipid unique to the germ cell plasma membrane and a synthetic peptide. These monoclonals have been generated using a novel modification of the in vitro stimulation procedure, which allows the rapid production of antibodies against remarkably small amounts of antigen.

In vitro studies have involved germ cells separated enzymatically from juvenile and adult mouse testes and the isolation of purified populations by unit gravity sedimentation. Cell viability and metabolism are evaluated by measuring ATP production and synthesis of total and individual proteins to determine optimum medium composition, isolation procedures, and culturing conditions for germ cells. Reasonable viability can be maintained for up to 48 hours. Sertoli cell-conditioned medium improves the maintenance of viability and ATP levels and studies are underway to identify the factor(s) responsible for this. Specific antibody probes will now be used to study in vitro synthesis of specific molecules, including cytoskeletal proteins in the sperm fagellum, apparently produced post-meiotically in spermatids, and glycoproteins which appear on the surface of germ cells early in meiotic prophase. Future studies will examine the genes responsible for production of germ cell-specific proteins.

Reproductive Neuroendocrinology Section

Recent research has focused on the mechanisms and interrelationships mediating neuroendocrine responses within the hypothalamic-pituitary-gonadal axis. Studies have been directed toward elucidating the cellular mechanisms involved in the peptide-peptide, peptide-monoamine and peptide-monoamine-steroid interactions governing the regulation of this axis in order to obtain valuable information in the area of neural regulation of endocrine, paracrine and/or autocrine functions. Other studies have been directed towards determining the site(s) and mechanism(s) of action of different endogenous secretagogues and neuromodulators which affect hormone secretion at either the hypothalamic, pituitary or gonadal level. In selected models, an in-depth in vitro exploration is carried out to elucidate the role of specific intracellular mediators responsible for the amplification of different transmembrane signals enhancing hormone secretion. These studies are coupled with in vivo paradigms in order to obtain

a measure of the relative physiological significance of these observations in key reproductive events.

One major focus of this research is examining the cellular and subcellular mechanisms regulating the release of luteinizing hormone-releasing hormone (LHRH) and other hypothalamic peptides participating in the modulation of pituitary hormone release. Studies are designed to elucidate the role of monoaminergic neurotransmitters in the release of LHRH from nerve terminals, the nature of the specific aminergic receptors involved in the neuronal activation that precedes LHRH release, the post-receptor event that participate in the peptide-release process, the role of arachidonate metabolites in amplifying the response to key neurotransmitters, and the additional role played by other intracellular messengers such as Ca^{+2} and other putative intracellular messengers derived from the metabolism of membrane phospholipids. The role of protein kinase C activation is being explored with the use of phorbol esters, diacylglycerol and phospholipase C.

Other studies have demonstrated that the pattern of hormone-release may be more important than the quantity in determining the response of the target tissue(s). The pulsatile pattern of luteinizing (LH) and follicle stimulating hormone (FSH) secretion were determined *in vivo*, and it was established that the pulsatile secretion of FSH is independent of endogenous LHRH. Studies are underway to evaluate the exact role of LHRH and other hypothalamic factors on FSH release. Further experiments will examine the effects of altering the parameters of pulsatile input signals on gonadotropin and pro-opiomelanocortin-derived peptide release from incubated pituitaries using a computer-controlled perifusion apparatus,

The recent discovery by Seeburg *et al.*, using recombinant DNA techniques of the structure and sequence of LHRH prohormone, has allowed the development in our Section of antibodies that recognize specific portions of the prohormone molecule distinct from the LHRH decapeptide sequence. Studies are now underway using a combination of RIA and immunocytochemistry to study in detail the synthesis and processing of this molecule.

Additional research involves the analysis of the cellular and molecular mechanisms mediating peptide hormone action. Studies using pituitary cell cultures evaluate the precise mechanisms through which peptidergic or aminergic secretagogues enhance or suppress peptide hormone release. Other studies explore the intratesticular effects of LHRH-analogs which are known to adversely affect both the endocrine and the gametogenic functions of the testis. The interaction of these LHRH-analogs with intrinsic peptidergic systems within the testis such as the pro-opiomelanocortin-derived peptides is also being explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70010-10 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Normal and Abnormal Embryonic Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. M. Pratt	Head, Experimental Teratogenesis Section	LRDT NIEHS
Others:	B. D. Abbott	NIH Postdoctoral Fellow	LRDT NIEHS
	K. S. Morgan	NIH Postdoctoral Fellow	LRDT NIEHS
	T. Watanabe	Visiting Fellow	LRDT NIEHS
	J. G. Zendegui	Senior Staff Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*
Department of Pediatrics
University of Washington, Seattle

Cancer Research Center
University of North Carolina, Chapel Hill

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Experimental Teratogenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

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

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

The objective of this research project is to understand at the morphological, cellular, and biochemical levels various aspects of normal and abnormal embryonic development, especially relating to craniofacial development. Retinoids are craniofacial teratogens in the human, and our results using mouse whole embryo culture demonstrate that they exert a direct effect on the embryo. Studies with neural crest cells indicate that this may be the target cell type for retinoid-induced craniofacial anomalies. Growth and differentiation of secondary palatal epithelial cells in culture are dependent upon epidermal growth factor (EGF) and a fibronectin-rich extracellular matrix (ECM) substrate. Cyclic AMP greatly enhances the EGF effect and transforming growth factor α (TGFα) substitutes for EGF with the palatal epithelial cells in culture. These observations reinforce our hypothesis that TGFα is an important growth factor during development. Retinoic acid influences the growth and differentiation of palatal epithelial and mesenchymal cells from the mouse as well as the human embryo. Retinoid treatment decreases proliferation of palatal mesenchymal cells and results in specific changes in protein synthesis. Retinoids, along with EGF, also modulate the growth and differentiation of palatal epithelial cells in cell and organ culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70060-13 LRDT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Developmental Biology/Toxicology of Estrogenic Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. A. McLachlan	Head, Devel. Endo. and Pharm. Section	LRDT NIEHS
Others:	R. R. Newbold	Biologist	LRDT NIEHS
	K. S. Korach	Research Endocrinologist	LRDT NIEHS
	Y. Tomooka	Visiting Fellow	LRDT NIEHS
	C. Bunyagidj	Visiting Fellow	LRDT NIEHS
	R. P. DiAugustine	Research Chemist	LRDT NIEHS
	C. T. Teng	Expert	LRDT NIEHS
	J. C. Barrett	Research Chemist	LPP NIEHS

COOPERATING UNITS *(if any)*

Bowman-Gray School of Medicine
Duke University Medical Center
Medical Foundation of Buffalo
University of North Carolina
University of Wurzburg

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	2.8	2.8

CHECK APPROPRIATE BOX(ES)

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

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

Studies have continued to determine the molecular and cellular targets of estrogenic chemicals and establish the mechanisms by which interactions of estrogens with developing genital tract target cells result in permanently altered differentiation, including dysmorphology and neoplasia. In the period covered by the report, the developmentally estrogenized mouse model was used effectively to suggest an etiology for paraovarian cysts in similarly exposed women. Tissue recombination experiments established the uterine epithelium as a site for estrogen-induced differentiation defects even with a normal stroma. Moreover, the ontogeny of the estrogen receptor (ER) was studied in the epithelial and stromal cells of the mouse uterus using monoclonal antibodies to the ER. A new technique for the determination of catechol estrogens using high performance liquid chromatography with electrochemical detection was developed and used to study the relative formation of catechol estrogens in liver and uterine microsomes of the mouse. In the absence of phenol red in the media, proliferative and differentiative (secretory) responses induced by estrogen could be documented in organ cultures of the mouse uterus. Control of proliferation in uterine epithelial cells grown in serum-free media was further studied and found to involve prostaglandin E_1 or E_2, as well as epidermal growth factor (EGF) and insulin.

GPO 914-919

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70065-10 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical-Receptor Interactions in Reproduction and Hormonal Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. S. Korach	Research Endocrinologist	LRDT NIEHS
Others:	F. Ogata	Visiting Fellow	LRDT NIEHS
	S. Yamashita	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Develop. Endo. and Pharm. Section	LRDT NIEHS
	R. P. DiAugustine	Research Chemist	LRDT NIEHS

COOPERATING UNITS *(if any)*
University of Würzburg
Laboratory of Molecular Biophysics, NIEHS
Medical Foundation of Buffalo
Burroughs Wellcome Research Labs
UNC Medical School
Duke University

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

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

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

Diethylstilbestrol (DES), a potent synthetic estrogen and reproductive toxicant, has been shown to be extensively metabolized. Some metabolites retain hormonal activity while others are biologically inactive. This assignment of activity is consistent with the receptor binding activity of these compounds. Besides reproductive tract effects, some of these metabolites also elicit neuroendocrine effects by suppressing LH secretion. Two groups of metabolites were found to have poor uterotropic activity although they bound very well to the receptor. Some of these compounds show a variety of differences compared to the intracellular responses of DES, including lack of receptor synthesis, poor nuclear translocation, excessive retention of the receptor complex in the nucleus and the inability to stimulate certain tissue responses such as DNA synthesis, mitosis, and induction of specific enzymes and proteins.

Estrogen stimulation of reproductive tract tissue involves a mechanism which includes binding to a receptor with subsequent activation and localization in the the nucleus. Nuclear translocation follows a bimodal temporal pattern consistent with the stimulation of certain tissue responses such as DNA synthesis or enzyme induction. Only biologically active compounds induce these nuclear receptor events. This pattern was demonstrated by both ligand binding assays and immunoassay with a monoclonal estrogen receptor antibody. Multiple receptor peaks were also present in other estrogen responsive target tissues such as the rat uterus and MCF-7 cell tumors. Cell cycle kinetic studies of uterine estrogen stimulation show that the second peak occurs at the beginning of S-phase. A major effect of estrogen on uterine cells was to shorten the cell cycle by contracting the G-phase. Characterization of the receptor in mouse uterus has indicated multiple forms which are proteolytic fragments and found in nuclear and cytoplasmic tissue fractions. The protease action results in a receptor form which has lost its ability to bind DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70067-03 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanism of Steroid Hormone in Sex Organ Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Christina T. Teng	Expert	LRDT NIEHS
Others:	B. T. Pentecost	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Devel. Endo. and Pharm. Section	LRDT NIEHS
	Y. H. Chen	Expert	LRDT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.2	1.3

CHECK APPROPRIATE BOX(ES)

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

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

The mouse uterus has provided a system for the study of estrogen since it contains estrogen receptors and depends on estrogen stimulation for action of physiological functions. We have previously purified an estrogen-induced secretory protein from mouse uterine luminal fluid by CM-Affi-Gel blue and reversed phase high-performance liquid chromatography. This protein shows a $M_r \sim 70,000$ both by SDS-polyacrylamide (with or without β-mercaptoethanol) gel electrophoresis and by gel filtration column chromatography indicating that it exists as a single chain polypeptide. Further analysis of the protein revealed that it is highly basic (pI > 10) and is a glycoprotein. The in vitro incorporation of ^{35}S-methionine into uterine proteins revealed that estrogen treatment of immature mice stimulates both synthesis and secretion of the 70 kDa protein. Rabbit polyclonal antibody raised against the purified 70 kDa protein demonstrated specificity for the 70 kDa protein by "Western Blot" analysis. An enzyme-linked immunosorbent assay with polyclonal antibody has been used to determine the tissue distribution of the protein. Tissues such as lung, brain, spleen, muscle, intestine, liver, kidney, and ovary of estrogen-treated mice did not have detectable amounts of the 70 kDa protein. Immunoreactivity was present in uterine and vaginal tissues from estrogen-treated animals. The 70 kDa protein was not induced by testosterone or progesterone. Currently, a cDNA to the message for this protein has been isolated, sequenced, and found to be a member of the transferrin gene family. A comparison of the properties of the uterine protein and serum transferrin indicates that they are distinct proteins. This has been confirmed by isolation of a cDNA to authentic mouse serum transferrin. Although the function of this uterine secretory protein is unknown, the possible involvement of this protein in the iron transport and the growth promotion is currently under investigation.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70069-04 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Peptide Growth Factors in Reproduction and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. P. DiAugustine	Research Chemist	LRDT NIEHS
Others:	Y. Tomooka	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Devel. Endo. and Pharm. Section	LRDT NIEHS
	C. T. Teng	Expert	LRDT NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina, Chapel Hill
Chiron Corporation, Emeryville, California

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Developmental Endocrinology and Pharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

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

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

Although epidermal growth factor (EGF) stimulates the growth of various cells in vitro, the biological role of this polypeptide is not known. Evidence that EGF may function as an autocrine or paracrine factor is suggested from previous studies demonstrating submaxillary gland preproEGF mRNA in other organs. We have shown that primary cultures of mouse uterine epithelial cells possess specific binding sites ($K_d \sim 1.8$ nM) for ^{125}I-EGF and approximately 50 x 10^3 receptors/cell. EGF stimulated proliferation of the uterine cells in vitro, whereas other known growth factors did not. Immunolocalization of EGF in pronase-treated sections of the mouse uterus revealed staining at the luminal aspect of epithelial cells. Localization required protease treatment and the material reactive with anti-EGF antiserum was apparently not estrogen-dependent since this pattern of staining was readily observed in uteri from animals ovariectomized for over two weeks. Protease-treatment of sections was also required for localization of EGF in epithelial cells of the mouse kidney and mammary gland (mid-pregnant). We have found that kidney EGF occurs predominantly as the apparent precursor form (140 kDa) bound to cell membranes. Thus, it is likely that the observed pattern of EGF localization in uterus represents a membrane-bound form of the precursor. Hybridization with a ^{32}P-labelled cDNA probe for submaxillary gland preproEGF mRNA occurred with samples of uterine mRNA, although at much lower levels than that of kidney and submaxillary gland. The level of preproEGF mRNA increased in immature mouse uteri following treatment with estrogen. Northern blot analysis of uterine A^+ mRNA from estrogen-treated mice revealed a single band of 4.9 kilobases, equivalent to that of submaxillary gland preproEGF mRNA. Studies are in progress to further understand the synthesis sorting and processing of the EGF precursor in different organs and the potential for various hormones to regulate these events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70076-02 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Germ Cell-Specific Molecules of Spermatozoa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Eddy	Head, Gamete Biology Section	LRDT NIEHS
Others:	R. L. Allen	Staff Fellow	LRDT NIEHS
	K. Toshimori	Visiting Fellow	LRDT NIEHS
	M. P. Hedger	Visiting Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*

U. of Alberta, Ontario, Canada
U. of Washington School of Medicine
U. of North Carolina, Chapel Hill
Fred Hutchison Cancer Res. Center, Seattle
The Hospital for Sick Children, Toronto
U. of Pennsylvania School of Medicine

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Gamete Biology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

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

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

Monoclonal antibodies prepared to spermatozoa, spermatogenic cells, or isolated antigens are being used to identify and characterize molecules specific to germ cells. The general hypothesis being tested is that germ cell-specific gene products are responsible for the unique structural and functional characteristics of spermatozoa. The fibrous sheath is a cytoskeletal structure which underlies the plasma membrane and surrounds the outer dense fibers and axoneme in the principal piece of the mammalian sperm flagellum. A monoclonal antibody was found to recognize a protein of apparent M_r 66.7 K and pI 8.5 using 2D SDS-PAGE and Western blotting procedures. Antigen appearance during spermatogenesis was analyzed using germ cells isolated from juvenile and adult mice. The 66.7 K antigen first appears in round spermatids. However, a 78 K antigen recognized by the same antibody and present throughout spermatocyte development disappears between the round spermatid and elongating spermatid stages. Use of other antibodies indicates that this is due to epitope modification rather than degradation and that this protein also becomes incorporated into the fibrous sheath. The hypothesis currently being tested is that the fibrous sheath protein is a germ cell-specific intermediate filament protein. Studies on sperm surface components involve a 31 K glycoprotein that appears during epididymal maturation. A monoclonal antibody has been used to show that the antigen appears in the corpus epididymidis on the plasma membrane of the flagellum. This antigen is shed from sperm under in vitro fertilization conditions but retained in the presence of epididymal fluid. The antigen stabilizing factor is being purified from epididymal fluid and from Sertoli cell conditioned medium to study the nature of the molecule and its role in epididymal maturation and capacitation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70078-03 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Stage-Specific Surface Antigens During Mouse Spermatogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. A. O'Brien	Senior Staff Fellow	LRDT NIEHS
Others:	E. M. Eddy	Head, Gamete Biology Section	LRDT NIEHS

COOPERATING UNITS *(if any)*
Harvard Medical School
University of Washington School of Medicine
University of Pennsylvania School of Medicine
Columbia University, College of Physicians & Surgeons

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Gamete Biology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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

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

During spermatogenesis, regional specialization of the plasma membrane occurs resulting in a mature gamete with distinctly polarized functional and biochemical properties. One feature of this differentiative process is the appearance of novel surface constituents in a precise temporal sequence. Both immunological and biochemical techniques have been used to characterize germ cell-specific constituents and monitor membrane assembly. Central to these studies are methods for the purification of germ cells at defined stages of spermatogenesis by enzymatic dissociation of adult or prepuberal testes followed by unit gravity sedimentation. Three areas of research have been pursued: (a) Polyclonal and monoclonal antibodies have been used to characterize macromolecules first appearing on the surface of pachytene spermatocytes, coincident with a period of maximal protein synthesis. These constituents are not shared by most somatic cells and include at least ten proteins, a probable lipid constituent, and large lactosaminoglycans. Some of these components are retained on sperm, restricted to distinct domains on the cell surface. Monoclonal antibodies have also been raised which recognize germ cell cytoplasmic antigens including an acrosomal constituent and potential cytoskeletal elements. (b) Conditions for the short term culture of adult and prepuberal spermatogenic cells have been refined to facilitate metabolic studies and the development of in vitro functional assays. (c) Protein synthesis in isolated spermatogenic cells has been examined by 2D PAGE and autoradiography following short term culture with [^{35}S]methionine. Synthetic profiles become more complex throughout meiosis. A number of proteins previously identified as surface antigens are synthesized in a stage-specific manner. Germ cell surface constituents exhibiting both tissue and stage specificity are candidates for further studies exploring cell-cell interactions during spermatogenesis and fertilization.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70090-03 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroendocrine and Neurochemical Regulation of Gonadal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Head, Reprod. Neuroendocrinology Section	LRDT NIEHS
Others:	C. A. Johnston	Staff Fellow	LRDT NIEHS
	M. D. Culler	Staff Fellow	LRDT NIEHS
	M. M. Valenca	Visiting Fellow	LRDT NIEHS
	C. Masotto	Visiting Fellow	LRDT NIEHS
	M. Ching	Expert	LRDT NIEHS
	W. D. Wetsel	Guest Researcher	LRDT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Neuroendocrinology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	0.0

CHECK APPROPRIATE BOX(ES)

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

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

The Reproductive Neuroendocrinology Section is carrying out studies focused primarily on the cellular and subcellular mechanisms regulating the release of luteinizing hormone-releasing hormone (LHRH) and other hypothalamic peptides that participate in the modulation of pituitary hormone release. Specific studies are designed to elucidate the role of monoaminergic neurotransmitters in the release of LHRH from nerve terminals, the nature of the specific aminergic receptors involved in the neuronal activation that precedes LHRH release, the clarification of the post-receptor events that participate in the peptide-release process, the involvement of arachidonate metabolites in amplifying or modifying the response to key neurotransmitters, and the additional role played by other intracellular messengers such as Ca^{+2}, protein kinase C, and other putative intracellular messengers derived from the metabolism of membrane phospholipids. Other parts of the project are directed to perform an in-depth analysis in vivo of the changes in LHRH prohormone levels and processing in discrete brain nuclei that are known to be involved in regulation of gonadal function. Different experimental paradigms are employed, to re-create situations calling for an enhanced (or altered) function of the hypothalamic-pituitary-gonadal axis, such as steroid-feedback manipulations, pregnancy, lactation, estrous cycle, stress, ablation of selected endocrine glands or brain areas, etc. Finally, a group of experiments are directed to evaluate the mechanisms underlying the effects on the reproductive sphere of neonatal neurotoxin treatment, as well as the developmental changes and the role of steroids on certain sexually dimorphic patterns of gonadotropin secretion. The results are integrated to provide a comprehensive hypothesis of the complex, multi-level regulatory mechanisms modulating gonadal function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70092-03 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular and Molecular Mechanisms Mediating Peptide Hormone Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Negro-Vilar	Head, Reprod. Neuroendocrinology Section	LRDT NIEHS
Others:	M. D. Culler	Staff Fellow	LRDT NIEHS
	C. A. Johnston	Staff Fellow	LRDT NIEHS
	M. M. Valenca	Visiting Fellow	LRDT NIEHS
	F. Romanelli	Guest Researcher	LRDT NIEHS
	J. R. Dominquez	Guest Researcher	LRDT NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina, Department of Anatomy
Yale University Medical School, Department of Obstetrics and Gynecology
The Wellcome Research Laboratories, Department of Molecular Biology

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Neuroendocrinology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.9	0.0

CHECK APPROPRIATE BOX(ES)

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

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

Analysis of the cellular and molecular mechanisms mediating peptide hormone action constitutes an important component of the research efforts of the Reproductive Neuroendocrinology Section. The close interrelationships mediating neuroendocrine responses within the hypothalamic-pituitary-gonadal system offer an excellent opportunity to analyze some unique characteristics of peptide-peptide, peptide-amine, and peptide-amine-steroid interactions. Studies using pituitary cell cultures are directed to evaluate the precise mechanisms through which peptidergic or aminergic secretagogues enhance or suppress peptide hormone release. A main target of our research efforts will be the role of input signal on modifying cellular responses, using a computerized perifusion system that can exquisitely regulate pulse delivery to cells or tissues (in vitro) of appropriately designed hormone signals. Other protocols are designed to evaluate characteristics of hormone-receptor interactions, post-receptor as well as transmembrane events involved in the hormone-release process, and definition of the specific intracellular messengers transducing the action of key hypothalamic peptides involved in pituitary hormone release.

At the testicular level, studies are designed to determine the intratesticular effects of LHRH-analogs known to adversely affect both the endocrine and the gametogenic functions of the testis. Since some of these analogs are presently being tested for use in human contraception, an understanding of their site(s) and mechanisms of action is of obvious significance. The interaction of these LHRH-A with intrinsic peptidergic systems within the testis, such as the proopiomelanocortin-derived peptides, is also being explored. The results may provide very significant advances to our knowledge of paracrine and/or autocrine effects of gonadal peptides.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70094-02 LRDT

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroendocrine Regulation of Prolactin and Pro-Opiomelanocortin-Derived Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. A. Johnston	Senior Staff Fellow	LRDT NIEHS
Others:	A. Negro-Vilar	Head, Reprod. Neuroendocrinology Section	LRDT NIEHS
	J. Dominguez	Guest Researcher	LRDT NIEHS

COOPERATING UNITS *(if any)*
AmGen, Thousand Oaks, California
Lab. Clin. Sci., NIMH, Bethesda, MD

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Reproductive Neuroendocrinology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The focus of experiments in this project has been to identify and understand the developmental maturation of the cellular and subcellular mechanisms involved in the regulation of pituitary hormone secretion, in particular, prolactin (PRL), adrenocorticotrophin (ACTH), β-endorphin (β-end) and luteinizing hormone (LH). Specific studies are designed to elucidate the individual roles and interactions of monoaminergic neurotransmitters and neuropeptides in the neuroendocrine secretory control of these hormones from the anterior (AP) and neurointermediate (NIL) lobes of the pituitary, the sequential arrangement and functional connectivity of that neuronal circuitry, the developmental maturation of that circuitry, and the possible interaction that the AP and NIL undertake to achieve that neuroendocrine regulation. We have characterized and utilized the selective requisite developmental maturation of the PRL response to ether stress in order to determine which neurochemical responses are tied to that hormonal response. Pharmacological agents and specific antisera as well as lesioning and surgical procedures which selectively affect particular neurotransmitter or neuropeptide neurons are used to determine the individual contributions, sites of interaction, and functional neuronal connectivity of those specific components in the physiological regulatation of these hormones. Both static in vitro incubations of AP, NIL, or whole pituitary tissues as well as dispersed AP cell procedures are utilized to determine the sites of action of the individual components as well as the possible interaction of NIL and AP in governing the release of the hormones. Measurements of neurotransmitter/neuropeptide function are made during experimental conditions when dynamic changes in the secretion of these hormones are occurring, including the proestrous surge, acute stressful and suckling stimuli, pharmacologically-induced changes in hormonal secretion, and experimentally-induced hyperprolactinemia.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70096-02 LRDT

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Regulation of Pulsatile Pituitary Hormone Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael D. Culler Senior Staff Fellow LRDT NIEHS

Others: Andrés Negro-Vilar Head, Rep. Neuroendocrinology Section LRDT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

It has recently become patently clear that the concept of "blood concentration" of a given pituitary hormone may be relatively meaningless in light of the discovery that these hormones are secreted in a rhythmic, pulsatile manner. The pattern of hormone release seems to be far more important than the quantity in determining the response of the target tissue(s). Yet despite the recognized importance of pulsatile hormone secretion, very little is known about the effects of altering the parameters (frequency, duration, shape, amplitude, etc.) of the pulsatile signal on the target response. The proposed projects examine several important aspects of pulsatile pituitary hormone secretion and will lay the groundwork for changes in the way endocrine problems are evaluated and treated. First, the pulsatile pattern of LH and FSH secretion will be characterized in freely moving, cannulated rats. An emphasis will be placed on elucidating separate control mechanisms for the pulsatile release of the two gonadotropins. Second, the effects of altering the parameters of pulsatile input signals on LH-FSH and ACTH-β-endorphin release from incubating pituitaries will be determined using a computer-controlled perifusion apparatus. Since we are one of the few, if not the only, laboratory in the world with this capability, our contribution to the knowledge of pulsatile hormone regulation will be quite unique. In addition, the possibility of spontaneous pituitary pulses (without external stimulation) will be examined. Third, the effect of altering hormonal input signals on the intracellular messengers regulating cellular responses will be studied using the computer assisted perifusion system and monolayer pituitary cultures. In particular, the phenomena of desensitization and sensitization will be examined to elucidate the responsible intracellular messenger system(s). Information gained from these studies should be almost immediately translatable into information for improving treatments of infertility and for future prospects of fertility regulation.

COMPARATIVE MEDICINE BRANCH
Summary Statement

Administrative Activities

The Comparative Medicine Branch (CMB) is primarily a technical-administrative-service function within NIEHS. Responsibilities extend to all three intramural research programs including the Biometry and Risk Assessment Program, the Intramural Research Program, and the Toxicology Research and Testing Program. Principal activities include animal procurement; animal facilities management; animal health diagnosis and measurement; glassware and media services; and miscellaneous functions relating to legal and policy requirements for the use of animals. CMB also has a heavy investment in technical assistance and training in the use of animals; controlled substances management; animal technology in the execution of experiments; rodent breeding; quality assurance of animal health, media, animal feed, etc.; and applied research relating to many of the foregoing efforts.

The Glassware/Media Section experienced difficulty during Fiscal Year 1986 because of delays in installing a new large glassware wash machine. This machine is expected to relieve the increasing glassware work load that presently occurs. The machine was to be installed by October 1985; however, mismatch of services requirements, particularly steam and site preparation layout, with machine design have necessitated revision of the services layout and resulted long delays. The machine is now scheduled for installation in late FY 86, but it is uncertain that site restoration will be complete before the end of FY 86. Financial failure of the on-site contractor during site revision further embarassed efforts to complete this work. Thus, the Glassware Unit has had to function with a major disruption in the form of temporary partitions, construction dirt, and space encroachment for approximately a year. In the meantime, it was found necessary to terminate the temporary second shift which was established to handle the increased load because of employee dissatisfaction with prolonged night shift duty and/or legal constraints on renewal of temporary work leader assignments. Presently, the work load excess is being handled by standard weekend overtime use. It should be emphasized that overtime and night shift work have been necessary because of the slow function and limited capacity of existing wash machine equipment, not because of personnel shortages.

The Glassware/Media Handbook which contains information for investigators on standard practices and quality assurance procedures employed for these services was reviewed in FY 86. It was decided that no revision would be necessary at this time since the booklet accurately reflects current standard procedures. It was also decided to wait until FY 1987 to reconvene an internal ad hoc review panel of NIEHS investigators to advise and comment on practices and function of the Glassware/Media Unit. Thus, it will be three years between reviews. The Quality Assurance Program for Glassware/Media was also reviewed by CMB. Records and level of compliance with the program specifications are of a high order. While this program appears to be highly effective and few problems appear to exist, there is virtually no comment from the scientific staff to the CMB Office or the Glassware/Media Section supervisor. No major changes in QA procedures will be made pending outcome of the planned review next year. One equipment

failure occurred during FY 86, which resulted in probable detergent contamination over a period of slightly less than two weeks. A method for detergent contamination is presently being evaluated.

The Glassware/Media Section continues to perform journeyman and exceptional service to the entire spectrum of Institute scientific effort.

The Animal Husbandry Section made important strides in meeting its objectives in FY 1986. Two much needed, qualified supervisors were recruited and recruitment was begun for a supervisor trainee. When the latter action is completed, Animal Husbandry will be in a posture to proceed with opening the last Module of the Bldg. 101 Animal Facilities. Preparations for activation of this Module were begun early in the calendar year and have progressed to the final stages. Two events will dictate the time of opening and use of this facility: determination of the sense of the Institute Scientific Community on the type of use this Module should be put; the actual recruitment and training of a supervisor. More importantly, Animal Husbandry achieved a new level of participation in Institute animal science projects through its Bio Aid Training program, the Technical Assistance program, and the Rodent Breeding Technology program. These efforts have contributed heavily in a relatively short period of time to the initiation and successful conclusion of numerous studies and have been distributed about equally among the various intramural programs (IRP, TRTP, BRAP) at NIEHS. Outstanding efforts have been contributed by Rodent Breeding Technology to studies of gene regulation of hepatic enzymes in LP; chromosome translocation (hamsters) in LPP; neonatal behavior, LBNT; toxicology, CTEB; and developmental and reproductive toxicology, LRDT. Outstanding efforts have also been contributed by the Bio Aid Training program which has executed a broad range of animal technologies in fundamental and toxicology research including dosing, measurements, necropsy, observation, etc. This program has broadened the scope of studies possible, and reduced the time in executing them. Further training is desirable to continue the expansion of this program and to extend the training concept to technicians and investigators outside of CMB. A curriculum outline has been prepared; implementation is targeted for 1987.

For a brief period in FY 86, the entire experimental rodent population in NIEHS appeared to be free of all epidemic rodent viruses; however, the Animal Husbandry program continues to experience reintroduction of epidemic rodent diseases from supplier sources. Most troublesome has been mouse hepatitis virus (MHV), currently the most universal and troublesome of all the rodent viruses. This virus was introduced twice in FY 86. It was quickly contained in the first episode; however, because of the type of mouse populations infected, was rapidly spread in the second episode. Suspect, but as yet unconfirmed sources were identified in both cases. The breadth of distribution of this virus in commercial and research colonies the world over make prevention of infection in outside sourced mouse colonies difficult to achieve and maintain. Internal sources of supply may be essential for some types of studies, or carefully managed and hence limited quarantine programs may be necessary. Alternatively, conventional facilities for non-restricted use may be desirable. Thus for the present, long term freedom from epidemic pathogens remains an objective.

The Diagnostic Laboratory (DL) experienced heavier demand for service and a closer interaction with the scientific staff than in previous years. The DL identified the presence of EDIM (epidemic diarrhea virus of infant mice) virus,

a rota virus in the large date-mating reproductive toxicology mouse colony. The source of the infection was again outside supplier. Information available suggests that this virus may have been present for an extended period of time (years). This virus may cause neonatal morbidity, mortality, and permanent stunting.

The Quality Assurance Laboratory (QAL) continued close assessment of the QA practices and media produced by the Media Unit. Together with Animal Husbandry it is responsible for the Sentinel Animal early warning program. This program successfully detected both MHV episodes which occurred this year before perturbations in experimental data occurred. Reassessment of the Sentinel Animal program is presently underway in an effort to increase its sensitivity and define the parameters necessary for closer management of sentinel animals. A revised standard operating procedure is currently employed and is under further discussion. The QAL has now collected enough data to make decisions on the scope, content and value of testing deionized water which is used for animal drinking water and media preparation. The QAL continues close surviellance of animal feed for pesticides, nutritional content, undesirable estrogenic activity, heavy metals, etc. During FY 1986 the QAL identified two shipments of animal feed with excessive malathion content. Based upon a consensus of knowledgeable scientific staff, one shipment was used and the supplier was consulted; however, autoclaving failed to hydrolize the malathion to the extent anticipated and the second shipment was rejected. No further contamination occurred. Thus in two years service, QAL has identified three shipments of feed which failed to meet specifications, the first being absence of Vitamin A, detected the preceding year.

Other administrative activities of CMB include the continued quarterly-to-triannually presentation of the Workshop on the Humane Use of Animals in Research; the review and preliminary approval of Applications for the Use of Animals in Institute research programs, administration of controlled substances acquisition and issuance; and continued development of a spectrum of administration computer programs with CTEB/BRAP. To date programs in use include media cost and volume, animal inventory, animal applications tracking, sentinel animals, animal costing, and procurement. Additional work is in progress to broaden the media program to include calculations capability for ingredient content; and to modify the animal inventory program to show inventory by investigator for distribution. AUTOWEI, a program to automatically record weights, food consumption, water consumption, and clinical observations is almost completed.

Research Activities

Research activities in CMB continue to be of the problem solving type. They presently consume less than 20% of professional staff time, but are viewed as essential to the vitality of the staff and CMB in general.

During FY 86 studies on use of the rodent bioassay to detect estrogenic or differential uterine growth promoting activity continued. Principal observations included confirmation of higher than expected (more or less than 4-6 ppb DES) growth promoting activity in the widely used semipurified rodent diet AIN-76. Since this diet contains large amounts (more than 50%) of carbohydrates, studies were conducted to see if carbohydrates and fats may account for differential

uterine weight increase. Both fats and carbohydrates were shown to cause differential uterine weight increase but the meaning is uncertain. No attempt has yet been made to determine which tissue compartment of the uterus is responsible for the increased weight. Two manuscripts were accepted for publication in FY 86 for rodent bioassay work, and a paper will be presented at a national meeting this fall. It is anticipated that this line of work will be concluded in FY 86 or FY 87.

Studies continued on the ability of Mycobacterium chelonae, a common water/soil contaminant to cause microgranulomata in mice. The significance of this lies in the high incidence of such lesions (unexplained) in laboratory mice, notably those seen in chronic study bioassays. Liver microgranulomata are easily reproduced in euthymic and athymic mice by IV inoculation of M. chelonae. Granulomata are detectable up to 50 days PI and organisms are recoverable up to 35 days PI in athymics. A differential agar medium consisting of TGE agar with 3 μg/ml vancomycin and 10 μg/ml polymyxin was identified for the selective growth of M. chelonae from water samples. A manuscript is planned on these studies.

Further work was conducted on the serological grouping of rabbit coronavirus (RbCV). Based on cross neutralization tests with anti -CCV, -FIP, -TGF, and previous studies with -CDCV and -human 229E as well as partial protection from CCV and FIP vaccines, the RbCV appears to be a Group II Coronavirus. The virus remains uncultured in vitro and final determination of relatedness awaits this event. The role that this virus may play in natural disease of rabbits is uncertain, although newer information suggests that one or more coronaviridae may be present in rabbit populations, at least some of which may cause disease under natural circumstances. We have previously described the experimental pathology of RbCV. This work on RbCV will be presented at the Third International Coronavirus Workshop in September 1986.

Only limited additional work was undertaken on the natural history of MHV in FY 86, although as described above there are compelling reasons to pursue this line of investigation. New data collected from the sentinel animal program demonstrate graphically the serious gaps in our knowledge of virus spread and virus shedding. Our principal efforts in FY 86 are to gain insight into these phenomena by a carefully orchestrated sentinel program and contrived means of spreading the virus to "force" transmission. We have also added direct IFA examination of test tissues for MHV to our diagnostic regimen but problems with nonspecific fluorescence remain to be solved, and infected tissue culture-test serum techniques remain to be added.

We observed fatal wasting disease in athymic nude sentinel mice caused by Pneumonia Virus of Mice (PVM) in FY 86. These observations have been confirmed by experimental reproduction of the disease, scanning and transmission EM, IFA, and serology. This is believed to be the first such observation reported and may offer several exciting research opportunities. PVM is one of only three viruses currently classified as Pneumovirus. The others are human respiratory syncitial virus (RSV) and bovine RSV (BRSV). PVM is less closely related than RSV and BRSV; however,RSV is an important childhood disease. We plan studies on target cell population, virus distribution in the host, patency periods, and the pathophysiology of the disease. Preliminary findings will be presented at a national meeting this fall. The PVM studies and MHV studies are both hampered by lack of a clinical-experimental virologist within CMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 22102-05 CMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of a Coronavirus from Rabbits

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. D. Small	Head, Diagnostic Laboratory	CMB, NIEHS
Others:	M. E. Clements	Biol. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch, TRTP, NIEHS, NIH (Dr. M. Thompson); Division of Comparative Medicine, Johns Hopkins School of Medicine (Drs. J. Strandberg and L. Aurelian); National Animal Disease Center, ARS, USDA, Ames, IA (Dr. R. D. Woods)

LAB/BRANCH
Comparative Medicine Branch

SECTION
Diagnostic Laboratory

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this project is to study the pathogenesis of rabbit coronavirus (RbCV), the rabbit's physiologic response to this virus, and the relatedness of RbCV to other members of the Coronaviridae. Serum neutralization studies against RbCV with antiserum to canine coronavirus (CCV), feline infectious peritonitis (FIP), and transmissible gastroenteritis (TGE) viruses in the intact rabbit were completed. Results were: CCV 2/3, FIP 2/3, TGE 1/3, RbCV 3/3 survived. RbCV alone was lethal. All rabbits receiving RbCV in combination with antiserum to one of the coronaviruses, including RbCV showed clinical signs of disease. As expected RbCV antiserum muted clinical signs the most. RbCV reacted with antisera to CCV, FIP, or TGE produced a typical clinical picture of disease. In some survivors the rectal temperature remaind above 40°C for 10-12 days and in others the temperature pattern closely followed that of rabbits receiving RbCV + RbCV antiserum. Gross lesions in those which died were similar to rabbits dying with RbCV alone. Gross lesions were not observed in survivors killed following recovery. Based on previous work showing a 2 way cross with coronavirus 229E (Human), partial cross protection from vaccination with CCV and FIP, and this new data, RbCV is most probably in Group II of the Coronaviridae. Nucleotide homology studies will be required to prove this supposition. Assessment of myocardial damage measuring creatine kinase isozymes are in progress. Further attempts will be made to adapt RbCV to tissue culture. The significance of this work lies in the ability to study a viral disease with a cardiotropism in an animal of sufficient but manageable size to allow sequential clinical and physiological observations. The damage to the rabbit heart by RbCV has a corollary in the human heart with the Coxsackie viruses, Mycoplasma pneumoniae, influenza virus, Herpes zoster, and possibly other infectious agents.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 22103-03 CMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural History of Mouse Hepatitis Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C. B. Richter	Chief	CMB, NIEHS
Others:	J. E. Thigpen	Head, Quality Assurance Lab	CMB, NIEHS
	E. H. Lebetkin	Biol. Lab. Tech., QAL	CMB, NIEHS
	M. L. Dawes	Biol. Lab. Tech., QAL	CMB, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Comparative Medicine Branch

SECTION
Office of Chief, Quality Assurance Laboratory

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

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

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

Transmission of mouse hepatitis virus (MHV) was studied in a "natural" animal room setting using euthymic and athymic mice; and in unnatural settings where experimentally infected cage mates served as time-controlled donors.

We have demonstrated that euthymic sentinel mice may not be as effective indicators of MHV infection in mouse populations as generally accepted because of slow, or low titre response under certain husbandry conditions. In a single companion study, athymic sentinels gave histologically predictive indications of incipient MHV at 7-11 weeks post placement, and fully developed MHV liver lesions at 13-17 weeks post placement. The nude sentinel concept must be approached cautiously because the long patent period of MHV infection in nude mice identifies them as contributors to the persistence of the virus within the colony. We have also shown that CD-1 weanling mice experimentally infected with a street strain of MHV shed virus between 12 and 18 days PI as measured by cohabitant seroconversion. It must be emphasized that these results are indicators for the CD-1 mouse only, and must be confirmed.

We are presently repeating the euthymic sentinel study in a carefully controlled circumstance where sentinels are examined biweekly and/or monthly in known infected and noninfected rooms.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22104-03 CMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of the Mouse Bioassay Test for Detecting Estrogenic Activity in Feed

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. E. Thigpen	Microbiologist	CMB, NIEHS
Others:	C. B. Richter	Chief	CMB, NIEHS
	E. H. Lebetkin	Biol. Lab. Tech.	CMB, NIEHS
	M. L. Dawes	Biol. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Comparative Medicine Branch

SECTION
Quality Assurance Laboratory

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

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

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

We have previously shown that mice fed the American Institute of Nutrition (AIN-76A) purified diet experience a significant increase in uterine-body weight (U:BW) ratios when compared to the U:BW ratios of mice fed Purina #5002, a closed formula natural ingredient diet. The AIN-76A diet contains 65% carbohydrate with 50% coming from sucrose or dextrose and 15% from corn starch. The objective of this study was to determine whether fat and carbohydrate content contribute to unexpected uterine growth promoting activity observed in mice fed the AIN-76A diet. Bioassays were performed using CD-1 mice weaned at 15 days of age and randomly assigned to #5002 diet fortified with corn starch, sucrose, dextrose, corn oil, or soybean oil at the 10% and/or 25% level and to #5002 diet containing 0, 4, or 6 ppb diethylstilbestrol (DES). Uterine:BW ratios were determined on 15 mice/diet at 5 and 7 days post feeding. The U:BW ratios of mice fed #5002 diet fortified with corn oil, soybean oil, sucrose, dextrose or cornstarch were similar to each other, and were higher than the U:BW ratios of mice fed the standard #5002 diet without added DES. This increase in U:BW ratios is similar to the U:BW ratios of mice fed the #5002 diet containing 4 ppb DES. It was concluded that both the fat and the carbohydrate content of the AIN-76A diet contributed to its uterine growth promoting activity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 22106-02 CMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of an Acid Fast Bacterium Isolated from Animal Drinking Water

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. D. Small	Head, Diagnostic Laboratory	CMB, NIEHS
Others:	M. E. Clements	Biol. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, TRTP, NIEHS, NIH (Dr. C. A. Montgomery, Jr.)

LAB/BRANCH
Comparative Medicine Branch

SECTION
Diagnostic Laboratory

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this project is to characterize and study the pathogenic potential of an acid fast (AF) bacterium repeatedly isolated from an ultrapure water source furnishing drinking water to some of the Institute's animals. The organism has been identified as Mycobacterium chelonae. Growth characteristics were compared in broth media and on solid phase media. Middlebrook 7H9 broth + albumin fraction V and dextrose gave no advantage over BHI or TSB which gave similar results. Nutrient broth was inferior. TGE agar was as effective or more so than TSA, Mueller-Hinton, and Middlebrook 7H9 broth as above + agar and is our standard agar. Sensitivity to several antimicrobials was determined. From these results the growth of M. chelonae on TGE agar containing vancomycin (3 µg/ml) + polymyxin (10 µg/ml) was examined. Preliminary results indicate that M. chelonae is not affected by these concentrations. The media (TGE+VP) should prove useful in examining water samples and animal specimens for the presence of M. chelonae. Athymic mice inoculated IV with M. chelonae developed focal granulomas detectable in the liver on post inoculation day (PID) 2 through PID 50. Intensity of lesions peaked by day 7. AF organisms were rarely seen in sections after day 11. AF bacteria were isolated from livers on PID 2, 4, 7 (5/5), 11 (3/5), 14 (1/5), 21 (2/5), 35 (2/5), 50 (0/5).

Spontaneous liver lesions will be searched for AF organisms. This work is significant because of the focal lesions of undetermined origin observed in livers of rodents on chronic bioassays conducted under the NTP. Identifying water-borne bacteria and a Mycobacterium sp. in particular as the cause would allow for corrections in management practices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 22107-01 CMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	C. B. Richter	Chief	CMB, NIEHS
Others:	J. E. Thigpen	Head, Quality Assurance Lab	CMB, NIEHS
	E. H. Lebetkin	Biol. Lab. Tech., QAL	CMB, NIEHS
	M. L. Dawes	Biol. Lab. Tech., QAL	CMB, NIEHS

COOPERATING UNITS *(if any)*
Center for Electron Microscopy, Dept. of Microbiology, School of Agriculture and Life Sciences, North Carolina State University (J. MacKenzie, C. S. Richter, D. Flynn)

LAB/BRANCH
Comparative Medicine Branch

SECTION
Office of Chief

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.2	.1

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this project is to study the natural history of pneumonia virus of mice (PVM), an endemic/epidemic respiratory pathogen of laboratory rodents including mice, rats, hamsters, guinea pigs, and probably others. Little is known about the pathology, patent period, epidemiology, and target cell population of this virus. PVM is currently classified as a Pneumovirus of the family Paramyxoviridae. It shares this classification with respiratory syncitial virus (RSV) of man, and bovine respiratory syncitial virus (BRSV) of cattle. Minor differences are seen in the electrophoretic mobility patterns of the polypeptides of various isolants of RSV. These differences are not distinguishable with human convalescent serum but are distinguishable with individually prepared antisera. Similar statements may be made about BRSV, although the information is not as strong. Furthermore, RSV and BRSV have similar polypeptide profiles indicating close relatedness. PVM on the other hand has several major differences. We have observed, and will report for the first time, fatal wasting disease in athymic nu/nu mice naturally infected with PVM. Immunofluorescence studies of lung material from index cases indicate that the target cell in late stage natural infection in the nu/nu mouse is located in the pulmonary parenchyma rather than the lung airways. Because of the broad spectrum of rodent hosts, PVM has the potential to influence many experimental results, notably, those studies involving the cell dynamics of pulmonary parenchyma.

BIOMETRY AND RISK ASSESSMENT PROGRAM

BIOMETRY AND RISK ASSESSMENT PROGRAM
Summary Statement

The Biometry and Risk Assessment Program (BRAP) plans and conducts basic and applied research in the areas of quantitative and biochemical risk assessment, statistics, biomathematics, and epidemiology. A major focus of this research effort is the qualitative and quantitative estimation of adverse health effects resulting from exposure to hazardous environmental agents, and the development of methodology useful in this estimation process. Attention is also directed toward the identification of environmental risk factors and the elucidation of the biological mechanisms that underlie their action. Due to the complexity of many of the issues under investigation, an increasing proportion of this research is being conducted on a program-wide basis, combining the scientific expertise found in BRAP's different organizational units.

In addition to conducting its own research effort, the BRAP also provides statistical, mathematical, data processing, and computer engineering and user support to other programs of the Institute. It assists the Office of the Director in addressing specific health issues that bear on the welfare of the general public; and maintains an active association with peer groups in other federal agencies, academic institutions and private organizations with similar research interests.

The Biometry and Risk Assessment Program is organized into an Applied Pathology Section and a Molecular Modeling Section within the Office of the Director, a Statistics and Biomathematics Branch (SBB), an Epidemiology Branch (EB), a Laboratory of Biochemical Risk Analysis (LBRA), and a Computer Technology Branch (CTB).

The Statistics and Biomathematics Branch conducts a broad research effort in a variety of areas such as statistical studies in carcinogenesis and genetic toxicology, biomathematical modeling, risk assessment methodology development, and the generation of biostatistical procedures applicable to epidemiology and toxicology. Branch scientists also provide a comprehensive consulting service for the research staff of the Institute.

The Epidemiology Branch carries out field studies of chronic disease which may be attributable to environmental pollutants; investigates the effects of environmental toxins on fertility, fetal and child development; and applies experimental laboratory methods in the monitoring of human populations.

The Laboratory of Biochemical Risk Analysis is primarily concerned with the development of laboratory procedures for quantifying exposures in terms of the biologically effective dose, and with the adaptation of these procedures for application to human populations and to the enhanced extrapolation of toxicologic outcomes across species.

The Computer Technology Branch operates the Institute's central Vax computer system; coordinates data communication activities; develops laboratory computers and related scientific software, administrative information systems,

and NTP scientific information systems; provides an extensive user services program for the Institute; manages the Institute's word processing and office automation activities; provides support for a variety of computer-based work stations; and manages the formal administrative systems associated with computing at the Institute.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43009-03 BRAP

PERIOD COVERED

October 1, 1985, to September 30, 1986

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

Role of Kidney & Nutritional Factors in Metabolism of Toxic & Essential Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert A. Goyer	Deputy Director	NIEHS
Others:	Winona Victery	Expert	EB, BRAP, NIEHS
	Chris R. Miller	Bio. Lab. Techn.	EB, BRAP, NIEHS
	Rong-fang Hu	Visiting Fellow	EB, BRAP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director, Biometry and Risk Assessment Program

SECTION

Applied Pathology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The cellular pathology of the kidney is studied in rats following exposure to toxic metals. A series of experiments is being conducted to determine the influence of essential trace metal metabolism on metal nephrotoxicity. Metabolic parameters and indicators of toxicity measured include cell ultrastructure, toxic and trace metal burden and excretion, changes in metal-binding protein, e.g., metallothionein.

Results to date indicate that:

- o Lead exposure increases urinary zinc in a dose and exposure length-dependent manner; urinary calcium is increased at the highest lead dose only.
- o Calcium content of kidney is normally low, but there is a discontinuous increase in calcium concentration, indicative of renal cell injury, if blood lead concentration exceeds 45 µg/dl.
- o Increased blood pressure occurs in rats after moderate exposure to lead for 12 months (mean blood lead, 45 µg/dl).
- o Animals injected with cadmium have increased metallothionein content of liver and kidney with liver metallothionein concentration two-fold greater than kidney. Zinc deficiency results in less increase in kidney metallothionein with cadmium exposure, greater increase in non-metallothionein bound cadmium, and increased susceptibility to cadmium nephrotoxicity.

The purpose of these studies is twofold: one is to determine biologic indicators of renal toxicity in response to toxic metal exposure. The second is to determine the role of metal binding proteins and essential metals on toxicity. From these studies the influence of various factors on risk to exposure to toxic metals may be estimated. The identification of indicators of exposure and biologic effect may have application in prevention of toxicity.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43010-01 BRAP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Macromolecular Modeling and Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Hoel	Director	BRAP
Others:	Marshall W. Anderson	Research Chemist	LBRA
	Tom Darden	Sr. Computer Scientist	BRAP
	Lee G. Pedersen	Research Chemist	BRAP

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Risk Assessment Program

SECTION

Office of the Program Director

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is concerned with exploring theoretical factors involved in mutagenesis and the initial steps in carcinogenesis. Recent experimental advances in the area of genetic engineering have provided new possibilities for studying the dependence of chemically induced mutational events on DNA sequence. We hope to use computer modeling to examine the physical chemical factors (charge distribution, chemical reactivity, and stereochemical and thermodynamic relationships) contributing to site specificity of chemical agents at the level of DNA damage. An additional area of interest is the effect of amino acid substitution on protein function.

GPO 914-918

COMPUTER TECHNOLOGY BRANCH
Summary Statement

As well as developing overall strategies for computing at the Institute, the Computer Technology Branch (1) manages and operates the Institute's central VAX computer system including hardware development and systems programming; (2) coordinates data communications activities associated with the central system, workstations, and remote facilities such as NIH/DCRT; (3) develops laboratory computers and scientific software to support Institute research; (4) develops administrative information systems (application software) for the Institute; (5) develops scientific information systems for the NTP; (6) provides services to VAX users including consulting, problem investigation, training, manuals, and information distribution; (7) manages the Institute's word processing and office automation activities; (8) provides support for a variety of workstations including text and graphics terminals, personal computers, and stand-alone word processors; and (9) manages the formal administrative systems associated with computing at the Institute.

Management of the central VAX computer facility includes hardware development, systems software development, and traditional operations functions for the VAX processors and peripherals including management of computer accounts, invoices, supplies, storage media, and disk space. Hardware design, procurement, installation, and maintenance are managed for computer equipment, power, air conditioning, and physical security. Computing capacity is continuously being increased to meet increased demand from the Institute. Systems software for the VAX processors is provided in the following categories: operating system, data communications software, compilers, mathematics and statistics packages, graphics packages, data management software, and office automation software. To provide this varied software, commercial software is evaluated, procured, installed, and maintained and in-house software such as the NEWS utility is designed, programmed and maintained. As part of the systems programming effort VAX performance is monitored, resource utilization is monitored, capacity needs are forecast, resources are reallocated, and the VMS operating system is tuned for improved performance as the computing resources and workload change. Approximately half the system programming support provided is consulting with management, applications programmers, user support groups, and end users regarding software issues.

Data communications support consists of the provision (analysis of requirements, design, selection, installation, and maintenance) of all hardware and software components necessary to fulfill the intra- as well as extra-Institute data transmission requirements of all Institute employees and contractors. A major task continues to be responding to numerous requests for information and assistance regarding the proper configuration and operation of various terminals, microcomputers, printers, modems, and related equipment. A major effort recently completed was the development of written documentation for recognizing, reporting, investigating, diagnosing, and resolving data communication problems. Major projects currently in progress include doubling the speed of dial-up data communications, providing high-speed dedicated (non-dial) access for the majority of Institute users, and improving (i.e., higher speed and better reliability) access to remote computer systems, including NIH/DCRT.

Laboratory computing support is provided to the Institute with a primary emphasis on consulting with researchers to determine functional needs and on performing risk/benefit analyses of possible applications of computers in the laboratory. The traditional effort of laboratory data acquisition continues with the design, implementation, and maintenance of five computer-assisted data acquisition and analysis systems for serially-interfaced radiometric analyzers. Development of a new computer-assisted animal weighing system is under way, following identification of a need for this function among several Institute laboratories. Custom circuitry has also been developed, for example to convert analog magnetic tape data containing infant EKG and respiratory signals into digital form for analysis by computer. The design and implementation of scientific software supporting a researcher's specific needs, such as radio-immunoassay and bacteria colony counting, has continued. The use of standard approaches, such as MICRO/PDP11 computers for data acquisition and RS/1 software for data analysis, has helped to simplify and streamline development and support for researchers with common needs.

The information systems development effort consists of projects for the development of medium to large automated systems for both the Institute and the National Toxicology Program (NTP). Institute projects include efforts on behalf of the Office of Administrative Management for NIH Budget Reporting, Personnel Action Tracking, Requisition and Contract Tracking, Self-Service Stores System, Agent Cashier System, Automated Inventory System and Travel Order and Voucher System. Systems have been developed for the Office of Facilities Engineering for Work Orders, Special Inventories, Space Management, and BPA, Requisition, and Contract Tracking. Special Contracts and Personnel Action Systems have been developed for the NTP.

Scientific information systems for the NTP include the Chemical Information and Tracking System (CHEMTRACK), the Carcinogenesis Bioassay Data System (CBDS), and, in cooperation with the National Center for Toxicological Research, the Toxicology Data Management System (TDMS). CHEMTRACK accomplishments include a significant increase in the use of batch reports and the on-line query by NTP personnel; a formalization of the lab milestones reporting mechanism which substantially increases the currency of the CHEMTRACK data files; and the completion of workload projection reports which provide project officers and discipline and audit team leaders with future work projections for resource allocation. CBDS accomplishments include continued support of the NTP carcinogenicity and toxicity bioassays with the generation of 126 chemical package reports consisting of tumor and non-tumor pathology tables, survival curves and weight gain tables for the NTP Technical Reports. One hundred twenty three special reports were also generated. TDMS is being converted to the ADABAS Data Base Management System and also being converted to run on a VAX 8600 at NIEHS. This should be completed in FY87. The major new accomplishment was the release of a new version of the micropathology data collection module which greatly improves the speed with which the pathologists can record their findings. New report software is available which generates formats needed in the NTP Technical Reports. At the end of FY86, the TDMS data base included data for two year studies for 83 chemicals and data for 90 day studies for 35 chemicals. Most chemicals were tested on two species and in some 90 day studies by multiple routes of exposure. Both in-life and pathology data are routinely updated and hundreds of reports are produced each month as needed by the laboratories and NTP scientists.

The word processing and office automation section has expanded its support from a base of thirty-two machines with seventy users to about one hundred and fifty machines with two hundred users. The group provides a variety of training classes, assists in development of small office applications, tests and delivers new software and documentation, and provides help in problem solving. Present efforts include providing word processing to a growing Institute audience and adding the office automation functions of electronic mail, desk and time management, and electronic filing through VAX-based tools.

The expanding demand for computing is illustrated by the growing number of workstations throughout the Institute. To date, there are about one hundred DEC personal computers, two hundred fifty terminals, and three hundred attached printers. In addition to draft document preparation, these workstations are used for a wide variety of scientific and administrative applications. During the past year, the Institute's first desk-top laser printers were acquired to be used for text and graphics. The development of training and promotion of user groups will be the emphasis for this audience in the coming year.

Through an Interagency Agreement with the General Services Administration (GSA) the branch acquires expertise of GSA contractors to perform much of the automatic data processing (ADP) and office automation (OA) service that is being delivered to the Institute. Of the approximately sixty contract personnel serving the Institute, most are on-site and working closely with the scientists and administrative personnel whose technical work is being done by the contractor. CTB in-house personnel direct and oversee the ADP and OA aspects of the work being performed.

EPIDEMIOLOGY BRANCH
Summary Statement

The health effects of chronic, low-dose environmental exposures are more diverse than any single epidemiologic strategy can encompass. The Epidemiology Branch has developed a broad program of research that emphasizes innovative methods and interdisciplinary collaborations. This effort draws on the expertise of laboratory scientists, clinical scientists, and statisticians in our own Program as well as outside. Within this broad scope there are three general areas of research: the study of reproduction and early development, the study of chronic disease, and the detection of human genetic damage. Each of these areas are discussed below, with specific examples of current studies in each area.

Reproduction and Early Development

The study of reproductive events is an epidemiologic specialty with special relevance to environmental research. Since the late 1970s, a number of reproductive and developmental problems have been linked to environmental chemicals. These include infertility and the pesticide DBCP, spontaneous abortion and the herbicide 2,4,5-T, low birthweight and toxic-dump effluents, and developmental abnormalities associated with PCB-contaminated rice oil.

Animal studies have reinforced the ideas that reproductive processes may be vulnerable to low-dose toxins. Gamete formation, fertilization and early fetal development in animal species can be damaged by levels of chemical exposures too low to produce acute toxicity. The accumulating body of animal literature, together with the several examples of human reproductive damage by low-dose exposures, have led to speculation that reproductive damage may prove to be a sentinel event in humans. The epidemiologic detection of reproductive damage may provide an early indicator of exposure to mutagens or teratogens that otherwise require a latency period before their damage is clinically detectable.

The Epidemiology Branch has approached reproductive research in two ways. One is to develop more specific and precise tools for measuring reproductive outcomes. For example, early pregnancy loss is undetectable in humans without special techniques. A new assay for human chorionic gonadotropin has been applied in a study of early pregnancy, allowing the risk of early loss to be accurately measured in humans for the first time. With further development, this method may be applicable in prospective studies of women exposed to suspected hazards.

Another example of improved methodology is a questionnaire method for measuring fertility. This method was used to test the hypothesis that adult fertility can be impaired by the adults' prenatal exposures. Specifically, animal studies have found a decreased fertility in rodents exposed prenatally to components of cigarette smoke. In a study of nearly 700 couples carried out by the Epidemiology Branch, this effect was not confirmed, although a woman's own smoking was related to a decrease in her fertility.

A second approach to reproductive studies has been to study populations that have been accidentally exposed to high levels of a toxin. An accidental poisoning of 2000 people in Taiwan presented an opportunity to study the offspring of poisoned mothers. One-hundred and eight exposed children and 106 controls were recently given neurologic, genetic, dental and dermatologic exams. Preliminary data indicate higher rates of respiratory disease and dental abnormalities among the

exposed children. These data may shed light on the possible effects of PCBs among children who have had lower exposures to this ubiquitous and persistent class of chemicals.

Chronic Disease

Chronic diseases contribute substantially to the morbidity of human populations and result in large expenditures of public health dollars. While environmental exposures may produce some of these diseases, potential links between many chronic diseases and toxic exposures have not been adequately pursued. Environmental exposures may affect a variety of body systems, and may produce a wide range of effects from asymptomatic biochemical changes or minor symptoms to serious disabling illness. Many conditions that might relate to environmental exposures, however, have been neglected by epidemiologists because appropriate tools to measure either disease or exposure are lacking. Focused attention on the study of chronic diseases may lead to earlier detection of potential hazards.

For example, chronic renal failure is a serious, debilitating and expensive disease that for the most part occurs without a known cause. A few specific environmental exposures such as lead are known to be toxic to the kidneys at high doses, but the contribution of low-dose environmental factors over many years is not known. A case-control study of 709 patients with chronic renal disease has been recently completed which investigates a broad range of environmental exposures for possible renal toxicity.

The epidemiology of certain chronic diseases can be further strengthened by improved diagnosis and classification. In a current study of adult acute leukemias, newly diagnosed cases are being classified cytogenetically. Previous case-reports have suggested that some risk factors, such as exposure to solvents, may be more strongly associated with specific cytogenetically defined leukemia subgroups. The current study will be the first large-scale leukemia study to look at a range of exposures in relation to cytogenetic subtypes.

Detection of Human Genetic Damage

The potential exposure of humans to mutagenic agents is wide, but the actual extent of this exposure and associated health effects are largely unknown. This paucity of data occurs in part because epidemiologic studies for genetic toxicity are limited by the lack of adequate means of assessing genetic damage in human populations. Some epidemiologic studies have employed laboratory assays, but the comparative biologic importance and effectiveness of each assay is unknown. There is a need to develop, validate, compare, and field-test a battery of laboratory tests to indicate exposure and damage.

Work in the Epidemiology Branch is proceeding in these four phases: (1) Assay Development. Promising assays are identified, further developed and evaluated for their feasibility, reliability, and biologic interpretability. (2) Assay Validation. A battery of tests purported to indicate genetic damage are applied to populations with known exposures to genetic toxins (such as patients undergoing cancer chemotherapy). Results are analyzed to indicate which assays respond to which sorts of compounds, the doses required, temporal aspects of that response, and correlations with known risk. (3) Assay Comparison. The results of different assays are analyzed statistically to indicate the amount of information provided by each assay, and to consider the cost of these assays relative to information

gained. These results will be used to devise the most effective battery of tests for incorporation into epidemiologic studies. (4) Field Testing. The utility of specific assays will be field tested in selected epidemiologic studies.

In one current project, blood specimens are being collected from women before and after chemotherapy for breast cancer. These specimens provide the means to validate and compare a number of tests for genotoxicity, including a sister-chromatid exchange assay,the glycophorin-A-locus assay, and chromosomal aberrations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43002-10 EB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Human Exposure to Halogenated Aromatic Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Walter J. Rogan	Acting Chief	EB	NIEHS
Others:	Beth C. Gladen	Statistician	SBB	NIEHS
	Gwen Collman	Staff Fellow	EB	NIEHS
	Karsten Lundgren	Visiting Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)* Statistics and Biomathematics Branch; Biochemical Risk Analysis Branch; Wake Area Health Education Center, Raleigh, NC; Durham Women's Clinic, Durham, NC; East Carolina School of Medicine, Greenville, NC; National Taiwan University Hospital, Taipei, Taiwan; Academica Sinica, Taipei, Taiwan.

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	0.00

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

Polychlorinated biphenyls (PCBs) and the DDT family are toxic, widespread hydrocarbons that are poorly understood in terms of their toxicity for human beings. In addition to their direct toxicity, both of these chemicals pass from mother to child through the placenta and by contaminating breast milk. This project includes a study of subjects exposed to low levels of these compounds in the US and two studies of subjects exposed to higher doses in Taiwan.

The Breast Milk and Formula study is a birth cohort follow up study of 856 North Carolina children. PCBs and DDE (the stored metabolite of DDT) are measured in breast milk and the children are followed medically over time. Most of the children have completed 5 years of observation and are now followed only by a birthday card registry. This study has shown that more than 90% of NC breast milk samples have PCBs and DDE detectable; levels of PCBs are as high in NC as in areas thought to have special exposures. Levels of both PCBs and DDE decline over the course of lactation, and levels are higher in first than in subsequent lactations. Children exposed to higher levels of DDE and PCBs transplacentally are more likely to have mild degrees of neurological impairment at birth. Women with higher levels of DDE breast feed for shorter lengths of time.

An epidemic of 2000 cases of PCB poisoning occurred in Taiwan in 1979. Rice oil was accidentally contaminated during manufacture. We did a survey of 108 children who were born to mothers who were poisoned, 40 of their older siblings, and 106 controls. All children received a physical examination and the mothers answered a questionnaire about their children's health. Another study of this outbreak used a modification of an assay for sister chromatid exchanges to evaluate the potential for long term, subtle genetic damage, as well as assays for cellular immune function (see also Z01 ES 46003-01 LBRA).

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 43004-08 EB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Other:	Walter J. Rogan	Acting Chief	EB	NIEHS

COOPERATING UNITS *(if any)*
Bowman Gray School of Medicine/Baptist Hospital, Duke University Medical Center, University of North Carolina Medical School, Charlotte Memorial Hospital, Food and Drug Administration, Centers for Disease Control

LAB/BRANCH
Epidemiology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.50	0.50

CHECK APPROPRIATE BOX(ES)

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

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

Exposure to environmental hazards may produce a variety of short and long-term effects, including many common chronic diseases. Surprisingly little attention has been paid to identifying associations between environmental exposures and many chronic conditions for which etiologic agents are not yet known. Identification of such associations is a first step towards possible prevention of substantial morbidity in human populations.

BRAP's program in environmental epidemiology addresses the role of environmental factors in the etiology of some less well studied chronic diseases. The program is developing methodologies appropriate to the epidemiologic study of chronic diseases which are often difficult to characterize or define precisely, and is adapting methodologies that have been used in studies of cancer for use in studies of nonmalignant diseases.

Current emphasis is on identifying risk factors for chronic renal disease which is likely to have a strong environmental component. Chronic renal disease has received little attention in epidemiologic studies, and presents numerous methodologic challenges. A multi-center case-control study of risk factors for chronic renal dysfunction has been completed as has a case-control study of risk factors for biopsy diagnosed IgA nephropathy. Preliminary findings suggest that consumption of certain analgesic medications and environmental exposure to certain solvents and metals may play a role in renal disease etiology. Related ongoing studies involve the development of a renal disease classification scheme for use in etiologic studies, and the analysis of vital statistics and other data to identify time trends, geographic patterns of renal disease, and occupations with potentially increased renal disease risk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43008-07 EB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Biochemical and Cellular Environmental Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard B. Everson	Medical Officer	EB	NIEHS
Other:	Walter J. Rogan	Acting Chief	EB	NIEHS

COOPERATING UNITS *(if any)* Columbia University, U.S. Department of Agriculture Western Regional Research Center, University of North Carolina at Chapel Hill, Duke University, Baylor College of Medicine, Laboratory of Biochemical Risk Assessment, NIEHS, Laboratory of Pharmacology, NIEHS, Medical University of South Carolina.

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project uses an approach integrating epidemiologic and laboratory methods to measure genetic damage and alterations in metabolism associated with human exposures to toxic chemicals. Laboratory tests for measuring genetic damage include assays for modifications at each of the three levels of organization of genetic material: chromosomal, gene or specific locus, and chemical. Tests for measuring alterations in metabolism include enzymatic and immunological assays for the activity of mono-oxygenase enzymes in human tissues and blood cells. Newly developed assays are initially used to study subjects with large, precisely defined exposures (e.g. medical exposure to cytotoxic drugs); if the assays prove sufficiently sensitive they are then used to study subjects with less intense and less well characterized exposures (e.g. occupational, lifestyle, and other environmental exposures). Groups under study include patients and workers exposed to cytotoxic drugs, individuals exposed to active and passive smoking, women accidentally exposed to large quantities of PCBs and their offspring, and children exposed to lead. The project emphasizes interdisciplinary development of study approaches with attention to details of both the laboratory procedures and the gathering and analysis of data concerning human subjects. These studies are designed to help evaluate and refine approaches that can be used to investigate both mechanisms involved in the etiology of cancer and other chronic diseases and effects of exposures that may be important to public health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 44003-09 EB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen J. Wilcox	Medical Officer	EB	NIEHS
Others:	Donna D. Baird	Senior Staff Fellow	EB	NIEHS
	Beth C. Gladen	Statistician	SBB	NIEHS
	Clarice R. Weinberg	Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*
Developmental Endocrinology Branch and Biometry Branch, National Institute of Child Health and Human Development, National Institute of Dental Research, Columbia University, Atlanta University, University of North Carolina

LAB/BRANCH
Epidemiology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.60	2.60	0.00

CHECK APPROPRIATE BOX(ES)

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

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

The reproductive epidemiology project emphasizes the development and application of methods for measuring human reproductive damage. Reproductive damage can include infertility, sub-clinical early fetal loss, spontaneous abortion, impaired fetal growth, and low birthweight. Each of these outcomes can be produced by environmental factors, and each represents a possible endpoint for detecting the effects of toxins on human reproduction. One major component of this project is the study of fertility. Time-to-pregnancy (that is, the number of cycles a couple takes to conceive) is being developed as a potentially sensitive measure of fertility. This approach will be used in a study of reproductive outcomes among dental technicians exposed to mercury. A second component of this project is the study of very early pregnancy loss. In a prospective study of 230 women who have stopped using birth control in order to become pregnant, daily urine specimens have been collected in order to test for evidence of pregnancy. This will provide an estimate of the extent of early pregnancy loss in humans. The risk of early loss will be studied in relation to common exposures in this population, such as use of alcohol, tobacco, caffeine beverages and medications. Work continues on the development of a method for the analysis of birthweight and perinatal mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 46002-02 EB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dale P. Sandler	Senior Staff Fellow	EB	NIEHS
Others:	Gwen Waldman Collman	Staff Fellow	EB	NIEHS
	Richard B. Everson	Medical Officer	EB	NIEHS

COOPERATING UNITS *(if any)* University of Minnesota, Harvard University, Cancer and Leukemia Group B member institutions, the Johns Hopkins University Training Center for Public Health Research, Laboratory of Biochemical Risk Analysis, NIEHS and the Department of Epidemiology, University of North Carolina

LAB/BRANCH
Epidemiology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

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

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

The study of cancer risk is a major component of environmental epidemiology. The Epidemiology Branch emphasizes the development of methods for assessing environmental exposures and, where feasible, uses biochemical measures of exposure or disease markers to evaluate cancer risk. Current studies focus on cancer risk from passive exposure to cigarette smoke and risk factors for leukemia.

Studies in the Branch have identified associations between passive exposure to cigarette smoke during childhood and adulthood and risk for cancer at several sites in adulthood. Current studies are aimed at confirming some of these associations, documenting passive exposure to cigarette smoke, and understanding mechanisms of cancer risk. A prospective study of mortality and cancer incidence among persons who lived with smokers in 1963 has been conducted. In another study, indicators of mutagenesis and genotoxicity were measured in nonsmokers, passive smokers and active smokers. In addition, data from the Branch's study of cancer risk from passive and transplacental exposure to cigarette smoke were used to evaluate the quality of retrospective questionnaire data on passive smoke exposure and to explore a possible association between passive cigarette smoke exposure and early age at menopause.

Risk factors for acute leukemias in adults are being evaluated in a cooperative case-control study. The study aims to identify risk factors for cytogenetically defined subgroups of acute leukemia. Some risk factors, such as exposure to solvents, may be more strongly associated with cytogenetically defined leukemia subgroups. Therefore, the results of bone marrow cytogenetics being done as part of other cancer treatment studies are being used to classify patients into potentially etiologically distinct subgroups. In a related project, the relationship between low-level background irradiation and leukemia risk is being explored in an ecologic study of radon in ground water and leukemia mortality.

LABORATORY OF BIOCHEMICAL RISK ANALYSIS
Summary Statement

Formation of the Laboratory of Biochemical Risk Analysis reflects the growing awareness that biochemical approaches can contribute a great deal to risk assessment and epidemiology. There exists considerable uncertainty in current methodology for making species-to-species extrapolations when attempts are made to correlate overall exposure of a chemical with a gross biological effect such as tumor incidence. Our experimental approaches are designed to remove some of the uncertainty by quantifying biochemical parameters which are directly involved in the mechanism of action of specific classes of chemicals. Such measurements would include receptor interactions, DNA adducts, DNA repair and activated oncogenes in accessible human samples as well as experimental animal models. In these studies we attempt to differentiate between biochemical/molecular changes that are intimately associated with the mechanism of action from those that are only indicators of exposure. These approaches should provide a more rational basis for making species-to-species extrapolations as well as enhancing our ability to predict groups at risk from exposures to specific classes of chemicals. The Laboratory of Biochemical Risk Analysis is the NIEHS focus for application of biochemical methods to risk assessment. With ready access to the broad range of basic and applied research resources within NIEHS, the Laboratory of Biochemical Risk Analysis is in a unique position to address many important public health questions related to human health hazards arising from exposure to toxic chemicals.

Receptor Toxicology Section

1. The two-stage model for hapatocarcinogenesis has been established in the rat using diethylnitrosamine (DEN) as the initiating agent and either 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 17α-ethynyl estradiol (EE_2) as the promoting agent. Time-course and dose-response studies have been conducted and a number of parameters are being evaluated including tumor incidence, histological changes, GGT-positive foci, TCDD or estrogen receptor, epidermal growth factor receptor (EGFR), cytochrome P-450 isozymes, covalent binding, oncogene activation and concentrations of TCDD or EE_2 in liver. Data have been generated to suggest that interactions of various receptor systems, especially EGFR, play a critical role in the promotion of hepatocarcinogenesis by estrogens or halogenated aromatics. Several lines of evidence suggest that ovarian hormones play a critical role in the hepatocarcinogenic actions of TCDD. Furthermore, a careful evaluation of the reversibility of preneoplastic lesions is being made within the framework of the experimental model. These studies, by quantifying biochemical parameters important to the carcinogenic process, are attempting to provide information useful to the possible classification of chemical carcinogens according to mechanism of action which would remove some of the uncertainty in risk assessment.

2. Placentas obtained from women in Taiwan who were accidentally exposed to rice oil contaminated with polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans had dramatically elevated concentrations of a specific enzyme system (one isozyme of cytochrome P-450) although analyses were conducted four years after the exposure had occurred. Clinical symptomology characteristic of exposure to toxic halogenated aromatics were present in many of the newborns as well as the mothers. Ongoing studies are evaluating the role of TCDD receptor and EGFR in individual variation in responsiveness to PCBs as well as the polychlorinated dibenzofurans (PCDFs), and we have measured concentrations of PCB and PCDF congeners in placenta and blood. These findings will permit the NIEHS to study mechanisms responsible for individual variation in responsiveness to PCBs and related compounds present in the rice oil which may lead to a rational process for identifying groups at risk to the toxic effects of compounds such as PCBs, PCDFs and TCDD.

Cellular Epidemiology Section

1. Cytogenetic studies have led to the development of an assay that greatly enhances our ability to detect exposure to cigarette smoke and halogenated aromatics including structural analogs of dioxin. Sister chromatid exchange (SCE) frequencies were evaluated in human lymphocytes from smokers or non-smokers following in vitro exposure to α-naphthoflavone (ANF). Although no difference in SCE frequency was detected between smokers and non-smokers in the absence of ANF, in vitro challenge with this chemical produced a large increase in lymphocyte SCE frequency from smokers. This assay allowed us to detect effects from smoking as few as 3-5 cigarettes per day. Studies on the mechanisms responsible for this effect suggested that lymphocytes from smokers possessed enhanced capacity to metabolically activate ANF to a mutagenic compound capable of binding covalently to DNA. In experimental animals, TCDD and structurally-related compounds, like the polychlorinated dibenzofurans (PCDFs), induce the same metabolic pathway as some of the toxic constituents of cigarette smoke. For this reason, lymphocytes from a PCB-PCDF exposed population in Taiwan were examined for cytogenetic damage using our modified SCE assay and revealed that, like the data on smoking, ANF increased SCE frequency in lymphocytes from exposed but not non-exposed individuals. These effects appeared to reflect the presence of PCDFs in exposed individuals. The PCDFs which are extremely toxic were present in small concentrations in the PCB-tainted rice oil. This assay provides a sensitive non-invasive method to monitor human populations for exposure to certain classes of chemicals which are often carcinogenic. Studies are continuing to address the mechanism responsible for the differential SCE effects so that rational predictions can be made concerning its utility for monitoring human exposures to different classes of chemicals and for evaluating dose-response relationships in humans.

2. Pharmacokinetic parameters such as metabolism play a critical role in regulating delivery of biologically-active compounds to the cellular target site. However these parameters do not provide direct information on the amount of chemical that interacts with cellular macromolecules such as DNA, RNA and protein. This interaction is termed the biologically-effective

dose and can be defined as "the concentration of active chemical (parent compound and/or metabolite) at the macromolecular target site which produces a biochemical lesion thereby initiating a sequence of events that ultimately results in the toxic effects characteristic of the chemical being studied". For many chemical carcinogens, DNA-adducts represent a biologically-effective dose and it is felt that the concentration of adducts in target tissues should be a better indicator of outcome as well as dose-response relationships than the administered dose or overall exposure. Postlabelling methods, utilizing ^{32}P-ATP, can provide extremely sensitive techniques to detect DNA adducts and we plan to apply this methodology to detect adducts in human lymphocytes from chemically-exposed populations or following *in vitro* exposure to selected carcinogens.

Molecular Toxicology Section

1. Chemically-induced and spontaneous tumors in rats and mice were examined for the presence of activated oncogenes. High molecular weight DNA was isolated from tumor tissue and transfected into NIH/3T3 mouse fibroblasts. We observed that 10 of 13 spontaneous mouse hepatocellular carcinomas were capable of inducing morphological transformation. Southern blot analysis showed that the transforming property of 8 of these tumors was due to the transfer of an activated cellular homolog of the H-*ras* oncogene. The activation is due to a mutation at the 61st codon. The characterization of the transforming gene in the other two tumors is under investigation. Mouse hepatocellular carcinomas induced by diethylnitrosamine and furfural also contained genes capable of transforming the NIH/3T3 fibroblasts in 9 of 10 and 6 of 7 tumors, respectively, Several other chemically-induced mouse liver tumors are currently being examined for activated oncogenes. We have also examined spontaneous and chemically-induced tumors in the Fischer 344 rat. In contrast to the spontaneous liver tumors in mice, the spontaneous tumors in rats which we examined, did not contain genes capable of transforming NIH/3T3 fibroblasts. The chemically-induced tumors in rat did contain activated oncogenes as detected by the transfection assay including tetranitromethane-induced pulmonary carcinomas, diethylnitrosamine-induced hepatocellular carcinomas, and a variety of 3,3'-dimethoxybenzidine-induced tumors. These and previous results enable us to begin to compare spontaneous and chemically-induced tumors in rodents for the presence of activated oncogenes. These comparisons and an assessment of possible tissue and/or chemical selectivity for the presence of activated oncogenes in rodent tumors may be helpful in the risk assessment of chemicals based on bioassay data in rodents. These data should result in enhanced sensitivity of the bioassay and assist regulatory agencies in evaluating human health hazards of marginal carcinogens.

2. Low dose accumulation of O^6-methylguanine in lung following multiple dose administrations of the tobacco-specific carcinogen 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has been demonstrated. Previous studies have shown that treatment of rats with NNK (100 mg/kg) for 12 days resulted in accumulation of the promutagenic adduct O^6-methylguanine (O^6MG) in lung. The purpose of this study was to determine the distribution of this adduct in specific lung cell populations and the dose response for O^6MG in lung

following multiple dose administrations of NNK. After 1 day of treatment with NNK (100 mg/kg, i.p.), concentrations of O^6MG were greatest in fractions enriched in Clara cells followed by macrophages, Alveolar Type II cells, and unidentified small cells. Treatment of rats for 4 days with NNK increased O^6MG by 3-4 fold in all lung cell populations indicating that the accumulation of this adduct in lung was not cell specific. O^6MG accumulated in lung following treatment of rats for 12 days with doses of NNK ranging from 0.3 to 100 mg/kg/day. The dose response was nonlinear with similar alkylation levels observed following treatment with 0.3, 1, 3, or 10 mg/kg/day. However, alkylation increased significantly in rats receiving 30 or 100 mg/kg/day NNK. The ratio of O^6MG to dose, an index for efficiency of alkylation, increased dramatically as the dose of NNK decreased. These data suggest that both high and low Km pathways exist in lung for the activation of NNK. Moreover, this study demonstrates that low doses of NNK, a carcinogen present in relatively high concentrations in tobacco products and smoke, is efficiently metabolized in lung to a methylating agent resulting in accumulation of the promutagenic adduct O^6MG.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 35005-07 LBRA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Carcinogen-Induced DNA Damage and Repair

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Marshall W. Anderson	Res. Mathematician	LBRA	NIEHS
	Dr. Steve Belinksy	NIH Postdoctoral	LBRA	NIEHS
Others:	Dr. Felix Romagna	Visiting Fellow	LBRA	NIEHS
	Ms. Catherine White	Bio. Lab. Tech.	LBRA	NIEHS
	Ms. Coleen Hunnicutt	Biologist	LBRA	NIEHS
	Dr. Claudia Thompson	Staff Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Dr. Richard Philpot and Ms. Teddy Devereux, Laboratory of Pharmacology
Dr. Jim Swenberg, CIIT

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Molecular Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

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

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

There is compelling evidence that many mutagens and carcinogens are able to react with cellular DNA either directly or following metabolic formation of reactive products. If DNA replication proceeds on such a modified template before altered bases or nucleotides are removed by enzymic repair processes, the mutations can be genetically fixed. Thus, the extent of carcinogen-induced promutagenic DNA damage and the capacity of cells to repair such damage represent critical events in the initiation of carcinogenesis. We are studying the in vivo formation and repair of carcinogen metabolite-DNA adducts in tissues and cells that are susceptible or resistant to carcinogen-induced neoplasia. We are concerned with the effects of dose of carcinogen on the amounts and types of adducts formed and on the subsequent repair of these adducts. Studies with benzo(a)-pyrene (BP) and 4-(N-Methyl-N-Nitrosamino)-1-(3-Pyridyl)-1-Butanone (NNK), a major nitrosamine found in tobacco smoke and products, demonstrate that extrapolation from high to low doses for the estimation of carcinogenic risk requires data on DNA adduct formed for the specific chemical in question. Moreover, data from the target organ may be insufficient for accurate risk assessment. For example, the Clara cell, although accounting for only 1% of the pulmonary cells in the lung of rat, was found to possess a 30-fold higher level of O^6-methylguanine adduct than lung tissue. The Clara cell is the purported progenitor cell for NNK-induced pulmonary neoplasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46003-02 LBRA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Lymphocyte markers for evaluating exposure and biologically-effective dose

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claudia Thompson	Staff Fellow	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	K. Lundgren	Visiting Fellow	LBRA	NIEHS [left 11/85]
	M. Andries	Visiting Fellow	LBRA	NIEHS
	M. Anderson	Res. Mathematician	LBRA	NIEHS
	I. Zajac	Chemist	LBRA	NIEHS
	O. McDaniel	Bio. Lab. Tech.	LBRA	NIEHS
	J. Lambert	Bio. Lab. Tech.	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, BRAP
Preventive Medicine Institute - Strang Clinic
New York City, NY

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

It is the long-range plan of this project to evaluate the relationship between the "biologically-effective dose" for a particular chemical and the effects of prolonged or acute exposure on cellular processes that may be important in tumorigenesis. Animal models and defined human populations exposed to environmental substances will be used to evaluate the quantitative relationship between exposure dose and DNA-adduct levels; to determine the utility of lymphocytes as a molecular dosimeter of environmental exposure; to identify biochemical, molecular and cytogenetic markers to distinguish chemically-exposed individuals from nonexposed; and to evaluate the role of genetic factors in modulating cellular processes that influence the effect of a given exposure. Currently, a procedure has been developed that distinguishes smokers from non-smokers. The mechanism for the differences observed between smokers and non-smokers and other chemically exposed persons (i.e., PCB exposure) will be further evaluated in human lymphocytes as well as in animal and cell-culture models. Methods are being developed to quantitate DNA adducts in exposed individuals by post-labeling. Individuals have been identified that have been exposed to substances of interest and biochemical assays are being developed to determine the relationship of adducts and exposure to cellular processes related to DNA damage and repair.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46004-02 LBRA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Receptor Interactions and Liver Tumor Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karen Nelson	Staff Fellow	LBRA	NIEHS
	George W. Lucier	Chief	LBRA	NIEHS
Others:	Tracy C. Sloop	Biologist	LBRA	NIEHS
	Alison Vickers	NIH Postdoctoral (GW)	LBRA	NIEHS
	Diane B. Campen	Res. Biologist	LBRA	NIEHS
	Geoffrey Sunahara	Visiting Fellow	LBRA	NIEHS
	Tamra Goodrow	Graduate Student (GW)	LBRA	NIEHS
	Zadock McCoy	Bio Lab Tech	LBRA	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina
Laboratory of Pharmacology, NIEHS

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Receptor Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	2.0	4.0

CHECK APPROPRIATE BOX(ES)

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

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

It is the long range plan of this project to evaluate actions of receptors for toxic halogenated aromatics and estrogenically-active chemicals in relation to hepatotoxic potency of these compounds. These studies focus on receptor mediated effects on gene expression critical to tumor promotion using the rat two-stage model for hepatocarcinogenesis. The compounds of special interest are 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), structurally-related polychlorinated dibenzodioxins and dibenzofurans, diethylstilbestrol, 17α-ethinylestradiol and α-zearalanol. The objectives of these studies are to evaluate the quantitative relationships between dose of tumor promoter, receptor interactions, critical changes in gene expression and histopathological alterations including preneoplastic lesions and tumor incidence. Furthermore, the time course of these changes are being investigated.

PHS 6040 (Rev. 1/84)

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46005-02 LBRA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Oncogene Activation and Expression in Rodent Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steve Reynolds	Staff Fellow	LBRA	NIEHS
	Marshall Anderson	Res. Chemist	LBRA	NIEHS
Others:	J. Stowers	Chemist	LBRA	NIEHS
	J. Angerman-Stewart	Biologist	LBRA	NIEHS
	R. Patterson	Microbiologist	LBRA	NIEHS
	Urs Candrian	Visiting Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Dr. Robert Maronpot, National Toxicology Program, NIEHS
Dr. Stuart Aaronson, National Cancer Institute
Dr. Roger Wiseman, University of Wisconsin

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Molecular Toxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

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

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

Recent evidence from two independent lines of investigation has merged to suggest that neoplasia results from the abnormal activation of a relatively small number of cellular genes. Certain retroviruses contain transduced cellular genes which infer transforming properties to the retrovirus. The retroviruses containing these cellular sequences in their genome can induce tumors in animals or transform cells in vitro. Subsequent studies have established that proto-oncogenes can also be activated as oncogenes in naturally occurring tumor cells by mechanisms completely independent of retroviral involvement. These genetic alterations range from point mutations to gross DNA rearrangements such as translocation and gene amplification. We have initiated studies to investigate oncogene activation and expression in spontaneous and chemical-induced tumors in rodents. Results to date have characterized the properties of activated oncogenes in spontaneous tumors as well as some chemically-induced tumors in rats and mice. These studies have provided data which show that oncogene activation in spontaneous tumors is different, in some cases, from that observed in chemically-induced tumors, thereby providing a possible approach to substantially increase sensitivity of rodent bioassays for detection and classification of carcinogens according to mechanism of action. This would enable us to more accurately estimate risk of cancer in humans exposed to specific classes of carcinogens by removing many of the uncertainties inherent in the process when more gross biological endpoints are used.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46006-02 LBRA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Placental Markers of Exposure to Halogenated Aromatics and Cigarette Smoke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	George W. Lucier	Chief	LBRA	NIEHS
Others:	T. Wong [left NIEHS on 12-31-85]	Staff Fellow	LBRA	NIEHS
	G. Sunahara	Visiting Fellow	LBRA	NIEHS
	K. Nelson	Staff Fellow	LBRA	NIEHS
	T. Sloop	Biologist	LBRA	NIEHS
	M. Anderson	Res. Mathematician	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, NIEHS
Laboratory of Pharmacology, NIEHS
Wright State University

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Receptor Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of this project are to evaluate the congener-dependency of some of the biochemical effects observed with human exposure to halogenated aromatics and the role of receptor(s) in mediating the biologic and toxic effects in placental tissue. This information will be critical in determining mechanisms of individual differences in responsiveness to the chlorinated chemicals. The metabolic activation and the role of receptor(s) in mediating the biochemical effects in human placentas are the central focus of this project. Study subjects were pregnant women identified from a registry of individuals accidentally exposed to PCBs, PCDFs, and PCQs; control subjects were age-matched to within three years of exposed subjects. Placental homogenates and microsomes from exposed subjects showed marked elevation of cytochrome P-450 monooxygenase activities when compared with samples from controls. "Western blots" of placental microsomes were found to contain a protein which cross-reacted with antibody raised to rabbit cytochrome form 6, an isozyme induced by polycyclic aromatic hydrocarbons. The elevation of P-450 dependent monooxygenase showed that the biologic effects resulting from this accidental exposure (in Taiwan in 1979) can persist for at least 5 years. No relationship between the biochemical markers studied and blood PCB levels and/or clinical symptoms of exposed subjects is apparent. A consistent finding is that birth weights of offspring were lower for exposed subjects than control subjects. Work is in progress to explore the role of receptors in mediating this metabolic alteration in placentas as well as in determining the persistent PCB and PCDF congeners contributing to these biologic effects.

STATISTICS AND BIOMATHEMATICS BRANCH
Summary Statement

The Statistics and Biomathematics Branch (SBB) plays a major role in BRAP's research efforts directed towards the qualitative and quantitative estimation of adverse health effects resulting from exposure to hazardous environmental agents, and the development of methodology useful in this estimation process. Research conducted within the Branch is concerned with statistical and mathematical issues in studies ranging from experiments at the molecular level to epidemiological investigations of health risk factors or disease etiology. This research can typically be described under the general categories of biostatistical applications, methodology development and mathematical modeling, which are, respectively, the focuses of the different sections into which the SBB is organized. While these sections clearly emphasize different aspects of biostatistical/mathematical research, specific projects may incorporate more than one aspect or research element. In addition, individual research projects are often a collaborative endeavor involving scientists from the Institute's Intramural Research Program and Toxicology Research and Testing Program as well as individuals within BRAP's Epidemiology Branch and Laboratory of Biochemical Risk Analysis.

Biostatistical Applications Section

The primary objective of the Biostatistical Applications Section is to carry out statistical research related to the application of statistical techniques to toxicological and epidemiological problems arising within the Institute's various research programs.

At the present time much of the applications research effort is concentrated in the area of toxicology/carcinogenesis, with special emphasis on the longterm carcinogenicity studies conducted by the Toxicology Research and Testing Program (TRTP). Research projects deal with a variety of issues ranging from evaluating the effectiveness of different experimental design protocols to developing innovative applications of statistical methodology for data analysis. The large data base of carcinogenicity studies generated by the TRTP also provides a unique opportunity to examine critical issues related to the interpretation of study results, such as the estimation of false positive rates, the effect of confounding variables (e.g., corn oil gavage, body weight differences), and the evaluation of tumor onset distributions and sources of variability in tumor incidence rates. These efforts are essential to increase understanding of the various factors that influence the results of carcinogenicity testing in laboratory animals and to enhance the utility of these studies in the overall assessment of human health risk.

Statistical applications research in the area of epidemiological or human studies is directed at gaining additional insight into the uses and limitations of existing study designs and related analysis methodologies, and investigating the adaptation and development of new statistical methods when such a need is indicated. For example, an assessment of some of the techniques that are customarily employed in the analysis of spontaneous abortion data is currently underway to determine whether these techniques adequately deal with the different biases involved in this type of data.

In addition to its research activities, the Biostatistical Applications Section provides general computational and statistical support for various research projects within the Institute. These activities span a spectrum from routine data analysis to more extensive, collaborative efforts that result in joint publications in subject matter journals.

Statistical Methodology Section

The Statistical Methodology Section conducts theoretical and methodological research on statistical topics that are motivated by environmental health issues. The intent is to produce results of broad interest to biometricians, but especially to those involved with human health concerns. The research of this Section may result in the development of entirely new statistical techniques, the improvement of existing statistical procedures, or, to a lesser extent, the adaptation of an existing statistical method to a new area of biological application. The Section also exploits large scientific data bases to aid and validate its research efforts.

An illustration of methodology development research, drawn from genetic toxicology, is the generation of an index of heterogeneity among sister chromatid exchange counts for human lymphocyte cells drawn from a single subject. This index, which has been adopted by a number of other researchers in the field, is particularly sensitive to damage exhibited by a subpopulation of a subject's lymphocytes. Another, epidemiologically motivated example is the formulation of a beta-geometric statistical model for the number of menstrual cycles required to achieve pregnancy by women who are trying to conceive, and the development of related analytical methods. This work grew out of research in fecundity, a topic of interest in its own right as well as a potentially important component in the study of abortion induced by environmental exposures.

The improvement of existing statistical procedures is exemplified by the development of a strategy to test nonparametically for an increasing dose-response relationship when a downturn in response at high doses is possible. The motivation for this research was provided by *in vitro* mutagenicity assays, such as the Ames test, where toxicity of a test chemical may depress an otherwise clearly mutagenic response.

Lastly, the adaptation of existing statistical methodology is illustrated by the demonstration that existing trend tests are more sensitive for the analysis of *in vitro* cytogenetics data than other statistical techniques in current use. As a corollary, it was concluded that a doubling of the number of cells scored per dose point in the current National Toxicology Program protocol for *in vitro* chromosome aberrations is worthy of serious consideration.

Mathematical Modeling Section

The main goal of the Mathematical Modeling Section is to explore the utilization of mathematical theory in addressing problems arising in the biological sciences, particularly in the modeling of biological processes. Much of the current research effort has been motivated by problems in theoretical population genetics and risk assessment.

The development of new technology for studying genetic variation at the molecular level has resulted in a virtual explosion of knowledge about the structure of genetic material of a cell, as well as an exponentially increasing data base of DNA sequences. The statistical methods used to analyze these data are based on population genetic models. Therefore, it is essential that these models be as realistic as possible. One of the primary objectives of the Mathematical Modeling Section in this area has been to incorporate the concepts of mutation and recombination into the models. Estimates of the rate of mutation and recombination are important for both theoretical and practical reasons.

The other main area of investigation is the development of methodology for estimating the risk associated with low levels of exposure to hazardous environmental agents. The fundamental problems with determining such estimates are how to extrapolate high dose results in laboratory animals to low dose exposures, and how to extrapolate low dose risk estimates across species. Low-dose extrapolation is usually based on some underlying model for which, hopefully, there is a biological rationale, whereas species-to-species extrapolation has relied primarily on the use of scaling factors to establish interspecies equivalency. An important goal of the Mathematical Modeling Section is to increase the validity of extrapolation models used for risk estimation by incorporating as much of the current knowledge about the underlying biological processes as possible. Specific issues that are presently under investigation include the evaluation of measures of carcinogenic potency and the exploration of risk assessment models for teratology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 40004-09 SBB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Beth Gladen	Statistician	SBB NIEHS
Clarice Weinberg	Mathematical Statistician	SBB NIEHS

COOPERATING UNITS *(if any)*
Epidemiology Branch, NIEHS

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

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

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

This project encompasses the development of new and the evaluation of both old and new statistical methods appropriate for the types of data arising in epidemiologic research. This year, statistical methods for the analysis of human fertility have been studied. New methods have been developed and evaluated. In the past, fertility has often been studied in a crude fashion by techniques like counting the number of live births a couple has or by measuring the intervals between births. These sorts of techniques are unreliable, because in a country where effective birth control is widely available, the influence of these contraceptives can be so strong as to mask any possible effect of environmental toxins on fertility. Thus, in cooperation with the Epidemiology Branch, we are continuing efforts to develop methods based on analysis of the number of non-contracepting menstrual cycles a couple requires to achieve pregnancy, which is a much more sensitive endpoint for study. We have previously developed a model for such data based on a beta mixture of geometric distributions. We have now investigated the use of such models in three types of designs: a prospective design where couples are followed from the start of their efforts to achieve pregnancy, a retrospective design where women who are pregnant are asked how long it took them to achieve pregnancy, and a cross-sectional design where couples who are currently trying to achieve pregnancy are asked how long they have been trying. The applicability of the beta-geometric model to each of these designs has been investigated. The influence of a possible subgroup of sterile couples in the first and third types of designs has been examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 40005-09 SBB

PERIOD COVERED: October 1, 1985 to September 30, 1986

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

Statistical Methodology and Analysis of Mutagenesis Testing Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barry H. Margolin	Mathematical Statistician	SBB	NIEHS
Ken Risko	Mathematical Statistician	SBB	NIEHS
Susan Murphy	Mathematical Statistician	SBB	NIEHS
Arnold Stromberg	Mathematical Statistician	SBB	NIEHS
Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Cellular Genetics and Toxicology Branch, TRTP

LAB/BRANCH: Statistics and Biomathematics Branch

SECTION:

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.9	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The study of genetic toxicology is receiving substantial scientific attention because of the potential major role of mutagenesis in such diverse human health problems as cancer, aging and birth defects. Research in genetic toxicology is, relatively speaking, still in its infancy. The precise implications for humans of results from these in vitro and in vivo assays are still vaguely understood. Exploration of the mechanistic role of mutagenesis in each individual health concern will be a lengthy and involved process. The need for sound, statistically based evaluation of genetic toxicity data as part of this learning process is recognized by most experts in the field. Responding to this need is, and will continue to be, the primary motivation for this project. Design and analysis of individual assay experiments, together with efforts at assay validation, are areas in which statistical methodology contributions from this project have been notable. Emphasis in project research is shifting from in vitro to in vivo assays; this is, in part, an acknowledgement that the in vivo chemical environment is closer to that of an intact mammal for purposes of risk assessment. Large databases derived from NTP and international collaborative studies continue to provide the empirical foundation upon which new statistical methodologies are constructed and evaluated.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 41001-12 SBB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Risk Assessment Methodology Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

David G. Hoel	Chief	SBB	NIEHS
Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Christopher J. Portier	Mathematical Statistican	SBB	NIEHS
Clarice R. Weinberg	Mathematical Statistican	SBB	NIEHS
Michael D. Hogan	Special Assistant to the Director, BRAP		NIEHS

COOPERATING UNITS *(if any)*

Department of Biostatistics, School of Public Health, University of North Carolina; Laboratory of Reproductive and Developmental Toxicology

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Mathematical Modeling Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is concerned with the development of statistical and mathematical methodology useful in the assessment of risks associated with exposures to potentially hazardous environmental and occupational agents. A major focal point is the generation of improved statistical techniques for estimating adverse human health effects from laboratory animal data, with particular emphasis being placed on dose-response modeling, low-dose extrapolation and extrapolation of toxicologic responses across species. Consideration is also given to the modeling of epidemiologic data in the risk assessment process. Current research efforts are concerned with the continuing evaluation of procedures for estimating carcinogenic potency, with the investigation of factors that appear to affect the correlation between background or natural radiation levels and various types of chronic disease mortality, and with the quantification of human teratogenic risk.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 44002-10 SBB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mathematical Modeling of Molecular Phenomena

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Norman L. Kaplan	Research Mathematician	SBB	NIEHS
Charles H. Langley	Research Chemist	SBB	NIEHS
Richard Hudson	Staff Fellow	SBB	NIEHS
Jotun Hein	Associate Fellow	SBB	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Animal Genetics, LRDT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research project is to develop and study mathematical models for certain biological phenomena at the molecular level. Current work has focused on (a) developing new estimates of the rate of recombination and determining their statistical properties, (b) developing models for the evolution of a family of highly repeated interspersed DNA sequences, (c) developing statistical tests of the neutral model using nucleotide sequence data, (d) studing the genealogic process associated with models for the evolution of a transposable element family, (e) analyzing the statistical properties of stratified sampling schemes when certain prior information is known, and (f) studying problems relating to the alignment of DNA sequences.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 45001-06 SBB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Design and Data Analysis Methodology for Animal Experiments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
Gregg E. Dinse	Senior Staff Fellow	SBB	NIEHS
Walter W. Piegorsch	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*

Department of Biostatistics, University of North Carolina, Chapel Hill, N.C.

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

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

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

This project is concerned with statistical methodology issues involved in the design, analysis, and interpretation of laboratory animal experiments in general and long-term carcinogenicity studies in particular. During the past year, considerable research was devoted to the derivation and utilization of survival-adjusted methods in the assessment of carcinogenic effects. These include consideration of important issues such as tumor lethality, the availability of cause of death information and mathematical modeling of the underlying response variable. The effect of various confounding variables (e.g., corn oil gavage; body weight differences) on the interpretation of carcinogenicity studies was also studied. Other biological/statistical issues that were the focus of research efforts during the past year were the relative merits of site-specific and overall (all sites) tumor incidence analyses, an evaluation of the frequency in which NTP carcinogenicity studies produce only benign neoplasia (i.e., with no supporting evidence of malignancy), and assessing the degree of synergistic effects in carcinogenicity studies in which two compounds are tested singly and in combination.

TOXICOLOGY RESEARCH AND TESTING PROGRAM

TOXICOLOGY RESEARCH AND TESTING PROGRAM
Summary Statement

The Toxicology Research and Testing Program (TRTP), the National Institute of Environmental Health Sciences (NIEHS) component of the National Toxicology Program (NTP), develops scientific information about potentially toxic and hazardous chemicals. [The two other components of the NTP are the National Center for Toxicologic Research (FDA) and the National Institute for Occupational Safety and Health (CDC)]. This toxicology information is used for protecting the health of the American people and for the primary prevention of chemically-induced diseases. TRTP concentrates activities in toxicology research, assessment of toxic potential of chemicals, development/validation/evaluation of toxicology methods and assays, and provides toxicological information to the Government research and regulatory agencies and to the scientific and public communities. Four specific and continuing aims center on:

- Expanding toxicological profiles of the chemicals nominated, selected, and studied.
- Increasing as necessary, and as funds permit, the number and rate of chemicals studied and evaluated toxicologically.
- Developing and validating a series of experimental designs, protocols, and biologic assays appropriate for research and regulatory needs.
- Using a coordinated communications network to collect, evaluate, share, and disseminate toxicological information.

To accomplish these major goals, the program segments are grouped into discipline-oriented yet fully coordinated branches: Carcinogenesis and Toxicologic Evaluation (Dr. J. Selkirk), Cellular and Genetic Toxicology (Dr. R. Tennant), Chemical Pathology (Dr. G. Boorman), Systemic Toxicology (Dr. B. Schwetz), and Data Management and Analysis (Dr. D. Hoel; this branch is part of the Biometry and Risk Assessment Program). In addition to basic and applied toxicologic research activities, these functions include scientific and technical oversight and monitoring of collaborating and contract laboratories. Each of these discipline areas and their accomplishments are described separately in the sections that follow this overview.

Individual NTP scientists serve as group or discipline leader for particular program areas or special projects and are responsible for developing (in collaboration with other NTP colleagues) the subprogram objectives and the implementation plans, as well as the coordination and supervision of the program work. Further, the program leaders are responsible for the development and supervision of contracts that extend these activities or that perform in-depth toxicologic characterization of chemicals.

The strategy for assay and protocol development and validation examines existing and emerging methodologies to identify those that may be adequately sensitive and reproducible. Those found to offer improvement over older methods are selected for further investigation and perhaps validation. When basic research findings suggest new areas of toxicology studies, TRTP undertakes the appropriate methods development and validation.

- For methods development and validation efforts, emphasis is given to in vivo mammalian and human somatic cell assay development including: (1) mouse bone marrow cytogenetics; (2) evaluation of the mouse erythrocyte micronucleus assay; and (3) protocol development and data collection assessing frequencies of chromosome aberrations and sister chromatid exchanges in human lymphocytes. Progress has been made in the use of in vitro and in vivo model systems to identify and characterize potential germ cell mutagens. A significant observation was that dose-rate effects of ethylene oxide on dominant lethal induction in rodents may carry implications for human exposure.

- A major effort this past year has been focused on an objective evaluation of the most prominent methodologies for using short term in vitro and in vivo systems that measure interaction with or damage to DNA by chemicals. Included are methods to measure mutagenicity in Salmonella, in rodent cells, and in Drosophila; chromosomal effects (aberrations, sister chromatid exchanges, aneuploidy), and DNA damage and neoplastic changes in eukaryotic cells. The major portion of the evaluation effort has been the development of reproducible results in the systems on a number of chemicals for which carcinogenicity and noncarcinogenicity in rodents have been determined. The long-term toxicity studies conducted by the National Toxicology Program in rodents represent one of the few sources worldwide where diverse chemicals have been studied at sufficient concentrations and for sufficient duration to be classified as not demonstrating evidence of carcinogenicity. Therefore, 73 of these chemicals have been selected for evaluation of effects on DNA based on having been adequately studied in rodents. A wide range of chemical structures and tumorigenicity patterns are represented as are a number of chemicals giving no evidence of carcinogenicity. All laboratories used protocols established to insure reproducibility of results. The data derived from these systems are currently being compiled and evaluated. Key questions that are being addressed include the relationship between the various endpoints of genetic toxicity and tumorigenicity patterns in rodents including tumor type, sex-species pattern, and relative potencies. It is anticipated that further detailed evaluation of these results will result in a more objective understanding of the advantages and limitations of genetic toxicity data. Further this evaluation will lead to development of strategies for the optimal use of genetic toxicity systems in the selection of chemicals for long-term toxicology studies in rodents.

- Efforts continue to refine rat liver tumor models for evaluating and interpreting the initiation and/or promotion mechanisms of chemicals known to be hepatocarcinogenic in rodents. Results suggest that the F344 rat is relatively insensitive to liver carcinogens and appears to be less responsive than the Sprague-Dawley rat.

- Major emphasis remains on designing broadened yet specifically tailored protocols for each chemical in the prechronic phases (usually toxicology studies of six months or less in duration) to include several select genetic toxicity assays, chemical disposition studies, measures of alterations in reproduction and fertility, and other target organ effects as well as clinical and morphological pathology.

- Nineteen draft Technical Reports on toxicology and carcinogenesis studies were presented for public review by the Board of Scientific Counselors' Peer Review Panel. Chemicals exhibiting clear evidence of carcinogenicity in rats

or mice in these studies included:
Dimethylvinyl chloride (a byproduct of pesticide manufacture)
1,4-Dichlorobenzene (an insect fumigant and deodorant)
Bromodichloromethane (a byproduct of water chlorination)
Ethylene Oxide (a sterilant)
Methyl Carbamate (a pesticide)

- Following the accidental release of methyl isocyanate (MIC) in Bhopal, India, the Department of State and the World Health Organization requested that the NTP examine in rodents both the immediate and long-term health effects from exposures to MIC similar to the burst exposures humans received in Bhopal. Animal studies were initiated in March of 1985. Rats and mice of both sexes were exposed to various vapor concentrations of MIC for either two hours on one occasion, or for six hours on four consecutive days. Exposure to MIC caused extensive injury to respiratory tissues resulting in deaths and persistent anatomical and functional lung changes.

- Ongoing initiatives include studies to explore the possible relationship of dietary oil or oils used as vehicles for oral studies and pancreatic proliferative lesions in male rats. Studies are being designed to assess the influence of various levels of dietary restriction on the occurrence of non-neoplastic (toxic) and neoplastic (carcinogenic) lesions in control animals. Continuing were short-term and long-term studies on the toxicology and carcinogenicity of chemicals and substances in foods, the environment, and the workplace.

- Studies are continuing on the evaluation of microencapsulation as a means to administer volatile, reactive, or unstable chemicals in feed. Microencapsulation is a process for enveloping solid particles or liquid droplets in a protective coating that separates the chemical from its environment. Feeding studies using microencapsulated trichloroethylene, 1,1,1-trichloroethane, cirtal, and 2-ethylhexanol are underway.

- Oncogene activation is being investigated by DNA transfection techniques in neoplasms taken from the B6C3F$_1$ mouse and Fischer 344/N rat. In rats there appears to be different oncogene expression between control and chemically induced tumors. Further studies are being done utilizing these rodent tumors from the long-term carcinogenicity studies.

- Nuclear magnetic resonance (NMR) imaging permits detailed examination of internal organs and structures based upon the property of certain atomic nuclei to align in an external magnetic field and to resonate (absorb and emit incident radiofrequency energy). High resolution pictures or images of biologic tissues can be produced. In addition to normal anatomic detail, various pathologic processes can be detected using NMR imaging. The ability to detect neoplasms and other lesions is an area of active collaborative research for TRTP, IRP and Duke University. The initial focus of research between TRTP and Duke University has been to refine experimental techniques, NMR equipment, imaging software and data handling to permit long-term studies involving small laboratory animals. Small hepatic and pituitary neoplasms in rats, microanatomic detail of the rat brain, kidney and liver and microscopic features of the developing chick embryo are examples of recent efforts. A unique aspect of this research is the ability to follow the growth or the disappearance of an abnormality (for example, a tumor) in the same animal as exposure is continued or altered. Potential benefits from this work include

the ability to detect the effects of treatment with a toxic compound at an early stage, to examine the biologic behavior of an induced abnormality throughout the course of an experiment, and to reduce the number of animals needed to define the effects of chemical exposure.

- Publications: during the last 18 months, TRTP staff members published 153 journal articles, 28 books or book chapters, and presented 208 abstracts at national meetings.

- A major effort on Good Laboratory Practice/Quality Assurance audits of experimental data continues. Four additional audit teams have been added which enable the TRTP to conduct more intensive GLP and in-life data audits. All toxicology and carcinogenesis studies receive an indepth examination of the chemistry, toxicology, and pathology records and data before TRTP/NIEHS draft technical reports are presented for public review to the NTP Board of Scientific Counselors Peer Review Panel. All studies except those from a single laboratory have been shown during data audits to support the data collected and evaluated in Draft Technical Reports. An inhouse Quality Assurance unit has been established at NIEHS to provide a program for tracking, inspecting, and auditing of all studies conducted by the TRTP.

- A conference jointly sponsored by industry and NIEHS was held November 18-21, 1985, on Managing the Conduct and Data Quality of Toxicology Studies. The Proceedings have been published, and the meeting provided a unique opportunity for participants to exchange current practices, concepts, and new ideas for managing the quality of toxicology studies which have a regulatory impact on both national and international levels.

- A pre-quality assurance review of pathology data was instituted to reduce the time, effort, and cost of this multistep process. Pathology data from the laboratories is now reviewed prior to submission of the wet tissues, paraffin blocks, and slides for histological review. If any discrepancies or inappropriate terminology is found, the data and findings are returned to the laboratory to make corrections. This has placed the responsibility for producing quality data more firmly upon the laboratory and has resulted in better quality data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21064-04 TRTP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Bioavailability of TCDD in Missouri Soil

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.E. McConnell			
OTHERS:	M.W. Harris	Biological Laboratory Technician	TRTP	NIEHS
	J.D. Allen	Biological Laboratory Technician	TRTP	NIEHS
	E. Haskins	Biological Laboratory Technician	TRTP	NIEHS

COOPERATING UNITS *(if any)*

Environmental Protection Agency
Laboratory of Molecular Biophysics, NIEHS

LAB/BRANCH

Office of the Director for Toxicology Research and Testing Program

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

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

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

TCDD (Dioxin) contaminated soil from two sites in Missouri is being investigated to determine the bioavailability in soil. Guinea pigs are being used in the investigation. Results suggest that there is high bioavailability of TCDD in dirt after ingestion. Studies on bioavailability via the skin are in progress in a second species.

Studies finished.

PHS 6040 (Rev. 1/84) GPO 914-918

CARCINOGENESIS AND TOXICOLOGY EVALUATION BRANCH
Summary Statement

The Carcinogenesis and Toxicology Evaluation Branch (CTEB) of the NIEHS conducts applied research studies designed to detect and characterize the toxic potentials of chemical agents. Although most of these studies are conducted in rodent species or other experimental model systems, the results are relevant to and are a major factor in estimating the toxic potentials of chemicals to humans. In addition to conducting chemical toxicity and carcinogenicity studies, the CTEB collaborates with other scientific staff at the Institute in developing and validating alternatives to standard toxicity tests, and conducts applied biological research in chemical toxicity.

The major effort of the CTEB staff during FY 1985 was the design, conduct, monitoring, evaluation, and reporting of toxicity studies completed off-site by contractual mechanisms. These studies encompass both prechronic (acute, short-term repeated-exposure, subchronic) and chronic (up to lifetime exposure) whole-animal tests employing morphological, clinical, functional and biochemical endpoints. Although many of these studies follow a standard protocol with regards to frequency of exposure and range of endpoints, each individual protocol is carefully tailored to the properties of the test chemical and the needs of the requesting agency.

The Collaborative Resources Group provides the essential aspects for the toxicology and carcinogenesis studies conducted by the National Toxicology Program, namely Analytical Chemistry and Chemical Health and Safety. Group staff procure, analyze and monitor chemicals for these studies. The chemical health and safety office monitors each study laboratory and each study within a facility for those factors which may adversely affect the proper research and study environment. Each resource is provided by in-house effort and supplemented by resource contracts.

The Group maintains a repository for over 1100 chemical compounds which are currently under study or which have completed studies in the various NTP programs.

The chemical health and safety aspects of the NTP are also the responsibility of the Collaborative Resources Group. Involvement consists of initial laboratory evaluation, follow-up site visits, program reviews, report monitoring, recommended changes in procedures, facilities design, etc., as well as response to problem emergency situations and concerns with eventual waste disposal and record archiving.

Extramural Research Highlights: Extramural research activities are conducted via contract mechanisms.

- The toxicity and carcinogenicity testing of approximately 250 chemicals is being performed under the NTP Master Agreement. This number includes chemicals in any of the stages from design through report preparation (CTEB staff).

- The Branch procured or synthesized and completely analyzed 88 chemicals for the general in vivo chronic toxicology studies. In addition, 38 chemicals were obtained and analyzed for other programs within the National Toxicology

Program such as teratology studies, immunotoxicology studies, reproductive toxicology studies, continuous breeding experiments, rat liver tumor model studies and in-house TRTP studies. Services were provided for the analysis of bulk chemical, chemical in test vehicles, methods development for quality assurance, including purity, stability (both bulk chemical and chemical/vehicle mixtures) and concentration determinations, chemical residue analysis of body tissues and fluids and special handling for residual and reprocured chemicals. In addition, tissue and body fluid residue analyses were developed and performed to enhance data from toxicity experiments of seven chemicals. Microencapsulation of study materials is actively being investigated as an alternative for administration of study chemicals in the feed rather than gavage of the neat chemical. Support was also provided to the Cellular and Genetic Toxicology Program by analyzing over 300 chemicals for purity and identity. The Chemistry Group provided staff support for data auditing activities of the TRTP. This included review of completed studies to assure the chemistry performed in support of these studies was adequate and accurately reported. The chemistry group was an active participant in the organization and running of the Symposium for Managing Conduct and Data Quality of Toxicology Studies held in FY 86.

- The technical effort of encapsulating volatile and/or reactive test chemicals in non-toxic microcapsules is underway. Microencapsulation will allow such chemicals to be administered experimentally as feed admixtures instead of by gavage (Dr. Jameson).

- Early results from studies of salicylazosulfapyridine, (SASP), a drug used in treatment of ulcerative colitis, indicate that this compound causes dramatic, dose-dependent morphological and functional changes in the pituitary and thyroid of rats. This is of interest because a metabolite of SASP is a structural analog to a compound known to cause metastasizing thyroid tumors. Results obtained by application of circulating hormone analysis combined with quantitative histochemistry are consistent with the interpretation that SASP or one of its metabolites causes a functional thyroid hormone deficiency thereby reducing the suppressive action of thyroxine on TSH production by the pituitary gland. These observations support the purported role of derangements in the pituitary-thyroid axis in the development of thyroid neoplasms (Dr. Kari).

- A study is being conducted to compare the sensitivities of carcinogenicity test models using post-weaning lifetime exposure only to those using in utero, perinatal, and post-weaning lifetime exposure. The test chemicals are phenytoin (diphenylhydantoin), ethylenethiourea, and polybrominated biphenyls (Firemaster FF-1®)(Dr. Chhabra).

- Under an interagency agreement with the EPA, the NTP is identifying and conducting the appropriate research and testing to eliminate "data gaps" for chemicals commonly found in waste disposal sites (Dr. Yang).

- Benzethonium Chloride is used in a wide variety of compounds as a germicide and disinfectant with major applications in pharmaceuticals and cosmetics. Consumer exposure is estimated to be 3,800 kg (about 8360 lbs) and skin is the usual route of contact. Fourteen-day and 90-day prechronic studies by skin paint have been completed and 2-year studies are planned by the same route of exposure. (Dr. Eastin)

- Thirteen-week toxicity studies with d-alpha-ticopheryl acetate (Vitamin E) in Fischer 344 rats and Sprague-Dawley rats were completed by NTP. Studies showed that Vitamin E at gavage doses ranging from 125 mg to 2000 mg/kg body weight caused hemorrhagic diathesis and interstitial inflammation and adenomatous hyperplasia of the lung. Lung lesions associated with the administration of excess amounts of this vitamin have not been reported previously. Additional studies are being designed to investigate the influence of diet (natural vs purified) and route of administration (corn oil gavage vs dosed feed) on the development of Vitamin E induced lung lesions. (Dr. Abdo)

- 8-Methoxypsoralen (8-MOP) is used in the treatment of vitiligo and psoriasis. The NTP is conducting a toxicity and carcinogenicity study of 8-MOP in F344/N rat. The toxic properties of 8-MOP followed by UV irradiation, in a regimen designed to mimic PUVA therapy in humans, are being studied in the hairless mouse (HRA/SKh strain). (Dr. Dunnick)

- We are currently preparing to report results of toxicity and carcinogenicity studies on two diuretics of major economic and therapeutic importance, furosemide and hydrochlorothiazide. Two-year studies on sodium fluoride, a chemical widely used in water fluoridation, are also underway. (Dr. Bucher)

Intramural Research Highlights: Intramural research is conducted at the NIEHS by CTEB staff and collaborating scientists.

Evaluation of microencapsulation as a means to administer chemicals in feed: Microencapsulation is a process for completely enveloping tiny masses of solid particles, or liquid droplets in a protective coating which separates the substance from its environment. Feeding studies using microencapsulated trichloroethylene, 1,1,1-trichloroethane, citral and 2-ethylhexanol are planned. (Dr. Jameson)

Mechanisms of phthalate ester toxicities in mammalian species: Phthalate esters are plasticizers incorporated into nearly all plastic materials. The biochemical and ultrastructural effects of di(2-ethylhexyl)phthalate (DEHP) and related chemicals are being studied in order to assess potential mechanisms of phthalate ester toxicity. (Dr. Melnick)

Inhalation toxicity studies on methyl isocyanate in rats and mice: Following the release of methyl isocyanate (MIC) from the Union Carbide plant in Bhopal, India, and the subsequent deaths of between 2 and 4,000 people, the Department of State, and the World Health Organization requested that the NTP examine the long-term health effects of short exposures to MIC. Animal studies were initiated in March of 1985 with exposure to MIC vapors. Rats and mice of both sexes were exposed to various concentrations of MIC for either two hours on one occasion, or for six hours on four consecutive days. Exposure to MIC caused extensive injury to respiratory tissues resulting in deaths and persistent anatomical and functional lung changes. (Dr. Bucher)

Cellular biochemistry studies on chemicals selected for evaluation by NTP: Mechanisms of cellular immunotoxicity were studied in rats or mice exposed to mercuric chloride, nickel sulfate or titanocene dichloride. Tissue accumulation of these metals in target organs indicated the sensitivity of the biochemical assays to evaluate target organ toxicity often preceeded gross, microscopic or clinical methods. (Dr. Dieter)

Bioavailability and toxicity studies of microencapsulated chemicals: Trichloroethylene (TCE) and 2,6-xylidine, two volatile chemicals, have been separately encapsulated in gelatin-sorbitol microcapsules. These formulations have been shown to provide sufficient stability to the chemicals to be useful in dosed-feed toxicology studies. (Dr. Melnick)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21050-03 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Evaluation of Microencapsulation As A Means to Administer Chemicals in Feed

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. W. Jameson	Head, Collaborative Resources Group	TRTP/CTEB	NIEHS
Others:	T. J. Goehl	Chemist	TRTP/CTEB	NIEHS
	R. L. Melnick	Head, Experimental Toxicology Unit	TRTP/CTEB	NIEHS
	A. Greenwell	Technician	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

Collaborative Resources Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Microencapsulation is a process for completely enveloping tiny masses of solid particles, or liquid droplets in a protective coating which separates the substance from its environment. The use of microencapsulated chemicals for toxicology studies presents a number of advantages, i.e. it permits testing volatile or chemically reactive compounds in the animal diet, minimizes problems with palatability, etc. Volatile and/or reactive chemicals have been encapsulated using a gelatin or gelatin/sorbitol matrix and determined to be stable when mixed with rodent feed. Relative bioavailability of the microencapsulated trichloroethylene compared to the neat test material indicates no significant difference in absorption after oral administration. Feeding studies using the microencapsulated trichloroethylene, 1,1,1-trichloroethane, citral and 2-ethylhexanol are planned.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21062-04 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanisms of Phthalate Ester Toxicities in Mammalian Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	TRTP/CTEB	NIEHS
Others:	Robert R. Maronpot	Pathologist	TRTP/CPB	NIEHS
	Scott Eustis	Pathologist	TRTP/CPB	NIEHS
	Elmer J. Rauckman	Expert	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

Carcinogenesis and Toxicology Evaluation Branch

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.375	.865	.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Phthalate esters are plasticizers incorporated into nearly all plastic materials. The biochemical and ultrastructural effects of di(2-ethylhexyl)phthalate (DEHP) and related chemicals are being studied to assess potential mechanisms of phthalate ester toxicity.

Since DEHP and other phthalates are also male chemosterilants and teratogenic in mice, studies are being conducted to determine the role of zinc in the pathophysiology of these reproductive effects and to discern no-observed toxic effect levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21067-02 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Inhalation Toxicity Studies on Methyl Isocyanate in Rats and Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John R. Bucher	Chemist	TRTP/CTEB	NIEHS
Others:	B.A. Schwetz	Chief	TRTP/STB	NIEHS
	E.E. McConnell	Director, TRTP	TRTP	NIEHS
	M.D. Shelby	Head, Mammalian Mutagenesis	TRTP/CGTB	NIEHS
	M. Luster	Head, Immunotoxicology	TRTP/STB	NIEHS
	B. Gupta	Staff Pathologist	TRTP/CPB	NIEHS
	C. Jameson	Head, Collaborative Resources	TRTP/CTEB	NIEHS
	C. Richter	Chief, Comparative Medicine Branch	CMB	NIEHS

COOPERATING UNITS *(if any)*

Pulmonary Physiology Testing Laboratory, EPA

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Following the release of methyl isocyanate (MIC) from the Union Carbide plant in Bhopal, India, and the subsequent deaths of between 2 and 4,000 people, the Department of State, and the World Health Organization requested that the NTP examine the long term health effects of short exposures to MIC. In response, the NTP and NIEHS assembled staff with expertise in inhalation, reproductive, immuno-, genetic and general toxicology, along with staff experienced in pulmonary and general pathology, to design and implement studies which would provide a data base on this chemical. Animal studies were initiated in March of 1985 with exposure to MIC vapors accomplished in the Building 14 inhalation facility by personel employed by Northrop, under contract NO1-ES-4-5044. Rats and mice of both sexes were exposed to various concentrations of MIC for either two hours on one occasion, or for six hours on four consecutive days. Studies of complete animal histopathology were performed immediately following the exposures and at periodic intervals during the subsequent 90 days. Pulmonary effects were examined by light and electron microscopy, and were correlated with results of pulmonary function tests performed by the Pulmonary Physiology Testing Laboratory at EPA. Reproductive effects were examined by mating trials, and by evaluation of offspring from late term pregnant mice exposed on gestation days 14-17. Evaluation of immunotoxicity included tests of humoral and cell mediated immunity. Genetic toxicity evaluations included a variety of in vitro assays, cytogenetic assays in vivo, and determinations of micronuclei, and dominant lethal assays in exposed mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21076-03 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cellular Biochemistry Studies on Chemicals Selected for Evaluation by NTP

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael P. Dieter	Physiologist	TRTP/CTEB	NIEHS
Others:	William Jameson	Chemist	TRTP/CTEB	NIEHS
	Gary A. Boorman	Pathologist	TRTP/CPB	NIEHS
	Michael I. Luster	Immunologist	TRTP/STB	NIEHS
	Linda S. Birnbaum	Pharmacologist	TRTP/STB	NIEHS
	John E. French	Physiologist	TRTP/CTEB	NIEHS
	Robert R. Maronpot	Pathologist	TRTP/CPB	NIEHS

COOPERATING UNITS *(if any)*

Systemic Toxicology Branch, TRTP
Chemical Pathology Branch, TRTP
Program Resources Branch, TRTP

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The effect of inorganic or organic metals and metal complexes is of particular interest to the NTP because of their prevalence in drinking water and industrial processes, use as constituents in anticancer drugs, and their diverse target organ toxicities.

Mechanisms of cellular immunotoxicity were studied in rats or mice exposed to mercuric chloride, nickel sulfate or titanocene dichloride. Tissue accumulation of these metals in target organs indicated the sensitivity of the biochemical assays to evaluate target organ toxicity which often preceeded gross, microscopic or clinical methods. Development of animal models to study Fischer rat leukemia revealed tumor markers for this disease that can be used to distinguish between age-induced and chemically-enhanced leukemogenesis. Urinary enzyme responses to nephrotoxic chemical insult were evaluated to use as a model to predict renal toxicity in chronic studies. Enzymatic method development to enhance stability, sensitivity, and efficiency of 7-ethoxycoumarin-ortho-demethylase was initiated to evaluate MFO-initiating activity of chemicals in minute amounts of rodent tissue.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21078-03 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Bioavailability and Toxicity Studies of Microencapsulated Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	TRTP/CTEB	NIEHS
Others:	C.W. Jameson	Chemist	TRTP/CTEB	NIEHS
	T. Goehl	Chemist	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

Midwest Research Institute, Kansas City, MO
Program Resources Branch, TRTP

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

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

Trichloroethylene (TCE) and 2,6-xylidine, two volatile chemicals, have been separately encapsulated in gelatin-sorbitol microcapsules. These formulations have been shown to provide sufficient stability to the chemicals so that they may be useful in dosed-feed toxicology studies. The objectives of this project are to compare the rates and extents of absorption of neat and microencapsulated chemicals in rats and mice, and to evaluate the feasibility of using micro-encapsulation as a means of incorporating unstable test chemicals into rodent feed for toxicology studies. The rates and extents of absorption of TCE, prepared either as a suspension of microencapsulated TCE in corn oil or as a solution of neat TCE in corn oil, administered by gavage to male Fischer 344 rats have been studied. The toxicity of microencapsulated TCE in rats has also been studied. A bioavailability study of microencapsulated 2-ethylhexanol is being developed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21079-03 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanism of Di(2-ethylhexyl)phthalate Hepatotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	TRTP/CTEB	NIEHS
Other:	K. Tomaszewski	Visiting Fellow	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In a two-year toxicology/carcinogenicity study conducted by the National Toxicology Program, di(2-ethylhexyl)phthalate (DEHP) was found to be hepatocarcinogenic in B6C3F_1 mice and F334 rats. Since DEHP also causes peroxisome proliferation, it has been suggested that the carcinogenicity of this chemical may be related to excessive peroxisomal production of H_2O_2. It is the objective of this project to examine the changes in H_2O_2 concentrations resulting from peroxisomal fatty acyl-CoA oxidation and catalase activity in livers of rats and mice treated with DEHP. Further assessment of an involvement of reactive intermediates of oxygen reduction in DEHP induced hepatotoxicity will be made from measurements of (a) activities of enzymes that eliminate toxic oxygen products (catalase, superoxide dismutase, glutathione peroxidase), (b) lipid peroxidation, and (c) superoxide anion radical production. New studies have begun to characterize the chemical requirements for induction of peroxisome proliferation in isolated rat hepatocytes.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30100-07 CTEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Toxic Effects of 1,2-Dibromo-3-chloropropane on the Urogenital System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Melnick	Chemist	TRTP/CTEB	NIEHS
Others:	Arnold Greenwell	Biologist	TRTP/CTEB	NIEHS
	Frank Harrington	Bio. Lab. Tech.	TRTP/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.25	.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The acute and subchronic toxic effects of the pesticide 1,2-dibromo-3-chloropropane (DBCP) and structurally-related compounds are studied from functional and mechanistic viewpoints. A reported chemo-sterilant in humans, DBCP is no longer manufactured in the U.S., but its presence in ground water and on edible imports and its illegal bulk transport into certain areas of the U.S. require its further toxicological characterization. Effects of DBCP on hepatic, renal, and reproductive functions and development are evaluated at several dose levels, after various treatment regimens and under differing conditions such as age, chemical or physical stress and the like.

The mechanism of DBCP inhibition of energy metabolism in sperm cells is being investigated.

GPO 914-918

CELLULAR AND GENETIC TOXICOLOGY BRANCH
Summary Statement

The field of Genetic Toxicology has evolved to deal with potential chemical hazards in a prospective manner. The discipline is predicated on the use of short-term in vitro and in vivo systems that can measure potential interaction with or damage to DNA by chemicals. A major portion of the effort the Cellular and Genetic Toxicology Branch over the past year has been focused on an objective evaluation of the most prominent methodologies. Included in this effort are methods to measure mutagenicity in Salmonella, rodent (L5178Y) cells and in Drosophila; chromosomal effects (aberrations, sister chromatid exchanges (SCEs) aneuploidy), DNA damage and neoplastic changes in eukaryotic cells. The major portion of the evaluation effort has been the development of reproducible test results in the above systems on a substantial number of chemicals for which rodent carcinogenicity and noncarcinogenicity have been defined. The chronic toxicity studies conducted in rodents by the National Toxicology Program represent one of the few sources worldwide where a number of chemicals have been studied in rodents at sufficient concentrations and for sufficient duration to be defined as not demonstrating evidence of carcinogenicity. Therefore, the Cellular and Genetic Toxicology Branch utilized approximately 73 chemicals that have been evaluated for tumorigenicity under the aegis of the National Toxicology Program. These chemicals were chosen only on the basis of having been adequately studied in rodents. A wide range of chemical structures and tumorigenicity patterns are represented as are a number of chemicals giving no evidence of carcinogenicity. Aliquots of these chemicals were sent from the chemical repository under code to contract laboratories which conducted the assays and provided results prior to decoding. All laboratories used protocols established to insure reproducibility of results. The data derived from these systems are currently being compiled and evaluated in a data management system. Key questions that are being addressed include the relationship between the various endpoints of genetic toxicity and multiple parameters of tumorgenicity including tumor type, sex/species pattern and relative potencies. It is anticipated that further detailed evaluation of these results will result in a much clearer and objective understanding of the uses and limitations of genetic toxicity data. It is also anticipated that this evaluation will lead to development of strategies for the optimal use of genetic toxicity systems in selection and prioritization of chemicals for chronic toxicity studies in rodents.

Progress has been made also in the use of in vitro and in vivo model systems to identify and characterize potential germ cell mutagens. A significant observation was that dose-rate effects of ethylene oxide on dominant lethal induction in rodents may carry implications for human exposure.

The intramural research efforts of the Cellular and Genetic Toxicology Branch continue to emphasize the role of DNA repair mechanisms in meiosis and identification of meiosis-specific gene functions in Saccharomyces cerevisiae, the genetic control of DNA repair and mutagenesis in Drosophila melanogaster; comparative organ and species specificity of chemical carcinogen metabolism in human and rodent cells in vitro; the regulation of retrotransposition in mammalian cells; the mechanism of bisulfite mutagenesis in Salmonella typhimurium; and peroxidase enzyme metabolism of mutagens in mouse lymphoma (L5178Y) cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21012-05 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Organ and Species Differences in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Langenbach	Microbiologist	CGTB NIEHS
Others:	K. Rudo	Biologist	CGTB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

Carcinogen Metabolism

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

An in vitro approach for studying human tissue metabolism and mutagenic activation of chemical carcinogens has been developed. Freshly isolated, intact cells from humans as well as rodents are used for metabolic activation. Reversion of S. typhimurium is used to assess biological activity of metabolites and HPLC analysis of metabolites have been conducted. Work during the past year has centered on developing a system for frequent human tissue acquisition. A human system has been developed to investigate differences between individual humans and differences between human and rodent liver, kidney and colon cell activation/ metabolism of aromatic amines. Metabolism, mutagenic activation and inter-individual variation has been measured and significant differences found between individual humans for these tissues. Comparisons of these human tissues to the respective rodent tissues are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21013-05 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. R. Boone	Senior Staff Fellow	CGTB	NIEHS
Others:	R. W. Tennant	Supervisory Microbiologist	CGTB	NIEHS
	P. L. Glover	Biological Lab Technician	CGTB	NIEHS
	C. L. Innes	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Wen K. Yang Biology Division, ORNL

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.2	1.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

During the past year we have initiated an investigation into the regulation of retrotransposition in mammalian cells. The experimental model is the mouse Fv-1 gene which dominantly restricts certain preintegration steps and possibly the integration step involved in the replication cycle of retroviruses. Retrovirus vectors with selectable markers but no viral structural genes are used in these experiments. These constructs are not capable of replication and are transferred to recipient cells by packaging into retrovirus virions. Colony formation of mouse fibroblasts in the presence of the antibiotic G418 is the basis of our assay system. This is superior in many ways to assay systems involving replication competent retroviruses. By rescue of the marker neo resistance with molecularly cloned retrovirus with known sensitivities to various Fv-1 alleles, we have established that the integration of the retrovirus vector is subject to restriction by the Fv-1 gene. Our results indicate that non Fv-1 related differences in sensitivity to retrovirus infection of certain cell lines may be due to the traditional assay system (XC plaque assay) and are less of a problem with the G418 resistant colony assay. Recombinant DNA constructs have been prepared to create helper virus free packaging cell lines with N-, B-, and NR- tropism. This will allow us to analyze the role of two hit kinetics and abrogation in the mechanism of retrotransposon restriction by the Fv-1 gene product.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21016-05 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Enzymes Involved in DNA Repair and Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Resnick	Supv. Research Geneticist	CGTB	NIEHS
Terry Chow	Visiting Associate	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The RAD52 gene in Saccharomyces cerevisiae controls the repair of ionizing radiation-induced DNA double-strand breaks, radiation-induced spontaneous mitotic recombination, and recombination during meiosis. Utilizing an antibody raised against a Neurospora crassa deoxyribonuclease, we had shown that an antigenically related enzyme could be identified from yeast and that this enzyme is controlled by the RAD52 gene. Using a λgt11 vector expression library that contains genomic yeast DNA and the antibody as a probe, we have identified a segment of DNA that codes for cross-reacting material. A sequence from a yeast genomic library has been identified that hybridizes with this segment. Introduction of this into a Rad+ strain leads to enhanced synthesis of cross-reacting material. Since this sequence contains the putative gene for the RAD52 controlled nuclease, the role of the gene in recombination, repair and normal growth will be determined by inactivating the corresponding gene in the genome and determining the consequences. In the process, it will also be possible to map the gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21032-02 CGTB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Peroxidase Oxidation Systems in Mutation Assays

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Caspary	Biochemist	CGTB NIEHS
Others:	D. Daston	Biologist	CGTB NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Molecular Biophysics, NIEHS

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION
Chemical Mutagenesis

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

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms of metabolism other than those mediated by the mixed function oxidases may be important in activating certain chemicals to their ultimate carcinogenic form. Recent evidence suggests that benzidine dyes are cleaved by intestinal bacteria, thereby liberating the parent benzidine and its congeners. There is evidence implicating peroxidases in the metabolism of benzidine dyes.

Prostaglandin H synthetase is being used to activate compounds in the mouse lymphoma forward mutation assay. Modifications of the standard assay are necessary due to a high level of toxicity in this sytem, and because confounding reactions with the media during treatment have been observed. In addition, various substrates are being utilized. The possible mechanisms responsible for the formation of mutagenic metabolites induced by various peroxidase enzyme systems are being investigated. Selective inhibitors of the peroxidase are used to aid in the elucidation of these mechanisms. Studies include the identification of metabolites induced by this activation system.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21035-02 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Structural Analysis of Meiotic Chromosome Behavior in Yeast and the Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Craig N. Giroux	Senior Staff Fellow	CGTB	NIEHS
Michael Dresser	NRC Biotechnology Associate (from 4/1/86)	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Montrose Moses, Duke University, Durham, North Carolina

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The focus of this project is to investigate at the molecular level the structural basis of meiotic chromosome metabolism and segregation in the yeast, Saccharomyces cerevisiae, and to compare it to that of the mouse and related mammalian species. Methods of isolation and identification by light and electron microscopy are being developed for meiosis specific structures in yeast based on surface spreading techniques combined with immunofluorescence. Whole-mount preparations have been used to demonstrate well-preserved synaptonemal complexes in preparations of yeast meiotic nuclei, as visualized both by light and electron microscopy. Additionally, these methods combined with anti-tubulin antibody staining have allowed the stages of chromosome movement along the meiotic spindle to be elucidated. Two monoclonal antibodies against the mouse synaptonemal complex, isolated by Dresser and Moses, appear in preliminary experiments to recognize a comparable structure in yeast meiotic nuclei. The specific antigens recognized by these antibodies in yeast preparations are being determined by SDS PAGE and subsequent Western blotting. A combined cytogenetic and immunochemical analysis is being used to identify protein components of the synaptonemal complex and to determine their spatial distribution and organization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21037-02 CGTB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Salmonella Mutagenicity Testing Results

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Barry Margolin	Mathematical Statistician	BRAP	NIEHS
Joseph Haseman	Research Mathematician	SBB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

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

The database from the testing of approximately 1200 chemicals for mutagenicity in Salmonella is being analyzed. Approximately 250 of these chemicals have also been tested for carcinogenicity by the NCI/NTP. There are also a large number of chemicals that have also been tested for their ability to mutate mouse lymphoma (L5178Y) cells, or produce chromosome damage in cultured Chinese hamster ovary (CHO) cells. The correlations between Salmonella mutagenicity and carcinogenicity are being analyzed, as well as the correlations between Salmonella mutagenicity and the responses in mammalian cells. A mutagenic response in Salmonella is highly predictive of carcinogenicity, whereas a negative response is not predictive of noncarcinogenicity. The correlation achieved between mutagenicity and carcinogenicity is highly dependent on the class of chemical examined. The other in vitro test systems, when used in concert with, or subsequent to Salmonella, do not appear to significantly enhance the predictivity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21039-02 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Genetic Control of Sister Chromatid Exchange in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Resnick	Supv. Research Geneticist	CGTB	NIEHS
Abdul Chaudhury	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In an attempt to understand the genetic control and molecular mechanisms of sister chromatid exchange in the yeast Saccharomyces cerevisiae, mutants with elevated levels of sister chromatid exchange have been isolated. These mutants were isolated in a strain that is deleted for the resident HIS3 gene and that contains two truncated copies of the HIS3 genes integrated near the centromere of chromosome IV; unequal sister chromatid exchange between homologous regions of the HIS3 gene can restore the functional gene. Mutants elevated for sister chromatid exchange were identified based on their increased ability to form HIS^+ prototrophs compared to the control unmutagenized clones. Three mutants (esr1, esr2, esr3) have been characterized extensively. Each of these mutants behaves like a single Mendelian locus. esr1 and esr2 have elevated levels of mitotic gene conversion. Experiments are underway to clone the esr1, esr2, and esr3 genes by complementation.

Using the same system, effects of known RAD, mutations, meiosis, and DNA damaging agents on SCR have been measured. We are also determining the influence of various repair genes on SCR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21045-04 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Analysis of SPO11, a Gene Required for the Early Events of Meiosis in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Craig N. Giroux	Senior Staff Fellow	CGTB	NIEHS
Howard F. Tiano	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The goal of this project is to identify and analyze the cellular functions which are required specifically for meiosis in the yeast, Saccharomyces cerevisiae. In particular, we are focusing on the analysis of the SPO11 gene of yeast which is required for recombination and proper chromosome segregation during meiosis. A general system has been developed to isolate meiosis specific genes of yeast for which mutants are available. Using this system, the SPO11+ wild type gene has been isolated following transformation and complementation of a spo11-1 mutant with a total genome clone bank. The function of the cloned gene has been examined during a detailed analysis of the complementation of the spo11-1 mutant by the isolated SPO11+ gene. The structure of the cloned gene has been determined by restriction enzyme analysis and subcloning. A restriction fragment of 2200 bases containing the SPO11+ gene has been isolated and its DNA sequence determined. An open reading frame of 398 amino acids has been identified as the tentative coding sequence of the SPO11 gene product. The candidate coding sequence predicts a 45 kilodalton basic protein. We are attempting to directly identify the SPO11 gene product by expression of the genetically engineered yeast gene in E. coli systems. The function of the cloned gene is being further characterized by in vitro mutagenesis of the cloned DNA and by substitution of the chromosomal SPO11+ gene by the in vitro engineered constructions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21048-03 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of a Molecular System to Study Mutagenesis in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Craig N. Giroux Senior Staff Fellow CGTB NIEHS

COOPERATING UNITS *(if any)*

Dr. Bernard Kunz, Biology Department, York University, Toronto, Ontario

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this project is to investigate the mechanisms whereby genetic information is transmitted to progeny somatic cells with fidelity: how mutagenesis occurs, and what mechanisms the cell employs to avoid mutation. Using a combination of classical genetic and recombinant DNA techniques, we have constructed a model system to examine the molecular basis of mutagenesis in the yeast, Saccharomyces cerevisiae. Construction of a yeast tester strain has been completed which allows the mutagenesis of a cloned SUP4-o tRNA suppressor gene to be assayed by direct genetic selection and DNA sequence analysis. This tester strain will allow the role in mutagenesis of the three genetically defined repair pathways of yeast to be examined using the same target plasmid. Using this system, the spontaneous mutation rate in the target gene has been determined to be 2.7×10^{-7} events per cell division. Isogenic strains are being constructed which will allow the role of the cloned rad1 gene, required for excision repair in yeast, to be examined in the distribution of mutations which arise following ultraviolet irradiation. The SUP4-o system is being developed as a rapid genetic test for the induction of all types of mutation occurring within a eukaryotic gene which will also allow determination of the mutagenic specificities of agents giving positive responses.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21049-04 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

DNA Synthesis and Metabolism During Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Resnick	Supv. Research Geneticist	CGTB	NIEHS
Akio Sugino	Visiting Scientist	LGM	NIEHS
Terry Chow	Visiting Associate	CGTB	NIEHS
John Nitiss	Guest Worker	CGTB	NIEHS
James Westmoreland	Biological Laboratory Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS.	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Unique DNA metabolic activities have been implicated during meiosis and following exposure of mitotic cells to DNA damaging agents. We have characterized both the DNA and the DNA metabolic enzymes at various times in meiosis in wild type and repair-deficient cells of yeast. No changes in the single-strand or double-strand size of chromosomal DNA are detected at anytime during meiosis while changes are observed in various mutants. Previously we had shown that polymerase I and II increase by a factor of two and a RAD52 nuclease increases nearly 10-fold implicatingit in meiotic recombination. Antibodies which had been raised against several proteins associated with replication or DNA metabolism are being used to probe DNA metabolic activities during meiosis. Using various mutants and conditions of high meiotic efficiency, it may be possible to determine the roles of these proteins during meiotic replication and recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21051-03 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cytogenetic Analysis of Mutagen-Sensitive Mutants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James M. Mason	Geneticist	CGTB	NIEHS
Akihiko H. Yamamoto	Visiting Fellow	CGTB	NIEHS
Masayoshi Watada	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Department of Genetics, University of California, Davis
Albert Einstein College of Medicine, Bronx, New York

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

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

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

Mutagen-sensitive mutants defective in DNA repair mechanisms have been collected in Drosophila melanogaster and characterized cytogenetically in order to gain a basic understanding of the genetic control of sensitivity to mutagenic agents. The tests used in the initial characterization of these mutants include genetic and cytogenetic mapping, complementation analysis, tests for sensitivity to unrelated mutagens, and tests for pleiotropic effects on related functions such as recombination. A genetic fine structure map of the mei-41 region has been constructed using several independently isolated alleles. This map confirms the large size of mei-41 found during mutational analysis. The mei-41 locus is estimated to cover approximately 30 kilobase pairs of DNA. The mei-41 locus is being cloned to investigate the regulation of this important gene. A nuclease has been identified and is being purified that is under the genetic control of mei-41. This nuclease is antigenically related to nucleases from yeast and Neurospora.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21052-04 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Metabolism of Xenobiotics to Mutagens Using Non-hepatic Microsomal Enzyme Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Dennis Pagano	Microbiologist	CGTB	NIEHS
Thomas Eling	Head, Prostaglandin Group	LPFT	NIEHS
Thomas Petry	Staff Fellow	LPFT	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Pulmonary Function and Toxicology

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Xenobiotic chemicals can be metabolized to mutagenic products by organs other than the liver. The prostaglandin endoperoxide synthetase (PES) system is found in a number of organs and is not dependent on cytochrome P-450. The metabolism of the cooked food-derived aromatic amines Trp-P-1 and Trp-P-2, and the imidazo-quinolines IQ and Methyl-IQ, which are activated to mutagens for Salmonella by the cytochrome system, at concentrations below which they are direct-acting mutagens, are not activated by PES at these same concentrations.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21053-03 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Genetic Control of Mutation in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James M. Mason	Geneticist	CGTB	NIEHS
Larry Champion	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Department of Zoology, University of Wisconsin, Madison
Department of Genetics, University of California at Davis
Department of Biology, Brown University
School of Biological Sciences, University of Kentucky

LAB/BRANCH

Cellular and Genetic Toicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

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

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

This project is designed to determine the relationship between DNA repair and mutagenesis in Drosophila melanogaster. Three approaches are being taken: (1) A mutant which increases the mutation frequency (a mutator) has been identified, mapped, and characterized. This mutator blocks repair of chromosome breaks specifically in oocytes, thereby allowing a previously undescribed repair process to be observed. In this process broken chromosomes are "healed", allowing the recovery of terminal deletions. (2) The interaction of DNA repair-defective mutants and transposable elements has been observed in double mutant combinations. None of the repair-defective mutants examined to date influence the rates of transposon-induced mutation or recombination, although mutants at the mei-41 locus prevent the transmission of transposon-bearing chromosomes. (3) Aneuploidy is being examined as a genetic endpoint. Chemicals that induce aneuploidy are being identified as probes to investigate mitosis and meiosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21054-03 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

DNA Damage and Repair in Centromeres of Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Resnick	Supv. Research Geneticist	CGTB	NIEHS
James Westmoreland	Biological Lab. Technician	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Kerry Bloom, Associate Professor, University of North Carolina, Chapel Hill

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The induction of DNA damage in chromosomal DNA would be expected to be dependent on the total structure of chromosomes within cells. Protein associations, folding, and extent of superhelicity of DNA within chromosomes could influence the induction and distribution of damage. Because of the role that centromeres play in chromosome segregation and the unique structure of the protein-DNA complex, we are examining the distribution of damage in the centromeric region of cellular DNA. Excision-defective and proficient strains of yeast are irradiated with UV, the DNA is gently extracted and treated with UV-endonuclease to produce nicks next to pyrimidine dimer sites. The chromosomal DNA is then probed with a probe specific for the centromere of chromosome III. Compared to DNA irradiated in vitro, the centromere sequence of DNA from cells irradiated in vitro is less sensitive to UV and shows a different pattern of UV-sensitivity. These results suggest that the centromere-associated proteins may influence sensitivity. There is a significant amount of lesions on opposite strands that are sufficiently close so as to lead to double-strand breaks when the DNA is treated with the UV-endonuclease. Although single-strand and double-strand damage occurs at sufficiently high frequencies in the centromere region to implicate them as potential inducers of chromosome loss, aneuploidy is not detected. One reason for this is that the repair which we have observed in the centromere removes all centromere inactivating lesions. Another reason is that the absence of transcription results in lesions being less mutagenic.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 21091-01 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effects of DNA Lesions on Untargeted DNA Metabolic Events

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Resnick	Supv. Research Geneticist	CGTB	NIEHS
Abdul Chaudhury	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In the yeast Saccharomyces cerevisiae, a system has been designed to study the effect of defined DNA double-strand breaks (DSB) in plasmids on recombination and repair at various chromosomal sites. In this system, a diploid yeast strain with a set of heteroallelic markers harbors two plasmids: a low copy CEN plasmid that carries a gene coding for the site-specific endonuclease HO under the control of the inducing promoter GAL, a high copy 2μ plasmid that carries a site at which the HO endonuclease cuts. Since the chromosomal HO-cut sites are deleted in this strain, derepression of HO should generate a defined multiple DSB only at HO-cut sites carried by the 2μ plasmids. Preliminary experiments indicate that the depression of the HO gene and subsequent DSBs in plasmids causes induction of recombination in the chromosome. The mechanism of this trans-acting effect of DSBs is being investigated in terms of effects on survival, mutation, and recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60102-08 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Testing of Chemicals of Interest in Salmonella

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Dennis Pagano	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

International Program on Chemical Safety, World Health Organization
Avishay A. Stark, Department of Biochemistry, Tel Aviv University, Israel

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Chemicals of interest have been tested for mutagenicity in Salmonella. The chemicals studied were sodium bisulfite, cyclic nitrosamines, glutathione, N-substituted phenanthreneimines, and p-nitrophenyl pentadienal (NPPD; "spy dust"). The mutagenicity of bisulfite appears to occur through a free-radical mediated mechanism, and the level of mutagenesis is dependent on the rate of autooxidation of the bisulfite ion. The mutagenicity of the nitrosamines in the presence of homogenates from various organs does not appear to be related to their organ specificity for carcinogenesis. Glutathione is mutagenic in Salmonella as a result of its metabolism by purified γ-glutamyl transferase. The mutagenicity of phenanthreneimines is related to the electron-attracting or releasing properties of the N-substituted moieties, and their alkylation via carbonium ions is inversely proportional to their mutagenicity. NPPD ("spy dust") and one of its metabolites is mutagenic in Salmonella. The mutagenicity appears to be dependent on nitroreduction by either bacterial or rodent liver nitroreductases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60122-07 CGTB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Molecular Mechanisms of DNA Repair in Yeast and Their Role in Meiosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Resnick	Supv. Research Geneticist	CGTB	NIEHS
J. Nitiss	Guest Researcher	CGTB	NIEHS

COOPERATING UNITS *(if any)*

J.C. Game, University of California, Berkely, Department of Genetics
R. Malone, Loyola University Medical School, Chicago, IL
R.M. Roth, Illinois Institute of Technology, Chicago, IL

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.2	1.3

CHECK APPROPRIATE BOX(ES)

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

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

DNA repair systems identified in mitotic cells of the yeast Saccharomyces cerevisiae are being examined for a) their protection of cells undergoing meiosis, and b) the role of the corresponding genes in normal meiosis. We have developed unique sucrose gradient techniques to examine repair after low doses of UV or ionizing radiation and to follow changes in meiotic DNA during meiosis.

The RAD50, RAD52 and RAD57 genes are essential in the repair of DNA double-strand breaks in mitotic cells. They are also required for meiosis. Mutations in these genes abolish normal meiotic recombination; RAD50 acts early in meiosis. Rare single-strand interruptions (SSIs) were observed in rad52 and rad57 strains shortly after the beginning of meiotic DNA synthesis and these appear to be related to recombination. Gentle isolation techniques have allowed the characterization of SSIs as breaks in DNA; many have 3' OH and 5' PO_4 termini. The SSIs do not appear to be randomly distributed, based on experiments involving probes for specific chromosomal regions, suggesting specific sites or regions involved in normal meiotic recombination.

While rad52 and rad57 mutants are defective in meiotic recombination, recombinants can be recovered prior to commitment to reductional division. The frequency in rad52 is much lower than in Rad^+ strains, but it is comparable in rad57 mutants. The recombinants differ qualitatively from those in Rad^+. When meiosis is arrested and rad52 or rad57 cells are exposed to growth medium, recombinants are not recovered. This is due to the extended time necessary for recombinants to form, suggesting that rad52 and rad57 are blocked at an intermediate step. The recombination intermediates are resolved slowly and growth prior to resolution prevents the appearance of recombinants. Both single-strand and double-strand interruptions are found in rad57 mutants. Based on genetic and biochemical changes, the order of gene function appears to be RAD50, RAD52, and RAD57.

CHEMICAL PATHOLOGY BRANCH
Summary Statement

The Chemical Pathology Branch functions to improve our understanding of the nature of the chemically induced lesions in rodents and to standardize our diagnosis, documentation and interpretation of these lesions. The Chemical Pathology Branch devotes a major share of its efforts to reviewing pathology data and histological diagnosis. The Branch is also responsible for the production, health status, and genetic integrity of the rodents used in the National Toxicology Program. In addition, the Branch conducts in-house studies and studies under contract, aimed at elucidating the nature and significance of pathological changes found in rodents. These efforts support the NTP mission to define the toxicity of chemicals of environmental concern.

An important goal in the past year was to improve the pathology data evaluation and to do this in a manner that would save time and money. To accomplish this, a pre-quality assurance data review was instituted. The pathology data from the laboratories is now reviewed prior to submission of the slides for histological review. If data duplication or inappropriate terminology is found, the data with the review results are returned to the laboratory and the laboratory is instructed to make corrections. This has placed the responsibility for producing quality data upon the laboratory and has resulted in submission of better quality data in subsequent studies.

A second change to save time and money is the elimination of review of random animals and tumor diagnosis from the intermediate dose animals. This saves approximately twenty-five percent in time and man-hours on the QA contract for each study completed. This does not jeopardize study results since all target tissues are reviewed, nor historical data, since all tumors in controls are reviewed.

A third change is to have the study pathologist present the day prior to the Pathology Working Group (PWG). The study pathologist can review the QA and PWG chairperson's opinion and make appropriate updates in the data prior to the PWG. This saves the PWG from reviewing cases that the study pathologist clearly agree need to be changed. This has allowed the PWG's to be shorter and yet devote more time to examining the treatment related changes and the controversial lesions. These management changes are resulting in better quality data in the NTP technical reports and yet are not costing additional time or money.

The Branch devotes considerable effort to understanding rodent lesions and developing criteria that reflect this understanding. A notable landmark in this regard is the publication of a classification scheme for rat liver tumors. For the past ten years, the term "neoplastic nodule" has been used to designate a lesion that may represent hyperplasia or a benign tumor. While a single term brought uniformity to classification of rat liver neoplasia, there has been considerable controversy for using a term that has different meaning to different pathologists. The Chemical Pathology Branch has reviewed numerous studies where the rat liver was the target organ and has gained more understanding on the type and nature of lesions found in the rat liver.

Dr. Maronpot, from our Branch, collected a study set of problematic rat liver lesions and had this set reviewed by a number of pathologists. He then devised a classification scheme using standard pathological terminology and had a group of pathologists use this scheme to classify the lesions. The proposed scheme and manuscript was widely reviewed and has considerable support prior to publication. We feel that this publication represents a significant progression in the classification of rat liver tumors.

The Branch continues to use the rat liver models both in-house and contract studies to augment our understanding of the rat liver tumors. These studies proved useful in our development of a classification scheme. The studies to date would suggest that the F344 rat is not overtly sensitive to carcinogens and may be less sensitive than the Sprague-Dawley rat.

The Branch, in collaboration with Dr. Marshall Anderson of the Biometry and Risk Assessment Program, has been studying the expression of oncogenes in spontaneous and chemically induced tumors in rats and mice. It appears that in rats there is different oncogene expesssion between spontaneous and induced tumors. This holds great promise for increasing our understanding of induced tumors in rodents. In mice there may be a difference between benign and malignant liver tumors. This may prove useful in evaluating our classification scheme that is currently being used.

The Chemical Pathology Branch provides support for NIEHS and NTP investigators. We have recently completed the pathology evaluation of rats and mice that were exposed to methyl isocyanate. The principal investigator for this study was Dr. John Bucher of the Carcinogenesis and Toxicology Evaluation Branch. Nearly one thousand animals were subjected to a histopathological evaluation. The necropsies and pathological evaluation were completed by Branch personnel. - The histopathological evaluation revealed that the lesions were confined to the respiratory tract and were characterized by necrosis and loss of the lining epithelium of the nasal passages and the airways. There was rapid regeneration but in the major bronchi in mice and in several generations of bronchi and bronchioles in rats, there was intraluminal fibrosis. The fibrotic lesions appeared to resolve slowly but were still present at the end of the 90-day study. In addition, an ultrastructural evaluation was completed for the nasal passages of the male rats and mice.

This study was interesting in that there was regeneration of the complex olfactory epithelium in the nasal passages that appeared to originate from cells of the Bowman's glands. This study provides additional information olfactory epithelial regeneration which has been difficult to study because of a lack of a suitable model. This toxic gas was a good model in that it caused complete loss of epithelium while leaving the underlying tissue intact.

In the past year the Branch has developed the capability to use immunohistochemical stains such as immunoperoxidase and immunogold to identify tissue antigens on various tumors. One example in this regard is the demonstration of laminin in yolk sac tumors in mice. This staining conclusively demonstrated the nature of the tumor, a conclusion that could not be reached by morphological evidence alone. It is planned to explore the use of this technique to demonstrate the presence of oncogenes in tumor tissues.

The Branch is also using Nuclear Magnetic Resonance (NMR) immaging to follow the progression or regression of hepatic lesions following treatment with a known carcinogen. This should provide useful information regarding the biological nature of liver lesions in the rat. Finally, the Branch is carefully reviewing the NTP experience using clinical pathology results to help evaluate the potential toxicity of environmental chemicals. Clinical pathology has been used to varying degrees for 90-day and two-year studies for the past ten years. The utility of the studies and the cost effectiveness has not been throughly reviewed. The Branch is having a review consisting of both government and industry pathologists to review our past experience and select the best options for the future. It is the goal of the Branch to continue to explore methods that improve toxicological evaluation of environmental chemicals in a cost effective manner.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21068-02 CPB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Methyl Bromide in the Rat Forestomach

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. A. Boorman	Chief	CPB	NIEHS
Others:	H. L. Hong	Biologist	CPB	NIEHS
	K. Yoshitomi	D.V.M., Ph.D.	CPB	NIEHS
	C. W. Jameson	Ph.D.	CTEB	NIEHS
	C. B. Richter	D.V.M.	CMB	NIEHS
	R. R. Maronpot	D.V.M.	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Chemical Pathology Branch

SECTION
Tumor Pathology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

Methyl bromide (MB) was dissolved in peanut oil and given by gavage at 50 mg/kg body weight to Wistar rats five times per week for 13 to 25 weeks. At 25 weeks nearly 100% of the rats receiving MB had hyperplastic lesions of the forestomach which were more severe than at 13 weeks. In the stop treatment group receiving MB for 13 weeks, there was regression of the stomach lesions. These results were accepted for publication in 1986.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21074-02 CPB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effects of Glycol Ethers on Bone Marrow Parameters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D.	CPB	NIEHS
	J. Canipe	Biological Lab.Tech.	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Ethylene glycol (EG) or ethylene glycol monomethyl ether (EGMME) was administered to both sexes of mice by gavage for 4 consecutive days. EGMME is more potent than EG on hematopoiesis in B6C3F1 mice. Female mice produced anemia and males caused leukopenia and testicular atrophy following EGMME exposure. These results were submitted in 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21080-02 CPB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Morrow B. Thompson	Veterinary Pathologist	CPB	NIEHS
Others:	R. R. Maronpot	Veterinary Pathologist	TRTP	NIEHS
	G. A. Johnson	Radiologist	Dept. of Radiology	Duke Univ. Med. Center

COOPERATING UNITS *(if any)*

Duke University Medical Center

LAB/BRANCH
Chemical Pathology

SECTION
Experimental Pathology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

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

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

During FY 1986, advances that were made in animal handling and in the acquisition, reconstruction and storage of magnetic resonance (MR) data will make possible experiments that are technically and experimentally complex. For example, initial MR images that were made 12 to 18 months ago of the liver of rats consisted of 1 image with a pixel size of 500 by 500 μm and a slice thickness of 5.0 mm. Because of the relatively long imaging time (30-60 mm), respiratory motion that was transferred through the diaphragm to the liver resulted in blurring of the image with some loss of resolution. Because of these inherent limitations, structures or lesions in the liver less than approximately 2 mm in diameter would probably not be detected. Recent developments, however, that will allow the production of multiple, contiguous images as thin as 1.25 mm and with a pixel size of 200 x 200 μm are as follows:

1. The synchronization of respiratory movements and signal detection. This will effectively eliminate motion artifacts produced by breathing.

2. The adaptation and installation of a 3-dimensional Fourier Transformation program for the imaging devise. This allows the selective excitation of a volume of tissue with the subsequent production of multiple, contiguous images from that region.

3. The change in imaging devise from the 1.00 meter, 1.5 Tesla research system to the 0.30 meter, 2.0 Tesla CSI system. This system (CSI) was designed for the imaging of small animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21082-01 CPB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Residual Marrow Effect from Ethylene Glycol Monomethyl Ether (EGMME) Exposure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: H.L. Hong	Biologist	CPB	NIEHS
Others: G. A. Boorman	D.V.M., Ph.D.	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies were performed to determine if there was residual marrow effect in sublethally irradiated B6C3F1 mice which were 15 weeks after EGMME exposure. EGMME significantly prolonged murine recovery from irradiation as assessed by colony formation assay.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21083-01 CPB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Myelotoxicity Induced in Female B6C3F1 Mice by Methyl Isocyanate Inhalation Exposure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D.	CPB	NIEHS
	J. Canipe	Biological Lab. Tech.	CPB	NIEHS
	J. Bucher	Ph.D.	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The effects of a 4-day inhalation exposure to methyl isocyanate (MIC) on bone marrow parameters in female mice were examined. The MIC exposure was associated with myelotoxicity as evidenced by hypocellularity, suppression of pluripotent stem cells (CFU-S), granulocyte macrophage progenitors (CFU-GM) and erythroid precursors (CFU-E) in both dose groups. These results were submitted in 1986.

PHS 6040 (Rev. 1/84)

GPO 914-918

SYSTEMIC TOXICOLOGY BRANCH
Summary Statement

Prediction of the potential for chemicals to adversely affect human health is best accomplished through extrapolation from toxicological data collected in laboratory animals. Programs within the Systemic Toxicology Branch (STB), in combination with those of other branches in the Toxicology Research and Testing Program (TRTP), are designed to collect data to help characterize the toxicological profile of chemicals and also to collect data which help improve the methods for toxicological evaluation as well as better understand the mechanisms of toxicity of selected chemicals.

The Systemic Toxicology Branch consists of five groups: Biochemical Toxicology, Chemical Disposition, Fertility and Reproduction, Immunotoxicology, and Inhalation Toxicology. Each section is summarized below; for more details and specific accomplishments, consult the individual presentations on the following pages.

Biochemical Toxicology: Structure-activity studies of chemicals are done to ascertain the mechanisms of action at the molecular and biochemical level. Major projects involve the identification and characterization of chemically-induced alterations in cytochrome P-450(s). These enzymes are responsible for metabolism of exogenous chemicals. Studies are in progress to examine changes in the genetic control of various subspecies of cytochrome P-450 in the rat after treatment with several different environmental chemicals.

Chemical Disposition: Studies of chemical disposition under the NTP are conducted through four contracts and an in-house program consisting of three senior scientists plus post doctoral, student and technical support. The immediate objective of chemical disposition studies is to provide information on absorption, distribution, metabolism and excretion of chemicals or chemical classes chosen for testing in the NTP Bioassay. This information is intended for use in design and interpretation of results of studies of toxicity and carcinogenicity of these chemicals. Long-range, but equally important, objectives of chemical disposition studies are to develop and publish data which will permit a better assessment of structure-activity relationship which influence chemical disposition or mechanisms of toxicity and provide basic information which will facilitate the extrapolation of laboratory data to man. Projects in Chemical Disposition include studies of metabolism and disposition of a variety of chemicals, investigations of mechanisms of toxicity and metabolism and studies of the effects of age, body composition and route of administration on chemical disposition. Metabolism and disposition studies have addressed the fate of both industrial and environmental chemicals as well as chemicals commonly used in consumer products. Studies of mechanisms of chemical toxicity have included metals, metal complexes, halogenated aromatics and acrylate monomers. Studies of the effects of age, body composition and route of administration on chemical disposition indicate that each of these factors can have very selective effects and that the importance of these effects vary with the chemical administered and the mechanisms necessary to the metabolism and clearance of the respective chemical. In addition to studies of the disposition of metals, experiments are also done to characterize the toxicity of metals. Based on knowledge of toxicity, studies are done to better understand the mode of action of metals and the body's defenses to protect against metal-induced

toxicity. Metals encountered in the microelectronics industry, including arsine, gallium, and metal complexes, are among those currently under evaluation.

Fertility and Reproduction: Studies were conducted in in-house laboratories to assess the effect of various chemicals on reproduction and fertility in males and females. These studies included such known reproductive toxins as glycol ethers and phthalate esters. Through contract mechanisms, studies continue which are designed to assess methods to detect adverse effects of chemicals on reproductive function or capacity. These include a program to assess continuous breeding trials as a means of assessing the effect of chemicals on fertility as well as evaluations on animals in subchronic toxicity studies to assess sperm morphology and sperm counts as well as vaginal cytology in rats. Studies were also conducted to characterize toxicity in neonatal rodents resulting from exposure to chemicals excreted in milk.

Immunotoxicology: Studies in this group continue to evaluate the influence of selected environmental chemicals on the immune system of animals, to relate alterations in immunological functions with both general toxicity and organ-specific toxicity, and to relate changes in immunological function with alterations in host resistance. This evaluation consists of a panel of immune and host resistance procedures which characterize immunotoxicity and correlate changes in immune function with altered host resistance. Data from these studies help characterize the toxicologic profile of chemicals, including those being evaluated for other toxicologic endpoints elsewhere in the NTP.

Inhalation Toxicology: The program of this group includes the design and execution of studies of compounds to which toxicologically significant exposure could be expected to be primarily by the inhalation route. Research is focused on manifestations of toxicity at the levels of tissues, organs, and organ systems. The in-house program is integrated with that of Northrop Services, Inc., an on-site contractor with responsibility for conducting research and testing by the inhalation route in an exposure facility within the in-house facility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21003-06 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	David W. Brewster	Guest Researcher	NRSA Postdoctoral Trainee	
	Laurie Couture	Guest Researcher	Graduate Student, UNC	

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2 0.5	

CHECK APPROPRIATE BOX(ES)

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

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

Halogenated dibenzofurans are found worldwide as environmental pollutants. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the degree and position of halogenation. This work has established that 2,3,7,8-tetrachlorodibenzofuran (TCDF), an extremely toxic isomer, is excreted only after metabolism and toxicity is inversely related to metabolic capability. The distribution to the fetus was examined after maternal exposure. The role of body composition on the disposition of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), the most toxic man-made compound known, has been examined in congenic mouse strains which are sensitive or resistant to TCDD toxicity and shown to be a major determinant of disposition. The disposition of octachlorodibenzodioxin (OCDD) was also studied in rats. Studies with 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) have begun in rats and monkeys.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21004-06 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	William C. Eastin	Research Physiologist	TRTP	NIEHS
	Susan Borghoff	Graduate Student	TRTP	NIEHS
	Charles Hebert	Guest Worker	UNC	

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.4	0.3

CHECK APPROPRIATE BOX(ES)

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

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

Age-related changes in many physiological parameters have long been known to occur. The basis for these alterations is, however, not well understood. Response to various stresses seems to decline with age. Changes in the ability to metabolize exogenous as well as endogenous compounds has been suggested as a cause of altered functions. This work will explore senescent changes in metabolism of several tissues--liver, lung, kidney, and small intestine tissues. Altered distribution and excretion of chemicals in aging animals is being studied in order to elucidate the basis for age-related changes in toxicological responses. Age-related alterations in gastrointestinal absorption are also being studied.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21009-05 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reproductive Effects in Males Exposed to Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. Chapin	Senior Staff Fellow	STB	NIEHS
Others:	J.K. Dunnick	Biologist	CTEB	NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch
Carcinogenesis and Toxicology Evaluation Branch

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION
Fertility and Reproduction Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Various environmental and industrial chemicals can disturb male reproductive function. The objective of these studies is to enhance our understanding of this toxic potential, and to elucidate the mechanism of action in chemicals found to be toxic. For FY 86, efforts have focused on in vitro primary cultures of Sertoli cells and their metabolic responses to toxicants whose in vivo spectrum of effects has been fairly well defined: mono-(2-ethylhexyl)-phthalate, 2,5-hexanedione, and acrylamide. Endpoints for this system include glycolytic and overall energy-balance analyses, protein synthesis (both cellular and secreted), and morphology. The emphasis has been on dose- and time-relationships between these endpoints. A method to differentiate peritubular cells from Sertoli cells in vitro was devised and evaluated. These studies are expected to yield information on the response of the Sertoli cell to toxicants which affect it in vivo. A series of studies are ongoing to evaluate the effects of acrylamide and some analogues on the testis of rats. Another set of studies examined the effects of methyl-DOPA on male reproductive function.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21024-05 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effects of Environmental Chemicals on Drug-Metabolizing Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joyce A. Goldstein	Pharmacologist	TRTP	NIEHS
OTHERS:	P. Linko	Chemist	TRTP	NIEHS
	P. McClellan-Green	Q-Appointment	TRTP	NIEHS
	H. Yeowell	Visiting Fellow	TRTP	NIEHS

COOPERATING UNITS *(if any)*

T. A. Gasciewiz, University of Rochester Medical School

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Biochemical Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.2	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of these studies were to study the effects of sex, age, strain, and environmental chemicals on cytochrome P-450 enzymes and relate these changes to the ability of the liver to metabolize steroids and foreign compounds.

1. Cytochrome P-450g is highly variable in outbred CD rats (high and low populations) and absent in the Fischer rat. It is male specific and found only after puberty. This enzyme metabolizes steroids but there were no significant differences in steroid hydroxylation between high and low phenotypes of CD rats.

2. Cytochrome P-450 UT-H (debrisoquine form) and cytochrome P-450 2c (a male specific isozyme) were decreased (40% and 90%) by a toxic PCB isomer, and to a lesser extent by 3-MC and phenobarbital. Metabolism of testosterone by liver microsomes (P-4502c mediated) at the 2- and 16- positions was also decreased dramatically. Decreases in 6β-hydroxylation of testosterone and increases in 7α-hydroxylation also occurred, and may be involved in changes in endocrine status of the PCB exposed rat.

3. The possible binding of hexachlorobenzene to the Ah receptor and its induction of P1-450 and P3-450 is being investigated. Some evidence for involvement of the Ah locus in the action of hexachlorobenzene is indicated.

The goal of this project is to better understand changes in the ability of the liver to metabolize hormones and foreign chemicals after exposure to environmental chemicals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21026-05 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Disposition of Hexabromonaphthalene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Other:	James D. McKinney	Research Chemist	LEC	NIEHS
	Christopher Miller	Guest Worker	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Bromonapthalenes have no known industrial use or application, but have been identified as contaminants of Firemaster BP-6, the toxic mixture of polybrominated biphenyls used as a fire retardant and involved in a major episode of environmental poisoning in Michigan. Structurally related to other halogenated aromatic xenobiotics, their toxicity and disposition seem to vary with the position of bromination. This work has studied the chemical disposition of a mixture of 2 hexabromonapthalenes (HBNs), previously identified as a single isomer, 1,2,3,4,6,7-HBN. The compound is incompletely absorbed after an oral dose. After iv treatment over 50% of the dose is excreted as metabolites within 3 days. However, the remainder of the dose seems to be extremely persistent, over 25% remaining in the liver after 35 days. These disposition results led to proof of the presence of two isomers by high resolution NMR, present in a ratio of 65:35 which have been identified as 1,2,3,4,6,7- and 2,3,4,5,6,7 -HBN. The difference in the fate of the two isomers has been proven by isolation and characterization by high resolution NMR of the HBN remaining in the liver 10 days after treatment. While the HBN dosed was in an isomeric ratio of 65:35 (1,2,3,4,6,7-:2,3,4,5,6,7-), the HBN in the liver 10 days after oral treatment was in the ratio of 20:80. The toxicity of this HBN mixture was examined in mice. A single oral dose as high as 1000 mg/kg had no toxic effects. However, repeat dose toxicity was detected at doses as low as 5 mg/kg for 7 days. The toxic response was toxicity similar to that seen for TCDD and related compounds. A complete teratology study was carried out and the teratogenic response was identical to that observed with TCDD, with the main endpoints being kidney anomalies and cleft palate at doses as low as 1 mg/kg.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21031-02 STB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Computer Simulation of Inhalation Exposures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Michael P. Moorman Engineering Officer TRTP (NTP) NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.15	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A computer simulation is being designed to model the distribution and disposition of compounds administered by inhalation. This will be used as a tool in the design of inhalation exposures and the interpretation of the resulting data.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21033-02 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP NIEHS
Others:	Usha Gundimeda	Visiting Fellow	TRTP NIEHS
	Janet Diliberto	Biologist	TRTP NIEHS
	Susan Heaney	Guest Worker	UNC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Pharmacokinetic factors can assist greatly in both dose-setting for toxicity studies and in interpretation of the results. Chemicals on-test by the NTP are nominated for disposition studies. The absorption (oral, dermal), distribution, metabolism, and excretion is studied in rats and other species as needed. The effect of dose is determined. In this way, the effects of chronic exposure may be predicted. The first chemicals to be studied in this project include o-benzyl-p-chlorophenol (BCP), and citral (oil of lemon). The dermal absorption of 4,4'-thiobis-(6-t-butyl-m-cresol) (TBBC) was also studied in rats and mice.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21034-02 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Evolution of Metallothionein-like Proteins in Non-Mammalian Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B.A. Fowler Research Biologist STB NIEHS

COOPERATING UNITS *(if any)*

I. Armitage, Yale University; D. H. Petering, University of Wisconsin-Milwaukee; C.F. Chignell and R. Hall; Laboratory of Molecular Biophysics; D. R. Winge, University of Utah; J. S. Garvey, Syracuse University

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies examining the nature of the SH-Mediated metal binding sites of the 45,000 dalton scallop kidney CdBP in relation to secondary structure are in progress. These data taken in concert with ongoing amino acid sequence and structural studies of oyster CdBP suggest that one evolutionary pathway for MT may involve the insertion of cysteine based sequences into a more ordered protein with concomitant changes in structure and metal-binding site formation or gene cleavage with production of a smaller more efficient molecule from a larger protein. In addition, studies focused on examining the role(s) of this protein in toxicity from multi-element exposure showed that its apparent induction by cadmium exposure ameliorated copper toxicity to the scallops but that this protective effect was associated with marked alterations of normal cytosolic metal-binding patterns for copper, zinc and cadmium.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21038-04 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. B. Matthews	Research Chemist	TRTP, NIEHS
OTHERS:	J. M. Sanders	Biological Lab. Technician	TRTP, NIEHS
	A. A. Nomeir	Senior Staff Fellow	TRTP, NIEHS
	S. C. Tsao	Visiting Fellow	TRTP, NIEHS
	Y. C. Kim	Visiting Fellow	TRTP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.2	0.6

CHECK APPROPRIATE BOX(ES)

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

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

Studies of chemical metabolism and disposition are designed to provide both applied knowledge in support of chronic toxicity tests conducted by the National Toxicology Program and basic knowledge of those chemical structure and property relationships which determine toxicity. Comparative studies of ethyl carbamate in rats and mice have indicated both species and dose related variations in metabolism and clearance which may account for the reported variations in toxicity. Studies of 2-butoxyethanol indicate that it is readily absorbed and rapidly excreted, but it has a unique pattern of age related hemolytic effects which may be related to its metabolism. An investigation of the sex related variations in sensitivity to the toxic effects of resorcinol indicate that at moderate doses both male and female rats metabolize and clear this compound similarly except for variations in the amounts of two minor metabolites.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21046-03 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lori A. Dostal	Senior Staff Fellow	STB	NIEHS
Others:	B.A. Schwetz	Supervisory Pharmacologist	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION
Fertility and Reproduction Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this study is to improve our ability to characterize the toxicity of drugs and chemicals to neonates relative to adults, and to explore the role of lactation in the induction of neonatal toxicity. Lactation is evaluated as a source of exposure to chemicals secreted into the milk and as the nutritional source for the newborn. A study was completed in which the toxicity of di(2-ethylhexyl) phthalate (DEHP) was examined in suckling and adult rats of different ages. Hepatic peroxisome proliferation, hypolipidemia, and histological testicular damage were determined as indices of toxicity. Administration of DEHP to lactating rats caused increases in hepatic peroxisomal enzyme activities in the dams and in the suckling pups indicating the transfer of DEHP or its metabolites through the milk. A gas chromatography method was developed to analyze DEHP and its metabolite, mono(2-ethylhexyl) phthalate (MEHP), in rat plasma and milk, and these methods will be used to determine the amount of DEHP and MEHP transferred through the milk of lactating rats. Another study was begun in which neonatal rats were exposed to DEHP, and testicular morphology was examined after a 4 week recovery period. A study is underway to determine the extent of intestinal hydrolysis of DEHP in neonatal and adult rats and its role in the age-related differences in toxicity of DEHP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 ES 21057-02 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Pharmacokinetics and Metabolism of Neurotoxic Chemicals in Various Species

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Amin A. Nomeir Senior Staff Fellow TRTP, NIEHS

COOPERATING UNITS *(if any)*

Duke University Medical Center

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

2,5-Hexanedione (2,5-HD) is believed to be the ultimate neurotoxic metabolite of the industrial neurotoxic solvents n-hexane and methyl-n-butyl ketone. Tri-o-cresyl phosphate (TOCP) is also a neurotoxic contaminant found in the commercial preparation tricresyl phosphate. These studies were initiated to investigate the absorption, distribution, excretion and metabolism of these neurotoxic chemicals in various species. It was also of interest to study the role of pharmacokinetics and metabolism in species sensitivity to neurotoxic agents. Analytical methods using capillary gas chromatography (GC) and high performance liquid chromatography (HPLC) were developed to analyze the parent compounds and their metabolites. Five metabolites of TOCP were synthesized and their structures were verified by various spectroscopic techniques. The metabolism of [^{14}C]2,5-HD in the chicken was investigated following a dermal application of 50 mg/kg dose. The metabolism and disposition of TOCP was investigated following dermal application of [^{14}C] labeled compound on the male cat. Also being investigated is the metabolism and disposition of orally administered TOCP to rats, cats and chickens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21059-02 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Gastric Toxicity of Acrylic Acid Esters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burhan I. Ghanayem	Staff Fellow	TRTP, NIEHS
Others:	H.B. Matthews	Research Chemist	TRTP, NIEHS
	Robert Maronpot	Pathologist	TRTP, NIEHS
	Gregory Eatmon	Biological Aid	TRTP, NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, TRTP, NIEHS

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Rats receiving 14 daily gavage doses of 100 or 200 mg/kg ethyl acrylate (EtAc) and killed at varying times following the end of dosing exhibited dose-dependent lesions and recovery from lesions in the forestomach. The glandular stomach which was previously shown to be affected by acute exposure to EtAc appeared to have adapted to resist EtAc toxicity with repeat exposure and appeared normal in all animals. Adaptation of the forestomach was characterized by increased papillomatous thickening with dose. Lesions observed in acute exposure to EtAc were still present with repeat dosing and were more pronounced at the high dose. Forestomachs of rats which received 100 mg/kg EtAc for 14 days were recovered to normal within 2 weeks following the last dose. Forestomachs of rats receiving 200 mg/kg EtAc still exhibited numerous lesions 2 weeks following the last dose, and mucosal hyperplasia was present in these forestomachs at 4 weeks post-exposure. Two lesions, submucosal fibrosis and foreign body reaction, became more prevalent in high-dose animals with time. Foreign body reaction was present in all high-dose animals 4 weeks postexposure.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21060-02 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ethyl Acrylate Metabolism and the Metabolic Basis of Gastric Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burhan I. Ghanayem	Staff Fellow	TRTP, NIEHS
Others:	L.T. Burka	Research Chemist	TRTP, NIEHS
	H.B. Matthews	Research Chemist	TRTP, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

The absorption, distribution, covalent binding and excretion of 2,3-^{14}C-ethyl acrylate (EtAc) was studied in F344 male rats. EtAc was readily absorbed, distributed to all tissues, metabolized and excreted mainly in the expired air as ^{14}C-CO_2 (70% of the dose in 24 hours). Three to 5% of the administered dose was excreted in the urine in 4 hours as mercapturic acids of EtAc and acrylic acid. Approximately 4% of the administered dose was excreted in the bile in 6 hours. The highest concentrations of radioactivity were found in the stomach, liver and kidneys respectively. Chemical fractionation of the fore-stomach and liver revealed that a major portion of the radioactivity in the stomach and liver was covalently bound to the protein fraction at 4 hours after treatment. Twenty-four hours after treatment, there was a significant decline in EtAc covalent protein binding in the liver, while there was no such decline in the stomach. No significant binding to nucleic acids was found in the stomach or the liver.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21070-03 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
TCDD Teratogenicity: Modulation in Mixtures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Linda S. Birnbaum	Research Microbiologist	TRTP	NIEHS
Others:	James C. Lamb	Research Biologist	TRTP	NIEHS
	James D. McKinney	Research Chemist	LMB	NIEHS
	Martha Harris	Head Technician	TRTP	NIEHS
	Robert M. Pratt	Research Biologist	LRDT	NIEHS
	Richard Morrissey	Biologist	TRTP	NIEHS
	Betty Barnhardt	Research Chemist		CDC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

TCDD (dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin) is one of the most toxic chemicals known to man. Progressive weight loss and thymic atrophy are two of its most frequent toxic symptoms. The induction of cleft palate and hydronephrosis characterize the teratogenic response of mice to TCDD. Because of the sensitivity of this response, we decided to use teratogenicity to measure the interaction of TCDD and other compounds with which it occurs in the environment. Such chemicals include polychlorinated dibenzofurans, polychlorinated biphenyls, hormones such as thyroxins and hydrocortisone, and drugs. TCDD interacts in an additive manner with 2,3,7,8-tetrachlorodibenzofuran and 2,3,3',4,4',5-hexachlorobiphenyl, synergistically with hydrocortisone. Its teratogenicity is potentiated by thyroid hormones. Polychlorinated dibenzofurans cause the same spectrum of teratogenic effects as TCDD and appear to interact additively with each other.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21075-03 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. T. Burka	Research Chemist	TRTP	NIEHS
Others:	H. B. Matthews	Research Chemist	TRTP	NIEHS
	B. I. Ghanayem	Staff Fellow	TRTP	NIEHS
	Y. C. Kim	Visiting Fellow	TRTP	NIEHS
	C. P. Kool	Chemist	TRTP	NIEHS
	J. M. Sanders	Laboratory Technician	TRTP	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Two major urinary metabolites of 3-chloro-2-methylpropene (DMVC) were isolated and identified. They were 2-amino-4-thia-5-heptene-1,7-dioic acid and the corresponding N-acetylated derivative. These compounds appear to arise from oxidation of one of the methyl groups to a carboxylic acid followed by GSH conjugation and subsequent metabolism of the GSH moiety.

The absorption and metabolism of 5-(4-nitrophenyl)-2,4-pentadienal (NPPD, Spydust) was investigated. The compound was readily absorbed by the gastrointestinal tract and rapidly metabolized and excreted. NPPD was not readily absorbed through the skin. A total of five metabolites were identified in urine, 4-nitrocinnamic acid, 4-acetamidobenzoic acid, 4-nitrobenzoic acid, 4-nitrohippuric acid and 4-acetamidocinnamic acid. These metabolites result from some combination of oxidative metabolism of the pentadienal side chain, reduction of the nitro group, and conjugation of the resulting carboxylic acid group with glycine or amino group with acetate.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21081-01 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reproductive Effects of Di-pentyl-phthalate in Male Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert E. Chapin	Senior Staff Fellow	STB	NIEHS
Others:	P. Lindstrom	Guest Worker	STB	NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch
Data Management and Analysis

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION
Fertility and Reproduction Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.25	1.25

CHECK APPROPRIATE BOX(ES)

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

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

Numerous environmental and industrial chemicals can alter male reproductive function. Our ability to detect subtle alterations in human reproductive function has lagged far behind our ability to detect these lesions in experimental animals, which can be sacrificed for analysis. This project examines the effects of a single dose of a widely used plasticizer, di-pentyl-phthalate, on organ weights, testicular and epididymal histology, fertility, and circulating levels of reproductive hormones and a specific testicular secretion, androgen binding protein (ABP). The objective is to correlate changes in fertility and the more readily measurable endpoints (sperm count and plasma hormone levels) with those of plasma ABP to determine if circulating ABP values will be a more sensitive index of altered reproductive function. By measuring all these endpoints concurrently, we can determine if ABP will be more, or less, sensitive than existing methods in rats.

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21084-01 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Association of Chemically Induced Forestomach Cell Proliferation & Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burhan I. Ghanayem	Staff Fellow	TRTP	NIEHS
Others:	H.B. Matthews	Research Chemist	TRTP	NIEHS
	Robert Maronpot	Pathologist	TRTP	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, TRTP, NIEHS

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

A number of chemicals have been shown to cause malignant neoplasms in the forestomach of Fischer 344 rats when administered chronically by gavage. The present study was designed to identify early forestomach lesions following two-week repeated gavage administration of some forestomach carcinogens. Groups of 8 or more male F344 rats received one of six reported forestomach carcinogens ethyl acrylate (EtAc), diglycidyl resorcinol ether (DGRE), 1,2-dibromoethane (DBE), 1,2-dibromo-3-chloropropane (DBCP), 1-chloro-2-methylpropene (dimethylvinyl chloride, DMVC), or 3-chloro-2-methylpropene (CMP), one of two structurally related chemicals (methyl methacrylate and dichloroethane) which were negative in chronic carcinogenicity studies or the vehicle (corn oil) alone 5 days/week for 2 weeks. Histopathologic examination of forestomachs of rats sacrificed 24 hr after the last dose indicated no significant difference in the incidence or severity of epithelial cell proliferation in the rat forestomach between the vehicle control group and the two negative control groups. In contrast, the incidence and severity of epithelial cell proliferation of the rat forestomach in every group treated with a forestomach carcinogen was significantly higher than the incidence in the vehicle or negative control groups.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21085-01 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Calcium Channel Blockers Protect Against Chemically-Induced Gastric Lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Burhan I. Ghanayem Staff Fellow TRTP, NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, TRTP, NIEHS

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

These studies were designed to investigate the effect of calcium channel blockers on chemically-induced gastric lesions in rats. Results of this study indicated that pretreatment of male F344 rats with the calcium channel blockers verapamil, diltiazem, or Mg^{++} significantly protected against ethanol- and indomethacin-induced gastric lesions as demonstrated by gross and histopathologic evaluation. Treatment of rats with calcium channel blockers prior to ethanol or indomethacin resulted in a significant decline in the mean number of lesions per glandular stomach, the damaged area of the glandular stomach and the severity of lesions. Calcium channel blockers also caused a significant decline in the incidence of indomethacin-induced gastric lesions, but had no effect on the incidence of ethanol-induced gastric lesions. These results offer the first evidence that calcium channel blockers may play an important role in protection against chemically-induced gastric lesions and thereby offer insight into the mechanism of gastric ulcer formation.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21086-01 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

2-Butoxyethanol Hematotoxicity: Effects of Age and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burhan I. Ghanayem	Staff Fellow	TRTP, NIEHS
OTHERS:	L. T. Burka	Research Chemist	TRTP, NIEHS
	H. B. Matthews	Research Chemist	TRTP, NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, TRTP, NIEHS

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	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 *(Use standard unreduced type. Do not exceed the space provided.)*

Acute gavage administration of a single dose of 2-butoxyethanol (BE) to male F344/N rats caused severe hemolytic anemia as evidenced by a decrease in the number of circulating red blood cells, hemoglobin concentration, and hematocrit. The hemolytic effects of BE were found to be dose-dependent. BE also caused hemoglobinuria and liver and kidney changes both of which were considered secondary to the hemolytic effects of BE. We have found that BE-toxicity is age related with young rats being significantly less sensitive than older rats. Gavage dosing with 125mg/kg BE for 1 or 2 days resulted in a significant increase in BE hematotoxicity in rats treated for 2 days. However, continued daily administration of BE beyond 2 days resulted in a gradual decrease in the BE-induced hematotoxicity which is indicative of tolerance development. Tolerance was attributed to a decline in the hemolytic effects of BE with multiple dosing. Further, we have investigated the metabolic basis of BE-induced hematotoxicity. Pretreatment of rats with pyrazole has protected rats against BE-induced hematotoxicity, which suggested that metabolic activation via the alcohol dehydrogenase enzyme is a prerequisite for the development of BE toxicity.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21087-01 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Toxicity and Residues of S,S,S-tri-n-butyl Phosphorotrithioate (DEF) in Fish

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Amin A. Nomeir, Senior Fellow, TRTP, NIEHS

COOPERATING UNITS *(if any)*

Duke University

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)

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

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

DEF is widely used as a cotton defoliant in California and the southern United States. DEF has produced delayed neurotoxic effects, acute cholinergic effects and late acute effect in some animal species. DEF is a frequent contaminant in water drained from agricultural land and has been shown to be toxic to fish at low concentration under acute exposure (ppm). With chronic exposure, DEF has been shown to adversely affect the survival of some species of fish at ppb levels. This study was undertaken to examine the comparative neurotoxic effects of acute DEF exposures to two aquatic species, a vertebrate, the channel cat fish and the blue crab, an invertebrate. Residues of DEF and its rate of disappearance were also determined in certain tissues of these animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21088-01 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Studies on the Species and Tissue Selectivity of Dimethyl Hydrogen Phosphite

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Amin A. Nomeir	Senior Staff Fellow	TRTP, NIEHS
Others:	H. B. Matthews	Research Chemist	TRTP, NIEHS
	Steven Vo	Biological Lab Technician	TRTP, NIEHS

COOPERATING UNITS *(if any)*

Systemic Toxicology Branch

LAB/BRANCH

Chemical Disposition

SECTION

NIEHS, NIH, Research Triangle Park, NC 27709

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Dimethyl hydrogen phosphite (DMHP) has been found by NTP to be a lung and forestomach carcinogen in Fischer 344/N rats but not in B6C3F1 mice. The objectives of this study are to examine various factors which may be involved in these tissue and species selectivities. DMHP was stable in aqueous solutions for a period of time before decomposition began. The period of stability and the rate of decomposition were dependent upon the pH, concen-tration of DMHP and the storage temperature. The degradation products were identified as methanol, monomethyl hydrogen phosphite and phosphorus acid. DMHP was metabolized in vitro to formaldehyde by the microsomal fraction of liver, lungs, kidneys, forestomach and glandular stomach. Daily treatment with DMHP at 200 mg/kg for 6 weeks resulted in the detection of pathological and biochemical changes in the lungs and forestomach but not in the liver, kidneys and glandular stomach of treated rats. There was a significant increase in the weight of the forestomach of treated rats which was associated with hyperplasia and hyperkeratosis. There was also a significant increase in the levels of nonprotein sulfhydrals in the forestomachs of daily treated animals. The activity of angeotensin converting enzyme in the serum of treated rats significantly increased suggesting early lung injury. Carboxylesterase activity significantly was decreased in the lungs and forestomach but no other tissues of daily treated rats. No treatment related effects were observed in the activities of the microsomal p-nitroanisole demethylase, soluble glutathione S-transferase and soluble superoxide dismutase in liver, lungs, kidneys, forestomach and glandular stomach. Rats treated with a single oral dose of 200 mg/kg of [^{14}C]DMHP expelled 50% of the dose as $^{14}CO_2$, excreted 30% of the dose in the urine while 20% remained in the tissues 24 hr after dosing.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21090-01 STB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanisms of Arsine Gas and Gallium Arsenide Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. A. Fowler	Research Biologist	TRTP	NIEHS
Others:	P. L. Goering	NRSA Fellow	TRTP	NIEHS
	G. Rosenthal	NRSA Fellow	TRTP	NIEHS
	G. Boorman	Chief, CPB	TRTP	NIEHS
	B. Schwetz	Chief, STB	TRTP	NIEHS
	R. Morrissey	Research Pharmacologist	TRTP	NIEHS
	M. Moorman	Engineering Officer	TRTP	NIEHS

COOPERATING UNITS *(if any)*

Northrop Services (Dr. Bernard Adkins)

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A. Studies have been initiated to evaluate the prechronic toxicity of arsine gas via inhalation. Fischer 344 rats, B6C3F1 and C57BL/6 mice, and golden hamsters will be exposed to arsine gas at concentrations of between 10-5000 ppb for 14 or 90 days. The animals are being evaluated by a number of ultrastructural/biochemical/teratological criteria for evidence of toxicity following prolonged exposure to tolerated doses of this gas alone or in combination with GaAs particles administered via intratracheal instillation. These studies are in progress.

B. Intratracheal administration of GaAs to CD-1 rats has been found to produce marked inhibition of δ-aminolevulinic acid dehydratase (ALAD) in blood and to a lesser extent in liver and kidney with resultant increases in the urinary excretion of aminolevulinic acid (ALA). In vitro studies showed that ALAD is highly sensitive to gallium and appears to be primarily responsible for the in vivo inhibition of this enzyme since inorganic arsenicals were without effect except at high concentrations. The mechanism of gallium inhibition of this enzyme appears to involve zinc displacement from the enzyme.

These data suggest that measurement of urinary ALA in combination with assays of blood ALAD activity may provide one approach to the development of early biological indicators for workers exposed to this binary chemical compound.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21092-01 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Activation of Environmental Chemicals by Hepatocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joyce A. Goldstein	Pharmacologist	TRTP NIEHS
Others:	B. Furlong	Staff Fellow	TRTP NIEHS
	R. Weaver	Biological Lab Tech	TRTP NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Biochemical Toxicology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this study are to examine the activation of precarcinogens to reactive DNA binding and mutagenic metabolites in hepatocytes and S9.

1. The hepatocarcinogen, 2,4-diaminotoluene(DAT), was activated to reactive metabolites which bound to DNA to a greater extent than the nonhepatocarcinogen, 2,6-DAT. The ability of activation to mutagens and DNA binding species was examined. Inhibitors of metabolism were also examined.

2. The ability of constitutive isozymes of P-450 to activate a variety of mutagens was compared.

3. We also evaluated a human cell line, Hep G2, using antibodies to several human P-450s to determine whether the cell line resembled normal human liver cells in its metabolic capability.

The goal of this project is to investigate the ability of various liver pathways to activate precarcinogens and to reactive DNA-binding and mutagenic species. The enzymatic composition of a human cell line was compared with that of normal liver to determine whether this human cell line could be expected to metabolize foreign chemicals in a manner similar to normal human liver.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30044-10 STB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Toxicology of Environmental Chemicals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. B.A. Schwetz Chief, STB TRTP (NTP) NIEHS

OTHERS: Michael P. Moorman Engineering Officer TRTP (NTP) NIEHS

COOPERATING UNITS *(if any)*

Northrop Services, Incorporated

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	-

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Exposure of strain A mice for 6 months to vinyl chloride, ethylene dibromide or ethylene oxide, cause concentration-related increases in numbers of pulmonary adenomas that were formed. This inexpensive model may be useful in helping to identify inhalant carcinogens. Mice were exposed to nitrogen dioxide following profiles with equal integrals of concentration with time but different maximum concentrations. The wet/dry lung weights were taken as a measure of pulmonary edema. The same study has been conducted with Acrolein as the exposure compound.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 30106-12 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effects of Environmental Pollutants on the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael I. Luster	Research Microbiologist	STB	NIEHS
Others:	J. Blank	Staff Fellow	STB	NIEHS
	M. Ackermann	Visiting Fellow	STB	NIEHS
	G. Rosenthal	NIH Post-Doctoral Fellow	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Systemic Toxicology Branch, TRTP

SECTION
Immunotoxicology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	4.0	3.5

CHECK APPROPRIATE BOX(ES)

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

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

The ongoing objectives of the immunological-toxicology group include the following interrelated efforts: (1) to evaluate and examine the influence of selected environmental chemicals on the immune response including cellular changes associated with chemical interactions in lymphoreticular cells; (2) to relate alterations in immunological functions with both general toxicity as well as specific organ toxicity; (3) to relate changes in immunological functions with altered host resistance following challenge with either syngeneic tumor cells or infectious agents employing a defined panel of infectivity models; and (4) to refine and validate a panel of immune and host resistance procedures in order to better define immunotoxicity and correlate changes in immune function with altered host resistance. Studies were performed in the following areas: (a) Attempts to elucidate the mechanisms of antibody suppression by antifolates (methotrexate and trimetrexate) and restoration with hypoxanthine. (b) Role of altered arachidonic acid metabolism in immunotoxicity of benzidine and other co-oxidative substrates. (c) Investigation into the modulation of B cell differentiation by TCDD and the identification of specific antagonists. (d) Immunotoxicity studies with methyl isocyanate. (e) Development of model systems to study neutrophil function following exposure to chemical xenobiotics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70200-12 STB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms for Regulating for Intracellular Bioavailability of Metals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. A. Fowler	Research Biologist	TRTP	NIEHS
Others:	D. Gilg	Visiting Fellow	TRTP	NIEHS
	P. Goering	NRSA Postdoctoral Fellow	TRTP	NIEHS
	G. DuVal	NRSA Postdoctoral Fellow	TRTP	NIEHS

COOPERATING UNITS *(if any)*

C. F. Chignell, Laboratory of Molecular Biophysics

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	1.5

CHECK APPROPRIATE BOX(ES)

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

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

Molecular mechanisms which regulate the intracellular bioavailability of metals such as lead and cadmium have been studied. High affinity cytosolic lead-binding proteins (PbBP) of 63,000 (63K) and 11,500 (11.5K) daltons from kidneys of rats have been purified by gel, hydrophobic interaction, and anion exchange chromatography, electrophoresis, and sucrose density gradient analysis. Cell-free nuclear translocation studies showed both time- and temperature-dependent uptake. Cd^{++} ions effectively blocked the nuclear uptake of ^{203}Pb but Zn^{++} stimulated uptake. In vivo Pb-injection studies showed a close temporal relationship between formation and loss of Pb intranuclear inclusions in renal proximal tubule cell nuclei and marked changes in renal 2-D gel ^{35}S-labelled protein synthesis patterns. The data indicate that these high affinity PbBP, which act as the initial cytosolic ligands for Pb in the kidney, are capable of mediating the intranuclear translocation of Pb and that the presence of Pb within renal nuclei is temporally associated with marked changes in renal gene expression. The 11.5K dalton protein, but not the 63K protein, was also found to regulate the inhibitory effects of Pb on the heme biosynthetic pathway enzyme δ-aminolevelinic acid dehydratase (ALAD). The data indicate that the 11.5K dalton protein confers partial resistance to Pb inhibition of liver ALAD in vitro and suggests a similar role for this protein in kidney with respect to the resistance of renal ALAD to Pb inhibition. PbBP chelation of Pb and donation of Zn to the ALAD were found to be the mechanisms of this effect. Replicate studies performed with purified Zn metallothionein and purified ALAD demonstrated identical effects.

GPO 914-918

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
INTRAMURAL PROJECT NUMBER LISTING

Z01 ES 10004-07 LMB
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