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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1987 INTRAMURAL RESEARCH PROJECTS

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OFFICE OF THE DIRECTOR

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The National Institute of Environmental Health Sciences celebrated its Twentieth Anniversary Year in 1987, simultaneous with its participation in the Centennial observances of the National Institutes of Health. Today the Institute has reached a critical mass of personnel and expertise to carry out a broad-based program in environmental health sciences. NIEHS has the principal responsibility among Federal agencies for the support of research and training of research manpower concerned with the effects of chemical, physical and biological factors on human health. The emphasis in this work is to find the mechanisms of toxicity of environmental agents at the molecular and cellular levels. This year, as in past ones, technology and science have continued to open new doors to the researchers who seek to elucidate the complex way the body maintains health or falls prey to disease following exposure to environmental agents.

Advances in technology such as computer enhanced magnetic resonance spectroscopy and imaging, the dazzling progress in the field of genetics, and the increasingly sophisticated application of x-ray and scanning electron microscopy, among many such examples, continually offer researchers new insights and opportunities. Since its inception, Institute investigators have published almost 4,000 articles in the scientific literature making the results of their research known to the scientific community at large, and adding greatly to the knowledge base. This information is used by regulatory agencies, the medical community and other concerned groups to develop and initiate appropriate standards for public health programs.

While the Institute took time to look back at its history, challenging opportunities for the future became apparent this year. The Superfund Amendments and Reauthorization of 1986 authorized two new grant-supported programs at NIEHS, one for university-based basic research and training, the other for health and safety training of workers at hazardous waste sites and for emergency response personnel. These programs have been developed and implemented, including conducting the technical/scientific merit review of 79 complex applications submitted in response to two solicitations.

This year, the Department of Health and Human Services approved the redesignation of all the major organizational components within NIEHS, elevating the four Program areas to Division status.

The Division of Intramural Research concentrates on understanding the myriad ways in which environmental factors enter the body and disrupt its normal functions. The research ranges from experiments with isolated molecules such as DNA using sophisticated molecular biology techniques, to monitoring the levels of calcium ion in the beating heart of a living animal. The latter studies utilize nuclear magnetic resonance (NMR) a procedure requiring instruments housed in special facilities devoid of ferrous-containing materials. A new NMR facility, completed this year, will greatly expand our capacity to carry out non-invasive research on whole animals.

The Division of Biometry and Risk Assessment utilizes epidemiological studies and mathematical models in conjunction with toxicological studies of the underlying biological mechanisms to evaluate the risks of various chemicals.

A major focus of the research effort is the qualitative and quantitative estimation of adverse health effects resulting from exposure to hazardous environmental agents, with particular emphasis on the development of methodology useful in this estimation process. Using recent advances in genetic engineering and sophisticated new computer modeling technologies scientists have been able to examine DNA interaction at the molecular level and look at the mutational "hotspots" within DNA sequences that appear to play an important role in causing cancer. Researchers in this Division are making exciting progress in delineating the characteristics and action of oncogenes (chemical causing genes). They are investigating how chemicals activate normal genes into oncogenes and how this activation process is involved in the induction of tumors. Such information will enhance the use of data from animal toxicology studies for risk assessment and improve the basis for decisions on regulating human exposure to workplace and other environmental chemicals.

The Division of Toxicology Research and Testing is the core of the National Toxicology Program (NTP) and through its basic toxicologic studies provides much of the scientific information used by regulatory and public health intervention and prevention agencies to understand which chemicals may be public health hazards and what types of efforts must be implemented to prevent or reduce harmful exposures. The NTP uses state-of-the-art methods and, on an ongoing basis, examines, develops, and validates new, improved technologies. Those found to offer improvement over older methods are selected for further investigation and perhaps validation. Scientists from this and another Division have published a landmark study that places in perspective the limits of short term toxicological tests as predictors of carcinogenicity in rodent studies. Their analyses produced strong evidence that such tests, at their current state of development, are not an efficacious substitute for whole animal research.

The Division of Extramural Research and Training funds research and training in numerous environmental science disciplines throughout the country. Utilizing investigator initiated research grants and program projects, centers, fellowships, training grants and other mechanisms of financial support, scientists at universities and research centers concentrate on the prevention, diagnosis and treatment of a variety of environmentally induced diseases. Many of the current standards for lead exposure have been based upon research supported by NIEHS. The information developed by NIEHS grantees has influenced legislation on lead-based paint, guidelines for screening of lead levels in children, and Federal regulations on lead in gasoline. Currently supported research is discovering subtle neurobehavioral effects in children from exposure to levels previously thought safe. The possible implications for a generation of children is frightening.

These NIEHS research programs will continue to evolve, taking advantage of new knowledge. Today, because of advances in the environmental health sciences, we understand more about the mechanisms by which toxins cause disease. We have broadened our inquiry from looking at specific diseases or conditions such as cancer, birth defects or target organ damage to looking at the whole person. Our scientists now know that potentially toxic chemicals can affect the entire biological system and that many of these effects are subtle ones, and much more far reaching than originally thought. It appears that when these chemical toxins interrupt any one of the normal body processes, they create problems in other parts of the system in ways that we do not yet fully understand. We have

learned that the human body is a complex communications network that cannot function properly when any one major piece is not working properly.

Through a continually improving understanding of environmental agents, and how they affect human health, wiser choices can be made about how we live and work. With an expanded knowledge of environmental health sciences, more prudent regulations, legislation and public health policy can protect the air, water, soil and food sources on which we depend. Errors made in the past and lack of understanding about the by-products of technology have proven to have a substantial effect in the present. The research conducted today, and the decisions based upon it may well prove crucial to the health and happiness of our own old age, and the futures of our children, grandchildren and their heirs.

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

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

I. INTERNATIONAL PROGRAMS

NIEHS conducts scientific exchanges and collaborative research through a variety of mechanisms that extend from informal contact between investigators in a number of countries to formal bilateral and multilateral agreements for cooperation between the U.S. and the governments of other countries.

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.

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 1986, 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. During 1987, NIEHS hosted an informal meeting of representatives of IPCS-PI's from the American Region. The Director, Environmental Health, WHO, and the Manager, IPCS, reviewed the activities of the IPCS and the PIs discussed their work related to the objectives of the IPCS. The meeting greatly facilitated an exchange of information and hopefully will lead to joint activities between PIs under the IPCS umbrella.

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. The Fifth PAC met in Geneva in FY 1987. The Director, NIEHS,

attended this meeting and the Assistant to the Director for International Programs served as Rapporteur. 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 also 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; (5) Approaches Needed to Establish the Role of Chemical Agents in the Etiopathogenesis of Certain Non-Communicable Diseases and (6) Immunotoxicology. Also, during 1987, NIEHS staff continued to review IPCS criteria documents, working papers, and proposed projects.

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 1987, NIEHS staff also participated in the International Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) dealing with methods to reduce injury due to chemical accidents. Also, the Director, NIEHS, and the Assistant to the Director, NIEHS, participated in a SGOMSEC planning meeting in 1987 to outline the workshop on "Methods to Assess the Adverse Effects of Pesticides on Non-Vector Targets," to be held in Czechoslovakia in 1988. 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 1987, 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. During 1987, the Director, NIEHS, was the DHHS delegate to the U.S.-P.R.C. Joint Commission on Science and Technology meeting held in Beijing. The status and progress of various collaborative areas were reviewed and potential new areas of cooperation identified.

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 Coordinating Council for North American Affairs. For the past five 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 related chemicals. Preliminary data show that the children have abnormalities of pigment, nails, teeth, and skin; and show a developmental delay on a number of dimensions. The data is being analyzed and further followup of this cohort is being considered.

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, provides 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.

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. Several NIEHS scientists have visited research and government organizations in India to discuss NTP's research findings on the toxicity of methyl isocyanate. NIEHS also hosted the visits of a number of scientists from India to discuss toxicological research activities.

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-tetrachlorodibenzodioxin, studies on the chemical contamination of drinking water, chemical selection procedures, and the design of two-year toxicity studies.

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. The Director, Division of Biometry and Risk Assessment, NIEHS, serves as a member of the Panel; and the Assistant to the Director for International Programs, NIEHS, serves as the Panel's program officer. 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 State Department appointed member of the Joint Committee which oversees the overall activities of the US-Japan Cooperative Medical Sciences Program. This Joint Committee meets annually, alternating between the U.S. and Japan. During FY 1987, the Director, NIEHS; and the Associate Director for Genetics, NIEHS; and the Assistant to the Director for

International Programs, NIEHS, participated in the 15th US-Japan Joint Environmental Panel Conference on "Approaches for Human Population Monitoring", held at Lawrence Livermore National Laboratory, Livermore, California. Also, during 1987, the Director, NIEHS, participated in the US-Japan Joint Subcommittee Meeting on Program Review and Planning, and the 23nd 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 1987, 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 have been initiated.

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 biological effects of low frequency electromagnetic radiation. 1987 was the fifteenth 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. Duplicate collaborative experiments 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 have been completed. A large amount of scientific information has been exchanged during these collaborative activities.

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 1987 on the evaluation of the genetic effects of low levels of environmental chemical mutagens in bacterial systems, and comparison with eukaryotic cells.

II. 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 also coordinates the NTP's review of the toxicological profiles prepared under the Superfund Amendments and Reauthorization Act of 1986, and is a member of the Technical Advisory Committee of the Governor's Waste Management Board of the State of North Carolina.

HEALTH AND SAFETY OFFICE
Summary Statement

The NIEHS Health and Safety Office is administratively located within the Office of the 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 185 active protocols for use of hazardous chemicals in force during FY87. 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 53 biological safety cabinets are in use at NIEHS. All biological safety cabinets are tested and certified annually by an independent testing firm.

During FY87 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. In FY86 a special sampling program was initiated for evaluating exposures to airborne acrylamide dust. This program was expanded and continued during FY87. Special air sampling studies were completed to evaluate hard wood dust exposures among animal bedding handlers. An independent study was completed to evaluate performance of necropsy hoods used at NIEHS for control of formaldehyde vapors. A wipe procedure for monitoring benzo(a)pyrene (BaP) residues on laboratory surfaces was designed and tested.

The Industrial Hygiene Laboratory continues to be an important component of Health and Safety Office programs. A quality control program for the industrial hygiene laboratory was expanded. This program includes written

procedures, preventive maintenance schedules for instrumentation, calibration procedures and recordkeeping requirements. As a part of the quality control program, NIEHS participates in the NIOSH Proficiency Analytical Program (PAT). During FY87 PAT samples for various organic solvents and asbestos were analyzed on a quarterly basis to evaluate laboratory quality control procedures. The NIEHS laboratory was rated proficient on each round.

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 76 occasional users of respirators at NIEHS during FY87. In addition to qualitative fit testing, respirator users are given a complete overview of the respiratory protection program. An annual 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 conducted a detailed chemical inventory and 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 available for NIEHS compliance was completed during FY86. During FY87 a detailed written hazard communication program was developed for NIEHS. Initial program implementation including supervisor training was completed in FY87.

Important components of the NIEHS hazard communication program are the chemical inventory and the file of company material safety data sheets. During FY87, a computer system for maintaining the chemical inventory was developed and initial data entry and editing completed. This inventory includes more than 60,000 entries with approximately 14,000 unique chemical compounds or mixtures. In order to assist NIEHS with material safety data sheet aquisition, a support contract was awarded during FY87. The contractor will request data sheets from suppliers and develop appropriate files and indices for use by NIEHS employees.

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 250 active protocols for the use of radioactive material. Approximately 1600 shipments of radioactive material were received in FY87.

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.

Incineration has been a vital method for radioactive waste reduction and treatment for several years and is becoming even more important as other alternatives are being reduced or eliminated. In FY87 the Health and Safety Office published the results of a study on the incineration of 3H and 14C labeled wastes and began a study on the incineration of 35S. A finger-tip dosimeter for beta radiation has been developed and an article submitted for publication. Additional studies were initiated in FY87 to evaluate our urine bioassay program and to evaluate the use of newly introduced biodegradable liquid scintillation fluids.

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. Hazard Communication training for laboratory personnel was initiated during FY87.

NIEHS Security Personnel received training in emergency medical response and fire extinguisher use. The certification program for forklift operators was expanded to include appropriate shops and maintenance personnel.

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 training programs include confined space entry and lockout/tagout procedures for Office of Facilities Engineering personnel. 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 FY87 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 and FY87 fire protection features were upgraded. All renovated buildings now have fire control panels that can eventually be tied into the guard station. The Durham County Fire Marshall continued annual fire inspections for all NIEHS

properties. Suggestions made by the Fire Marshall were implemented. During FY87 the NIEHS Occupant Emergency Plan was reviewed and updated as necessary. The building evacuation team was expanded to include all NIEHS buildings.

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 during FY86 and FY87. 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. During FY87 a second allergy questionnaire was administered to evaluate allergy incidence. A clinical surveillance program for NIEHS animal handlers was initiated in FY87 and includes allergy questionnaires, RAST testing and skin prick testing for atopy.

The Employee Assistance Program which was developed and implemented in FY86 continued during FY87. This program provides employees with assistance in dealing with psychological or emotional problems potentially affecting work performance. During FY87 a program to assist employees to stop smoking was provided. In conjunction with the Executive Officer, investigations concerning development of an official NIEHS fitness program were initiated.

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 FY87 NIEHS injury and illness data for FY86 were analyzed. NIEHS injury and illness incidence rates were found to be 3.8 OSHA reportable cases per 100 employees and 1.5 lost work day cases per 100 employees. These rates are significantly lower than comparable rates for the Federal sector although slightly higher than NIEHS rates for FY85. 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. An expanded hazardous waste disposal contract was awarded in FY87.

New small quantity generator regulations promulgated in March, 1986 require that facilities that treat hazardous waste on-site to obtain a Part B waste permit. In FY87 NIEHS notified the State of North Carolina concerning its on-site waste treatment activities and is currently operating under interim status as an incinerator of hazardous wastes. NIEHS will submit Part B of the permit application in first quarter of FY88. The Part B application is an

extensive document consisting of maps, diagrams, plans and facility design information. In addition, to demonstrate appropriate incinerator performance, NIEHS will conduct a trial burn and submit results to the State of North Carolina for review.

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 protocols for detailed environmental audits were developed and reviewed. These protocols provide a suitable framework for evaluating and documenting compliance with the wide range of environmental regulations with which NIEHS must comply. During FY87 the following audits were conducted: air emissions, solid and hazardous waste management, bioenvironmental hazard/toxic material handling and disposal and hazardous materials management. In general, NIEHS was found to be in compliance with applicable regulations. Problem areas which were identified have been addressed and audit protocols updated where necessary.

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, all NIEHS underground storage tanks were tested for leaks. Several tanks on the North and South Campuses have not passed the initial leak test and were further investigated. These investigatons suggested a long standing problem with the six 20,000 gallon power house tanks requiring remedial action. Remedial actions plans were developed and implemented. An above ground oil storage facility was proposed and design initiated in 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 17,600 books on environmental health, participation in a nation-wide network for interlibrary loan and cataloging, procurement of 2,100 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 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 FY87, Library personnel performed comprehensive multi-database searches on some 3,300 topics for Institute investigators and administrators. This was an increase of 32% over last year. Besides online searches, 12,000 other reference questions were answered. The most heavily used online databases continued to be TOXLINE, MEDLINE, Hazardous Substances Data Bank, Biological Abstracts, and Chemical Abstracts. Examples of search requests include the following:

- Information on AIDS, the ability of the virus to cross the blood/brain barrier, and the synthesis and pharmacology of AZT. (For DIR)
- The areas of high technology where DHHS conducts or sponsors research or makes use of it in the course of day-to-day activities. (For Dr. Rall and OPPE)
- Product evaluation summaries for HASP and other computer programs. (For DBRA)
- The adverse effects of nitrous oxide on animals and humans. (For DIR and DBRA)
- The relationship between chemical exposure and leukemia in rats. (For DTRT)
- Identification of DNA adducts or protein-bound products of 2-acetylamino-fluorine from hepatic or liver microsomes. (For DTRT)

The Library staff began the third year of training investigators to do their own online literature searches. The number of trainees increased from forty to eighty on the PaperChase System, a user-friendly version of MEDLINE. Now, NIEHS scientists can choose between having searches done by Library Search Analysts or doing the routine 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 FY87 and ordered 400 subscriptions for the various laboratories. Issues were checked in and missing ones claimed using a new microcomputer system. The Library continued to bind journals selectively or replace them with microfilm to save space; 711 reels of microfilm of older volumes were purchased to extend the collection which now includes 15,300 journal volumes and 2,289 microfilm reels. The last free space was used for adding more shelves.

Book Collection: Continuing the development of the book collection, the Library ordered 2,100 books in FY87, of which 37% were ordered for the Library and 63% for the laboratories. The Library also ordered more than 1,000 government reports.

Computer Catalog: FY87 was the fifth 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 FY87 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 stabilized in FY87 at 21,270. For the fifth year in a row, more of the requests were filled from the Library collection (62%) than from other libraries through interlibrary loan (38%). 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. Also, the Library initiated a new program called INTERLIB on the Institute's VAX computer. Scientists can use this program to send interlibrary loan or photocopy requests to the Library by electronic mail. 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 1,300 loans or photocopies to other libraries or to individuals in the Research Triangle Park area. This 100% increase over last year 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 725 manuscripts were written by NIEHS scientists during the year. The Library published the 1986 NIEHS Bibliography, a catalog of the papers published by Institute personnel during NIEHS' first twenty years.

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 managed the DBRA branch library.

Offices and Meetings: Dav Robertson maintained close contact with various library and information organizations in FY87. He served as Secretary of the N.C. Chapter of the Special Libraries Association (SLA) and Chairman of the Nominating Committee for the Environmental Information Division of the national SLA. He was Past President of the RTP Association of Librarians and Information Specialists (TRI-LIBS). He also served on the Advisory Council for the N.C. Library Staff Development Program centered at N.C. Central University. Mr. Robertson represented NIEHS at the national meeting of SLA in Anaheim and the quarterly chapter meetings in North Carolina. He presented a paper on using microcomputers to communicate with mainframes at the C.L. Systems Users Group meeting in Boston, and he chaired a session at their meeting in Orlando. Larry Wright represented NIEHS at the National Online Meeting for computer search specialists in New York, and he was elected to the Board of Directors of the N.C. Chapter of SLA. He also served on the Nominating Committee for TRI-LIBS, and he gave a lecture on searching the Chemical Abstracts Online System to the UNC School of Library Science.

OFFICE OF FACILITIES ENGINEERING
Summary Statement

Responsibilities:

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, and storage and supply of construction and operations materials.

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

OFE is divided into four functional, cohesive sections under the Office of the Chief.

(1) The Facilities Management Section (FMS) is the coordination point for all service requests and work orders, providing planning, estimating, scheduling, expediting, 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 Special Projects Section (SPS) coordinates planning and design for construction of an addition to Building 101, related site improvements and utilities systems expansion on the South Campus, as well as other special design and construction projects.

(4) The Facilities Maintenance and Operations Section provides construction, renovation, repair service and maintenance and storage of shops materials and parts for all NIEHS buildings, utilities systems and installed equipment at both North and South Campuses. Trades include carpentry, locksmith, masonry, plumbing, pipefitting, sheet metal, welding, painting, utility system repair/operator, boiler plant equipment mechanic, and electrical. This section operates and maintains the NIEHS power plant at South Campus. The power plant houses two 40 million BTUH High Temperature Hot Water boilers and two 2500 ton air conditioning chillers. These systems are in continuous operation and provide heating, cooling, and humidity control for all South Campus facilities.

The Maintenance and Operations Section also provides 24 hour a day service to South Campus buildings, monitoring all environmental systems through the JC-80 computer system.

Accomplishments:

North Campus improvements: Our largest single construction project of FY 87 is complete; i.e., the new Magnetic Resonance Imaging (MRI) facility. The 4,850 square foot wooden structure, completed at a cost of $485,080, was occupied in March 1987. Buildings 2, 4, and 15 also received extensive renovations. Alterations will commence in late FY 87 in Building 14, thus completing our major renovation program of North Campus facilities.

South Campus improvements: A bulk CO_2 system was installed at that site to replace the cumbersome and costly individual cylinders previously used. Additionally, the open basement of "A" module was converted to usable space for ultimate occupancy by the NIEHS Library. A new sidewalk was installed between the large parking lot and Module "E" and Building 101's roof was repaired. Backflow prevention devices were installed in fire protection water lines throughout the campus to preclude possible potable water contamination.

Design is underway for repair/replacement of defective valves in the basement of Building 101, which will enable large pumps to be isolated for repair and allow the various modules to be isolated for maintenance or repair, thus avoiding the entire complex being shut down. Also under design is a project to rework the campus high temperature hot water and chilled water distribution system pumps for much more efficiency, as well as construction of a laboratory floor in the existing high bay space within "E" module, thus providing another 22 laboratories for program research.

OFE has made many accomplishments in the routine administrative functions it performs for continued service requirements to the Institute. We have established and "fine tuned" some of the paperwork flow which had bottle necks in routine matters. Expansion of the automated system for paperwork has improved a lot of the time consuming data base type work.

The following addresses the accomplishments and future goals for OFE computer applications and usage.

The OFE Work Order computer system was expanded to include use by the foremen and supervisors of eight craft shops and support departments of OFE. Standing Work Order processing was also instituted for the maintenance shops to increase the performance of work for minor, non-planned work requests. A work order scheduling feature was implemented.

The OFE Record-of-Call computer system was enhanced with a new sub-system for contract tracking. These two systems, the Contract Tracking and Record-of-Call systems, provide automated support for OFE contract information and allow for complete generation and tracking of Records-of-Call and 2345 payment authorization documents.

The Records-of-Call and 2345's are monitored completely through their processing cycle.

The Space Management system has been of tremendous importance for answering questions and satisfying information requests. Information has been provided to all levels of NIEHS and NIH management.

OFE has recently completed a two year inventory project for the facility that includes all building equipment that supports the NIEHS research facility. This information has been automated into the OFE Equipment Inventory computer system that allows for complete update and display capability. In addition, there are sub-systems to the equipment inventory system to support preventive maintenance and equipment check information that was also gathered during this two-year project.

The data maintained by this system and the related sub-systems represent the base information that will be used in a comprehensive maintenance management system.

Plans:

For FY 88, we look forward to contruction of 22 laboratories in Module E, the revalving of Building 101 to preclude large shutdowns of utility services, improvements to the variable air volume (VAV) system in Modules "A" and "B", and continuation of minor office and laboratory renovations with our Task Order contractor.

Design for the additions to Building 101 and support facilities will be completed in the summer of 1988, and construction contracts awarded that fall if funds continue to be appropriated for the project. The additions to Building 101 will add some 57,500 square feet of laboratory facilities and 29,700 square feet of office space.

OFE is planning to obtain a software product to help manage the maintenance requirements at the Institute. This comprehensive system will support and enhance existing and planned maintenance activities as well as the preventive maintenance activities expected in the future.

Institute bar code processing techniques in the Stockroom system will greatly enhance the operation and function of the storeroom that maintains an inventory of material and tools.

DIVISION OF INTRAMURAL RESEARCH

DIVISION OF INTRAMURAL RESEARCH
Summary Statement

Scientists in the Division of Intramural Research (DIR) of the National Institute of Environmental Health Sciences (NIEHS) have the primary mission of investigating how living organisms, especially the human, react and adapt to a constantly changing and, in many respects, hostile environment. Considering the complexity of earth's environment and the additional potentially dangerous materials introduced into the environment by humans, it is not surprising that the mission of DIR requires an array of scientific disciplines encompassing nearly every field of biology and chemistry. Unique among the NIH intramural divisions, DIR has no clinical responsibilities; its purpose is not disease-oriented. On the other hand, to the extent that the environment poses risks to human health, DIR scientists develop the basic knowledge necessary for colleagues in other divisions of NIEHS to test and evaluate chemicals and other substances for their risk to human health.

For their investigations, DIR scientists use whatever biological systems or materials they deem appropriate. The research ranges from experiments with isolated molecules such as DNA using sophisticated molecular biology techniques, to monitoring the levels of calcium ion in the beating heart of a living animal. The latter studies utilize nuclear magnetic resonance, a procedure requiring instruments housed in special facilities devoid of ferrous-containing materials. A new NMR facility, completed in 1987, will greatly expand the capacity of DIR to carry out research on whole animals.

DIR is also richly endowed with other sophisticated instruments designed for detecting free radicals (an important factor in the production of toxic chemicals) and for characterizing structure (Mass Spectrometry). DIR is fortunate to have Dr. Kenneth Tomer as the new Head of the Mass Spectrometry Unit. Recently acquired is a fluorescent spectrometer that permits sensitive detection of rapid changes in intracellular calcium ion concentrations. This instrument will facilitate studies on calcium ion regulation in the newly organized Laboratory of Pharmacology under the direction of Dr. James Putney (see below).

Research involving the heavy use of animals (mostly rodents), such as in neurotoxicology and reproductive biology, requires animals that are healthy, untraumatized by their immediate environment, and which can be bred to give the needed varieties and stages of development of the animals. The Comparative Medicine Branch (CMB) of DIR under Dr. Herbert Amyx, its new Chief, has one of the finest animal resource facilities in this country. Under his strong leadership, CMB not only maintains and breeds animals, it trains scientists and technicians within NIEHS to properly handle and care for animals during their experiments.

Basic research in DIR is conducted in six Laboratories which encompass the following general research disciplines: Behavioral and Neurological Toxicology (LBNT), Pharmacology (LP), Pulmonary Pathobiology (LPP), Reproductive and Developmental Toxicology (LRDT), Molecular Biophysics (LMB), and Genetics (LG). The summary reports provided by the Laboratory Chiefs are a capsule overview of the substantive research efforts recorded in the individual progress reports. These reports show a remarkable diversity of research effort reflecting the broad interests and expertise of DIR scientists. There are underlying themes, however, that suggest an emerging pattern of thought and effort seen in

contemporary biology across institutions and international boundaries. Although certainly oversimplified, the pattern that emerges is an effort to understand how biological systems integrate the processing of information at all levels including the basic genomic structure which is the repository of all cellular information. Some illustrative examples of this pattern are distilled from the resumes of the six Laboratories.

Using Drosophila as model biological systems, it seems that transposons (retrovirus-like material) play a critical role in the structure and regulation of gene expression through their insertion at critical regions of the Drosophila genome. When inserted, regulatory proteins interact with these critical regions causing mutations that depend on the relative positions of the transposons, the sites of promotion of gene transcription, and other factors that probably reflect the architecture of the chromosomes. "Trans" acting regulatory proteins seem to play several roles in the mutation process, including suppression and activation of transposon-induced mutations. Clearly, the direction of research in this area is to understand the complex architecture of chromosomes and how various protein factors interplay with these structures to allow regulation of insertion and action of transposons, important elements in mutational events. Since both survival and adaptational changes to the environment depend on the types and sites of action of transposon elements, this area of research is critically important for understanding naturally occurring mutations.

Mutations are also influenced by the fidelity of DNA synthesis carried out by DNA polymerases of various types. Fidelity is determined partly by the selective discrimination of base-pair insertion during polymerization (termed base-selection) and removal by exonucleases of misinsertions or misalignments after bond formation (proofreading). With the use of a selectively modified bacterial DNA polymerase lacking the exonuclease activity necessary for proofreading, it has been possible to study in a more precise manner the contributions to fidelity of base-selection and proofreading. The powerful technique of site-directed mutations in genes allows for the first time the production of polymerases having selective changes in activities and thus the means of determining the precise role of different polymerases and exonucleases in DNA fidelity. Future studies of this type should provide new insights into the nature and frequency of mutational events.

Reproductive and developmental processes in biology are also largely dictated by the regulation of gene structure and read-out. For example, estrogens and various growth factors (positive and negative) act at discrete phases of cell or organ development through receptors that control gene expression and ultimately the types of proteins synthesized and secreted by the target cells. Recent studies now indicate that growth factors mediate certain actions of estrogens on mammary tissue. Estrogen-induced growth and tumorigenicity seem to reflect mechanisms independent of estrogen receptors but through parallel pathways involving growth factors. Of general interest are findings that estrogen receptor-deficient cells may be most susceptible to cancer induction.

Increasingly, research on these subjects tend toward isolation of genes and their protein products. One of the ultimate goals is to localize both the genes responsible for specific reproductive and developmental events and where the products are acting topologically and with respect to cell type. In such research, antibodies raised against the gene products have proven valuable for determining developmental patterns and for identifying cell types during, example, spermatogenesis. In addition, polyclonal antibodies against P-450 enzyme species are being used, particularly when coupled to gold particles, for

determining intracellular distribution as well as cellular distribution of these enzymes and their subtypes. From such research, there is now a better understanding of where the various P-450 isozymes are located in lung, liver, and other cells. Most importantly, it appears that the enzymes are localized in membranes, particularly the outer surface structures. Androgens, such as testosterone, have been demonstrated to regulate the read-out of genes for P-450 enzymes in liver and kidney where two isozymes are present in reciprocal concentrations depending on the sex of the animal (mouse). Thus, for the first time, an explanation is at hand for the long-known sex determined levels of certain enzymes in specific cell types. Clearly, the direction of research is how and where in the animal sex hormones regulate the production of P-450 enzymes critically involved in the metabolism of numerous toxic chemicals. These are a few examples of new investigative procedures being widely used by DIR scientists in their quest for resolving important issues in cellular biology.

Important discoveries have been made this year regarding the effects of particles (asbestos, silica) on the pathobiology of lung. For example, it is now clear that macrophage involvement in asbestosis involves the production of growth factors resembling or identical to platelet derived growth factor (PDGF). This growth promoting substance enhances the production of fibroblasts, a process that probably accounts for the early inducement of pulmonary fibrosis after administration of appropriate sized and charged particles. In a related study, silica has been shown to cause marked accumulation of lung surfactants in rabbits, producing a disease state reminiscent of alveolar proteinosis. The nature of the factors that regulate pulmonary surfactant levels is clearly a high priority subject for future investigation.

In addition to growth factor-induced production of fibroblasts, asbestos and other particles can induce tumors in epithelial cells. Employing transformed Syrian hamster embryo (SHE) cells, DIR scientists have shed new light on factors involved in carcinogenesis that may relate to particle-induced tumors. One basic thesis is that cells contain "suppressor" genes that control growth by suppressing the activities of growth-promoting factors, including carcinogens and mixtures of oncogenes. They now have evidence suggesting that monosomy of chromosome 15 in SHE cells is an essential result of asbestos (and other carcinogenic materials such as diethylstilbesterol) treatment, the suggestion being that disruption of chromosome structure rather than gene mutation is responsible for initiating the carcinogenic process. Moreover, it is possible that chromosome aneuploidy is the cause of suppressor gene loss in cells exposed to carcinogens such as asbestos. Adding further credence to the multi-stage features of carcinogenesis is the finding that a specialized oncogene, fms, related to hematopoietic growth factor is increasingly expressed at late stages of tumorigenesis in SHE cells. Hence, these studies once again emphasize the directions of research in DIR toward fundamental studies on the cellular and molecular biology of the regulatory components involved in normal and abnormal proliferation of cells.

DIR has made this year two organizational changes that should profoundly affect its ability to expand its efforts to understand the basic biology of regulatory processes. Dr. James Putney, appointed a year ago to head a Calcium Regulation Group, has been made Acting Chief of the Laboratory of Pharmacology. Under his direction, emphasis will be placed on the mechanisms of signal transduction, particularly as they apply to translocations of ions (calcium, potassium, etc.) which are known to be involved in cellular growth and differentiation. The other major change in leadership is the reassignment of Dr. Andres Negro-Vilar

from LRDT to Acting Chief, LBNT. Emphasis will be placed on signal transduction pathways in the complex neurological systems. Dr. Negro-Vilar's laboratory has made important discoveries in the area of neuropeptide secretion by the hypothalamic-pituitary axis. His laboratory has made large strides in understanding the pulsatile nature of neuropeptide secretion and action.

This past year has also seen a major increase in communications among scientists in DIR. A series of three "retreats" were held at Quail Roost Conference Center, Rougemont, North Carolina in which 14 scientists each spent two days discussing their research in an informal manner. By all accounts, the retreats were a success; this has been exemplified by several new collaborative studies established by the participating scientists as a result of their interactions at the retreats. Selected scientists have also given 30 minute "lay" presentations of their work before the Division Leaders and the Director of NIEHS during their weekly meetings. These presentations have increased the awareness of the types of high quality basic research carried out at NIEHS.

The additions noted above of Drs. Amyx, Putney, and Tomer to our permanent staff, have already had a major impact on the morale and direction of research in their groups. The inauguration of the Intramural Research Training Act now enables DIR to attract young postdoctorate trained in the U.S. On balance, therefore, 1987 seems to have been an excellent year from the viewpoint of science and personnel performance.

One major tragedy was the untimely death of Dr Robert M. Pratt, Head of the Experimental Teratogenesis Workgroup in LRDT. An eminent scientist in his field, he is irreplaceable. Perhaps even more importantly to NIEHS, he was dedicated to NIH and its principles of high quality research and intellectual freedom. He was the Head of the DIR Committee on Promotions responsible for the peer review of investigator promotions, conversion and appointment, a most difficult and important DIR committee. He worked hard to keep its standards high and to be fair toward his colleagues. He will be remembered always for his wonderful personal qualities. His death is deeply mourned by all.

As a final note, the Scientific Director was elected as Member of the U.S. National Academy of Science, and awarded jointly by that body and the French Academy of Science its most prestigious scientific award, the Lounsbery Award for Medical Science of 1987.

LABORATORY OF BEHAVIORAL AND NEUROLOGICAL TOXICOLOGY
Summary Statement

Research in the Laboratory of Behavioral and Neurological Toxicology is designed to acquire basic information concerning the structure and function of the nervous system. The ultimate goals of the program are to stimulate new research trends in neurobiology and make available newly discovered principles and observations and appropriate research procedures to detect and study behavioral and neurological abnormalities associated with environmentally related diseases. The research is predicated on the principle that alterations in neurobehavioral and neurophysiological function are associated with changes in the dynamic balance of neurotransmitters, neuromodulators, and neurohumoral factors, all of which ultimately affect the signal transduction process. The methods and approaches are frequently interdisciplinary in nature. Studies at the neurobehavioral level focus on the development of models of neurodegenerative processes, including alterations in cognitive and motor function. Neurophysiological studies investigate the biophysical and molecular neurobiology of excitable cells and mechanisms of neuronal plasticity. The Neuropharmacology group studies the regulation and interaction between neuropeptides and neurotransmitters at cellular and molecular levels and their functional roles in neurological diseases, such as extrapyramidal disorders or epilepsy. The Peptide Neurochemistry Group isolates neuropeptides and studies their pharmacological and physiological roles in the neuronal function of peripheral events and correlates central action with receptor analyses.

Neurobehavioral Group

Research in this group focuses on two main topics: 1) development of animal models of neurological disease by utilizing neurotoxicants to mimic specific aspects of disease processes, including cognitive and motor dysfunction. 2) study of neurobiological mechanisms involved in restoration of function at the neurobehavioral and neurochemical levels.

Results obtained in the last year characterized further the use of intracerebral administration of colchicine as a model of cognitive dysfunction. Colchicine applied into the hippocampal dentate gyrus destroys granule cells and mossy fibers, depletes levels of neuropeptides and induces cholinergic hyperinnervation of the hippocampus, all of which are associated with deficits in learning and memory. Pharmacological manipulations, which are designed to improve function by restoring the dynamic balance at the signal transduction level, found that the performance of colchicine-exposed animals is improved by cholinomimetics. Additional studies found increased levels of acetylcholine transferase and a decreased number of muscarinic receptors in the hippocampus of colchicine-treated animals, suggesting a hyperinnervation of the hippocampus. Neuronotrophic factors, such as nerve growth factor or gangliosides, given systemically or directly to the damaged area, did not affect colchicine-induced damage to the dentate gyrus. Systemic administration of gangliosides, however, did attenuate the neurochemical effects of 6-hydroxydopamine, a neurotoxic agent used in models of Parkinson's Disease. In other studies, a suspension of undifferentiated fetal cells was injected into the damaged area to promote regeneration and/or recovery of function; attenuation of colchicine-induced behavioral effects was observed. Colchicine was also injected into the area of the nucleus basalis, an area frequently affected by neurodegenerative diseases, and deficits in learning and memory associated with loss of presynaptic cholinergic marker in

the frontal cortex was observed. In other experiments concerning models of motor dysfunction, it was observed that brainstem-mediated tremor and hyperreflexia could be attentuated by alpha-1 adrenergic receptor antagonists and accentuated by 5-HT1 receptor agonists. These and other studies indicate that descending catecholaminergic and serotonergic fibers have an excitatory influence on spinal motoneurons.

Neuropharmacology Group

Research in this group focuses on two major areas: 1) molecular mechanisms underlying the regulation of brain opioid peptides and tachykinins by neurotransmitters or psychoactive compounds, and 2) seizure-induced alteration in the metabolism of hippocampal opioid peptides and their roles in the expression of seizures.

Results obtained in the last year clearly demonstrated that the dopamine (DA) system exerted potent regulation of the metabolism of enkephalin,dynorphin, and tachykinins, the three most abundant neuropeptides in the basal ganglia. Long-term blockade of DA transmission by a receptor blocker (haloperidol) or degeneration of DA neurons (lesion by 6-hydroxydopamine) increased the biosynthesis of enkephalin, but decreased that of dynorphin and tachykinins. In contrast, long-term enhancement of DA transmission by a receptor agonist (apomorphine) or by a DA releaser (d-amphetamine) produced the opposite effects. This finding reveals an important characteristic of peptide regulation, i.e., a long-term perturbation of neurotransmission is required to alter the biosynthesis of neuropeptides. Since most psychoactive compounds require chronic dosing to achieve clinical efficacy, this study raises an interesting possibility that gene expression may be an important site of action for certain psychiatric treatments.

The adrenal gland was used as a model to study molecular mechanisms underlying the regulation of gene expression of enkephalin and catecholamine systems which are co-stored in chromaffin cells. We have found evidence indicating that enkephalin biosynthesis is under transynaptic regulation. Enhanced transmission by reflex activation or direct electrical stimulation of the splanchnic nerve caused an increase in the synthesis of enkephalin. Using chromaffin cell cultures, we have demonstrated that activation of the cyclic AMP system increased the abundance of mRNA coding for tyrosine hydroxylase, but decreased that of phenylethanolamine-N-methyltransferase. This differential regulation of catecholamine synthesizing enzymes suggests an important function in the regulation of catecholamine secretion.

Other research studied the relationship between opioid peptides and seizures. Seizure activity induced by different experimental procedures alterated the release and biosynthesis of enkephalin and dynorphin in the hippocampus. Other evidence suggested that changes in the activity of these opioid peptides may participate in the development of seizures or mediate postictal behaviors. In addition, we have provided a neurochemical basis for the epileptogenic activity of enkephalin in the hippocampus by showing that disinhibition of GABA by enkephalin is a key event leading to the activation of pyramidal cells. 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.

Neurophysiology Group

Research in the Neurophysiology Group seeks to understand basic mechanisms underlying neuronal function. The hippocampal formation has been chosen as a "model" for these studies since it has been well defined anatomically, physiologically, and neurochemically, and it exhibits neuronal plasticity. The research focuses on two major topics: (1) modulation of neuronal function by neuropeptides (especially opioid peptides) and steroid 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. Two general approaches are used, including: (1) investigation of the biophysics and molecular biology of excitable cells, and (2) studies on the feedforward and feedback mechanisms influencing neuronal plasticity in the three major fiber tracts (perforant path, mossy fibers and Schaffer collaterals) within the hippocampal formation.

Current research has succeeded in developing a method for electrical stimulation of the perforant path (PP) which will elicit "wet dog shakes" (WDS) consistently and repeatedly in the absence of an overt seizure. Such stimulation significantly decreases hippocampal levels of enkephalin and dynorphin and increases GABA. Levels of these substances are only altered by stimulus parameters which elicit WDS. Moreover, intraventricular injection of either an opioid mu receptor (β-FNA) or delta receptor (ICI 174864) antagonist reduced the number of WDS elicited by PP stimulation. These data provide the first evidence that endogenous opioids are released by PP stimulation and lend further support to the notion that they play a role in regulation of hippocampal excitability.

Peptide Neurochemistry Group

Research in this group studies three interrelated areas: (1) chemistry and biochemistry of neuropeptides, including (a) the isolation, chemical characterization and structural analysis of new peptides from mammalian sources and the production of high affinity, site directed antibodies, and (b) the interaction of neuropeptides with specific receptor sites in synaptosomes; (2) pharmacology of neuropeptides which entails studies designed to determine the spectrum of bioactivity of neuropeptides; and (3) physiology of neuropeptides, which incorporates investigations on the central mechanism of action of neuropeptides on peripheral events.

Cation-exchange HPLC and copper chelation chromatography have been used to separate uterokinin active peptides from bovine milk into two populations, both exceeding 1800 daltons. Pharmacological assessment of the uterokinin activity of the milk-associated peptide indicated a similarity in action on rat uterus to bombesin, vasopressin and oxytocin. These milk peptides, in contrast to bombesin, fail to contract guinea-pig ileum.

Physiological studies suggest that both bombesin and lithium apparently alter prostaglandin biosynthesis since their suppression of gastric secretion is blocked by several cyclooxygenase inhibitors. Dermorphin and its dimeric analogs regulate gastric secretion through μ receptors in the brain. Interestingly, the action of the dimeric analogs are differentiated from dermorphin. The effect of calcitonin, which is more active peripherally than centrally, involves a complex mode and mechanism of action involving both central and peripheral sites. Work on the central modulation of the immune system has been initiated and the effect of neuropeptides on induced ulcer formation is proceeding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50015-13 LBNT

PERIOD COVERED

October, 1986 to September 30, 1987

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

Effects of Microwaves on Neural Response

PRINCIPAL INVESTIGATOR *(List other professional personnel 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:
2.0	0.75	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.)*

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^+-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 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. This project is being terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90033-05 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	William E. Wilson	Research Chemist	LBNT	NIEHS
Others:	L. H. Lazarus	Research Chemist	LBNT	NIEHS
	B. J. Irons	Biologist	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:
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

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

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

Efforts to purify bovine milk bombesin have revealed that it appears to comprise two chromatographically separable types of molecules: one type cross-reacts with an antiserum that recognizes the peptide sequence -Gly-Asp-Leu-Trp- of bombesin (residues 5-8); the second type comprises the milk uterokinins (m-uks), which stimulates contractility, as does bombesin, oxytocin, or vasopressin, on uterine smooth muscle while they do not contract guinea-pig ileum. The following physical and chemical properties of the m-uks have been determined: 1) they exhibit an apparent Mr in excess of 1800 daltons and, thus, are larger than bombesin; 2) m-uks possess cationic, but no free anionic, functionality; 3) at least two populations of m-uks are separable by cation exchange chromatography; 4) copper-chelation chromatography results indicate the m-uks may contain histidine, tryptophan, free amino groups, and/or cysteine; and 5) uv-absorption spectra of the most highly purified preparations are compatible with the possibility that m-uks contain tryptophan and histidine. Efforts to achieve final purification are continuing with the objective of elucidating the structures of these compounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90034-04 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Rabbit Stomach Peptide [Physalaemin-like Material (PHLIM)] 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. J. Irons	Bio. Lab. Tech.	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS
	C. Hamm	Electronics Tech.	LBNT	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, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

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

Physalaemin-like immunoreactive material (PHLIM) is the designation of the relatively prominent rabbit stomach macromolecule(s) whose structure(s) is/are not yet known. PHLIM appears to be glycoprotein and is presumed to be the metabolic precursor of the physalaemin-like octapeptide, PHLIP-8: <Glu-Val-Asp-Pro-Asn-Ile-Gln-Ala. Prior immunohistochemical studies, using antiserum to the amphibian peptide physalaemin, had indicated widespread distribution of cross-reactivity in the rat; thus, we proposed repeating that type of investigation using an appropriate antiserum to PHLIP-8. An attempt to develop an antiserum to [Lys^{0}]-PHLIP-8 was undertaken; however, problems encountered in obtaining either a suitable isotope-labelled analog or antigen, or both, have delayed our reexamination of the immunohistochemical profile of the physalaemin-like pepides in the mammal. This project will be continued by coupling [Lys^{0}]-PHLIP-8 to hemocyanin as the source of the antigen and the preparation of the new analog polyclonal antibodies. This approach will provide antibodies that recognize the N-terminal portion of PHLIP-8 (which has homology to physalaemin). This study directly impinges upon oat-cell carcinoma (which contains a physalamin-related peptide), diseases involving glycoproteins, and PHLIM function throughout the mammalian digestive and respiratory tracts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90037-04 LBNT

PERIOD COVERED

October 1, 1986 to January 1, 1987

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:	R. M. Booze	Neuropharmacologist	Dept. of Pharmacology	Duke Univ.

COOPERATING UNITS *(if any)*

Duke University

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research is to: 1) characterize the development of specific behavioral and neural functions accompanying perturbations of the hypothalamic-pituitary-adrenal axis (HPAA), 2) determine the physiological mechanisms by which abnormalities are expressed, and 3) identify specific environmental or pharmacological treatments which can mitigate the consequences of such physiological injury. Perturbation of the HPAA with chlordecone, an organochlorine which has estrogenic-like activity, had an organizational effect on sexual differentiation of neural tissue in rats. If dosing with chlordecone occurred on day 4 neonatally, the optimal time of sexual differentiation, sexually dimorphic alterations in functional and structural measurements (body weight regulation, gross motor movements, and general motor activity) were observed, suggesting an organizational effect. Treatment with chlordecone on day 11 of age had little or no sexually dimorphic effect, indicating a lack of an activational effect. It was also confirmed that chlordecone produces a generalized activation of adrenal steroids, as determined by the presence of circulating adrenal steroids at various times after treatment and an increased adrenal weight attributable to cellular hypertrophy/hyperplasia. These effects may explain long-term changes in circulating steroids in adult animals exposed neonatally to chlordecone. These data indicate that developmental exposure to agents having steroidal activity or those that stress the neonate causing a release of steroids can have long-term effects on the HPAA at adulthood. This is the final report for this project and no further work is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90038-04 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

PI:	Hugh A. Tilson	Pharmacologist	LBNT	NIEHS
Others:	L. Cook	Guest Worker	LBNT	NIEHS
	S. Shaw	Biological Aid	LBNT	NIEHS
	R. McLamb	Bio. Lab. Tech.	LBNT	NIEHS
	K. Nanry	Psychologist	LBNT	NIEHS

COOPERATING UNITS *(if any)*

North Carolina State University

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Neurobehavioral

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.25	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research is to characterize the neurological effects of organochlorine compounds, including chlordecone, p,p'-DDT and lindane. A single exposure to lindane interfered with the learning and memory capabilities of rats, supporting the observation that lindane has effects on long-term potentiation in the hippocampus. Posttraining administration of lindane did not affect retention at a later time, indicating that the process of memory consolidation was not altered. Our results also indicate that chlordecone and p,p'-DDT have few consistent effects on learning and memory. Chlordiazepoxide and phenobarbital, which have effects on GABAergic function, attenuated lindane-induced learning deficits. Phenytoin, which has little GABA activity, had no effect in lindane-treated rats. Similar pharmacological antagonism was seen for lindane-induced seizure activity. These data are consistent with the observation that lindane is a competitive inhibitor at the picrotoxin binding site within the GABA receptor-ionophore complex. In other experiments, the neuropharmacological mechanism of chlordecone-induced hypothermia was determined. Initial studies found an association between chlordecone-induced hypothermia and cutaneous vasodilation. Subsequent experiments found that direct administration of chlordecone into the fourth, but not third or lateral ventricle, produced hypothermia. Chlordecone-induced hypothermia could be attenuated by administration of phenoxybenzamine or phentolamine, but not propranolol or atenolol, into the fourth ventricle. These data are consistent with the hypothesis that chlordecone causes the release of norepinephrine in the area of the brain stem mediating peripheral vasomotor control. Activation of an alpha-1 adrenergic receptor in that area produces vasodilation, which may cause an increase in skin temperature and a decrease in core temperature. This is the final report of 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 90039-04 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

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

PI:	Jau-Shyong Hong	Pharmacologist	LBNT	NIEHS
Others:	S. P. Sivam	Senior Staff Fellow	LBNT	NIEHS
	S. Li	Visiting Fellow	LBNT	NIEHS
	P. Hudson	Bio. Lab. Tech.	LBNT	NIEHS
	M. Stachowiak	Senior Staff Fellow	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, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	0.3	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 objectives of this project were: 1) to understand the basic biosynthetic process of opioid peptide and tachykinin systems by employing molecular biological and immunochemical techniques, 2) to elucidate how the classical neurotransmitter systems such as dopamine (DA) interact with opioid peptides and tachykinins in the basal ganglia following the perturbation of dopaminergic transmission. The methods used include the quantitation of specific mRNA and the precursor content as well as the steady state peptide concentration. Long-term blockade of DA receptors with a DA antagonist, haloperidol (an antipsychotic drug), caused interesting changes in the expression of neuropeptides in the striatum: increases in [Met^5]-enkephalin (ME) but decreases in tachykinin. This finding not only suggests that the DA system exerts important regulation on these two peptide systems, but also raises an interesting concept that gene expression of neuropeptide systems may be a potential site of action for antipsychotic drugs. The effects of haloperidol on these two peptides were mimicked by intranigral injection of 6-hydroxydopamine which selectively destroys the nigral-striatal DA pathway. These results add further credence to the concept that DA plays an important role in regulating the metabolism of ME and tachykinin. To determine if enhancement of dopaminergic transmission would produce opposite effects on these peptides, apomorphine, a DA agonist, was employed. Repeated injections of apomorphine increased the abundance of mRNA and peptide content of tachykinin and dynorphin, but failed to alter ME content in the basal ganglia. This finding plus the aforementioned results strongly suggest the following: 1) DA exerts strong inhibitory influence on the biosynthesis of ME and this tonic inhibitory effect is maximal under physiological conditions, 2) DA enhances the biosynthesis of tachykinin and dynorphin and this tonic excitatory effect is not maximal under physiological conditions. Striatal cell cultures will be employed to determine the subcellular events which link the DA receptor activation to gene expression of opioid peptides and tachykinins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90042-02 LBNT

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Models of Neurodegenerative 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
	J. S. Hong	Pharmacologist	LBNT	NIEHS
Others:	R. McLamb	Bio. Lab. Tech.	LBNT	NIEHS
	P. Pediaditakis	Biological Aid	LBNT	NIEHS
	J. Peterson	Bio. Lab. Tech.	LBNT	NIEHS
	B. Mundy	Staff Fellow	LBNT	NIEHS
	C. Hamm	Electronics Tech.	LBNT	NIEHS
	J. Harry	Guest Worker	LBNT	NIEHS
	[illegible]	Biologist	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:
4.25	1.25	3.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research is to: 1) develop animal models of neurodegeneration using neurotoxicants to mimic neurological disorders, with an emphasis on understanding the adaptive and compensatory changes associated with insult to the nervous system; 2) establish neurobiological principles concerning restoration of function. Intradentate hippocampal infusion of colchicine causes dose- and time-dependent loss of granule cells and mossy fibers, decreases in neuropeptides and deficits in spatial, short-term memory. We also found that the memory deficits observed in colchicine-treated rats were attenuated by cholinomimetics, agents reported to be effective in some patients with Alzheimer's Disease (AD). Noncholinergic agents, such as naloxone, piracetam and vasopressin had no effect. Colchicine-treated rats were also less sensitive to the pharmacological effects of scopolamine, a muscarinic receptor antagonist; this effect was associated with an increase in a presynaptic marker for acetylcholine and a decreased number of muscarinic receptors in the hippocampus, suggesting a cholinergic hyperinnervation. In an attempt to promote regeneration or reactive synaptogenesis, direct application of neurotrophic factors such as nerve growth factor or gangliosides at the time of lesion did not affect colchicine-induced damage. In other studies, a suspension of undifferentiated fetal hippocampal cells was injected into the hippocampus of colchicine-treated rats and an attenuation of colchicine-induced behavioral effects was observed. Colchicine injected into the nucleus basalis, an area known to be affected in AD, also produced deficits in learning and memory, which were associated with a loss of a presynaptic cholinergic marker in the frontal cortex. The performance of these rats was also improved by cholinergic agents. We plan to investigate the conditions necessary to reestablish connections following implantation of undifferentiated cells into damaged areas and, because of the observed hyperinnervation, to determine changes in cholinergic stimulation of phosphoinositol metabolism in lesioned areas of the central nervous system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90043-02 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 effects of KA. When given 15 minutes after the subcutaneous injection of KA, it markedly potentiates KA activity. Dithizone also produces a transient decrease in hippocampal levels of enkephalin and dynorphin. It also produces transient increases in the hippocampal levels of a number of amino acids (viz., taurine, glutamate, glutamine, and GABA). It appears, then, that dithizone may prove to be a useful tool for exploring the actions of zinc on the hippocampus. Work in progress involves: (1) further characterization of the changes in peptide and amino acids induced by dithizone, and (2) examination of the 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 90044-02 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 this laboratory has focused on the role of enkephalin and dynorphin 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 has also implicated the dentate granule cells (DGCs) as being necessary for the elicitation of WDS at least with respect to induction by kainic acid or by stimulation of the perforant path (PP). The first objective of this project was to develop a method of electrical stimulation of the PP which would elicit WDS consistently and repeatedly in the absence of an overt seizure. Using this method, we have demonstrated that stimulation of the PP under conditions which elicit WDS produces a significant decrease in hippocampal levels of enkephalin and dynorphin. Levels of these substances are not altered by stimulus parameters insufficient to elicit WDS. Moreover, intraventricular injection of either an opioid mu receptor (β-FNA) or delta receptor (ICI174864) antagonist reduced the number of WDS elicited by PP stimulation. These data provide the first evidence that endogenous opioids are released by PP stimulation and lend further support to the notion that they play a role in regulation of hippocampal excitability. 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 (1) the relative contribution of the dorsal vs. ventral portions of the hippocampal formation in the phenomenon of WDS, and (2) whether or not there is a loss of recurrent inhibition on the DGCs before WDS can be elicited. Also, the effect of enkephalin on DGCs and basket cells will be examined in hippocampal slices and in hippocampal cells grown in 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 90045-02 LBNT

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	P. Lee	Visiting Fellow	LBNT	NIEHS
	D. Zhao	Visiting Fellow	LBNT	NIEHS
	C. L. Mitchell	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, 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.)*

The purposes of this project were: 1) the determine alterations of the metabolism of enkephalin and dynorphin in the limbic-basal ganglia regions after electroconvulsive shock (ECS) or amygdaloid kindling; 2) to study the possible roles of brain opioid peptides in the development or maintenance of kindling-induced seizures. Repeated ECS produced an increase in the tissue level of both enkephalin and dynorphin in most of the limbic-basal ganglia regions, e.g. hypothalamus, amygdala and striatum. However, the most interesting alteration after ECS was in the hippocampus: an increase in enkephalin level but a drastic decrease in dynorphin level. Measurement of mRNA abundance by Northern blot indicated that repeated ECS increased the biosynthesis of enkephalin but decreased that of dynorphin. This differential regulation of these two opioid peptides may be related to the changes in opioid receptor mechanism in the hippocampus after repeated ECS. It is believed that enkephalin has a high affinity in binding to delta receptors whereas dynorphin-(1-8) mainly acts on mu receptors. Repeated ECS-induced up-regulation in mu receptors and down-regulation in delta receptors may be a consequence of a decrease in the activity of dynorphin-containing neurons and an increase in the activity of enkephalin-containing neurons. These differential modulations of opioid peptides and receptors after repeated ECS strongly suggest that opioid mechanisms play an important role in regulating the neuronal excitability of the hippocampus. In comparison to the effect of repeated ECS, amygdaloid kindling produced changes in the level of enkephalin and dynorphin in limited regions, such as amygdala, substantia nigra and hippocampus. The kindling-induced differential changes in enkephalin and dynorphin in the hippocampus was comparable to those of repeated ECS, suggesting that the hippocampal opioid systems are sensitive to seizures. Pretreatment with opiate receptor antagonists facilitate the rate of kindling suggesting a role of opioid peptides in the initiation of kindling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90046-02 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Nervous Systems 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 evoked 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 evoked 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. This project is being terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90047-02 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Bio. Lab. Tech.	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina, Chapel Hill
University of Kyoto, Japan
Karolinska Institute, Stockholm, Sweden

LAB/BRANCH

Laboratory of Behavioral and Neurological Toxicology

SECTION

Peptide Neurochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.5	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Neuromedin B is the only known mammalian peptide of the ranatensin/litorin subgroup of the bombesin family and contains 70% homology with bombesin. The three amino acid substitutions are sufficient to provide the peptide with a unique set of physiological characteristics. Neuromedin B administered icv in pylorus-ligated rats failed to reduce gastric secretion even though it is a true neuropeptide with wide distribution in brain tissue. In extracts of dissected brain regions, the peptide was found by radioimmunoassays to be widly distributed: the pituitary gland (posterior lobe > anterior portion), the limbic system (hypothalamus > hippocampus > thalamus), and other brain regions (caudate, cerebral cortex, substantia nigra, brain stem). These results were further confirmed by the immunohistochemical localization of neuromedin B in nerve cells throughout rat brain. Preliminary data indicate the presence of a neuromedin B staining cell layer in the coronal region of the cerebral cortex with scattered cells in the underlying layers, individual cells dispersed among the pyramidal region of the hippocampus, a nucleus in the ventromedial hypothalamus, and dispersed cell bodies with long axons in the reticular net of the brain stem. Application of four polyclonal antibodies revealed that the amount of peptide detected depends upon the site-directed specificity of a particular antibody as well as the extraction conditions employed. Isocratic HPLC further revealed that each polyclonal antibody recognized different molecular forms of immunoreactive neuromedin B in rat spinal cord. Data also indicate that neuromedin B levels in the pituitary and limbic region decrease dramatically in lactating females and rise slowly during weaning, except in the hypothalamus which remains at the depressed state. In toto, the evidence suggests that pattern and distribution of neuromedin B defines a new neuronal pathway which may be involved in a regulatory capacity in the autonomic nervous system and subject to hormonal control.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90048-02 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Bio. Lab. Tech.	LBNT	NIEHS
	A. Guglietta	Visiting Fellow	LBNT	NIEHS

COOPERATING UNITS *(if any)*

University of Rome, Italy
University of North Carolina, Chapel Hill, NC
Farmitalia, Milan, Italy

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:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

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

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

The central neuroregulation of peripheral events by peptide hormones is a relatively new field. The intracerebral ventricular (icv) administration of bombesin, dermorphin (a frog-skin derived opiate heptapeptide), calcitonin (salmon) and lithium (the drug of choice for manic depression) effectively decreased all parameters of gastric secretion in pylorus-ligated fasted rats: neutralized acidic pH, reduced hydrogen ion concentration, and decreased volume of stomach secretions. However, subcutaneous (sc) injections of these peptides yielded differential effects relative to icv: bombesin was very weakly active (without a dose-response curve), while dermorphin was 0.0003 as potent; calcitonin, on the other hand, exhibited twice the activity sc as icv. Lithium chloride was effective both icv and sc, although the reduced response by sc is in keeping with the dilution of lithium throughout the body. Both bombesin and lithium appear to act centrally, perhaps through a common mechanism, on gastric secretion via products of arachidonate metabolism (e.g., prostaglandins) since their effects can be blocked by several cyclooxygenase inhibitors (indomethacin, diclofenac, naproxen, ketoprofen). Lithium and bombesin, which produce a similar effect and involve prostaglandins, do so through different routes; bombesin levels in the hypothalamus are unaffected by icv administered lithium. Several dimeric analogues of dermorphin (2 chains of dermorphin linked through the C-terminal amide nitrogen either directly or through methylene bridges) were tested for their ability to suppress gastric secretion and correlate that effect with an analysis of δ and μ receptors in brain synaptosomes. Only those analogues exhibiting μ-selectivity were active in vivo and their spectrum of bioactivity on gastric secretion were distinct from dermorphin. Although dermorphin requires central μ-type opiate receptors for gastric secretion, the role of calcitonin is less clear and may involve multiple interacting mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90049-01 LBNT

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Regulation of the Hormonal Output from Adrenomedullary Chromaffin Cells

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

PI:	Michal K. Stachowiak	Senior Staff Fellow	LBNT	NIEHS
Others:	Jau-Shyong Hong	Pharmacologist	LBNT	NIEHS
	P. Lee	Visiting Fellow	LBNT	NIEHS
	L. Thai	Biological Lab. Tech.	LBNT	NIEHS
	O.H. Viveros	Scientist	Burroughs Wellcome	
	R. Rigual	Postdoctoral Fellow	Burroughs Wellcome	

COOPERATING UNITS *(if any)*

Burroughs Wellcome Research Laboratories, Research Triangle Park, NC

LAB/BRANCH
LBNT

SECTION
Neuropharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

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

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

Adrenal chromaffin cells contain multiple hormones: epinephrine, norepinephrine, and enkephalins, each capable of producing unique physiological effects. Recently we have begun to investigate mechanisms which determine hormonal output from chromaffin cells. We have shown that the enhanced hormone release involves changes in mRNA levels of proenkephalin (pEK) and catecholamine synthetizing enzymes: tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltransferase (PNMT). The purposes of this project were: 1) to examine nature of extracellular and intracellular signals involved in the control of TH, PNMT and pEK mRNA levels; 2) to examine role of transcription and post-transcriptional mechanisms in these processes; 3) to determine whether expression of TH, PNMT and pEK genes could be differentially regulated. To address these questions we have used 3 experimental models: 1) in situ electrical stimulation of decentralized splanchnic nerves; 2)hypophysectomy and systemic administration of glucocorticoids; 3) primary culture of bovine adrenomedullary chromaffin cells. TH, PNMT and pEK mRNA levels were found differentially regulated by hormonal and neuronal inputs, by adenylate cyclase and intracellular catecholamines. We are currently investigating whether changes in mRNA levels are produced by transcriptional or post-transcriptional mechanisms, and whether differential regulations of individual mRNAs may provide bases for selective control of hormonal output from chromaffin cells. In the future, we intend to explore the role of different transmitters, hormones, growth factors, and second messenger systems in the control of TH, PNMT and pEK mRNA levels. To study coregulation of the expression of TH, PNMT, and pEK genes in individual cells we are currently developing in situ hybridization assay. We also plan to obtain clones of PNMT gene to further examine mechanisms which control transcription and stability of its products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90050-01 LBNT

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Roles of Opioid Peptides in the Regulation of Hippocampal Excitability

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. Lee	Visiting Fellow	LBNT	NIEHS
	P. M. Hudson	Bio. Lab. Tech.	LBNT	NIEHS
	J. Obie	Bio. Lab. Tech.	LBNT	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Behavioral and Neurological Toxicology

SECTION
Neuropharmacology

INSTITUTE AND LOCATION
NIEHS, NIH, Research Triangle Park, NC 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.)*

Roles of opioid peptides in the regulation of hippocampal excitability are a subject of intensive study since the discovery of endogenous opiates in the brain. Opiate peptides produce wet dog shakes (WDS) and epileptiform discharges in rats when administered intraventricularly. The purpose of this study was to obtain further information to support the hypothesis that opioid peptides in the hippocampus may play a role in mediating WDS and seizures activities. Injections of specific mu receptor agonist, [N-MePhe^3, D-Pro]-morphiceptin (PLO17), and delta receptor agonist, [D-Ala^2, D-Leu^5]-enkephalin (DADLE), to the ventral hippocampus produced convulsive seizures and numerous WDS, suggesting that both receptors are involved in the mediation of these behaviors. The levels of hippocampal glycine and gamma-aminobutyric acid (GABA) were increased but aspartate was reduced after PLO17 injection. Pretreatment with beta-funaltrexamine hydrochloride (B-FNA), an irreversible mu receptor blocker, attenuated PLO17-induced WDS and convulsions. It also restored the PLO17-induced changes in the levels of hippocampal amino acids to that of control values. These results suggest that opiate-induced WDS and convulsive seizures are receptor mediated and may be acting through a disinhibition mechanism by attenuating the release of inhibitory amino acids in the hippocampus. Nevertheless, the degeneration of hippocampal granule cells by intrahippocampal injection of colchicine attenuated PL 017-induced WDS but potentiated the severity of convulsions, therefore suggesting that these two behaviors may be mediated by different pathways in the hippocampus. These data give further evidence to the idea that opioid peptides in the hippocampus may play an important role in regulating hippocampal excitability. Studies are planned to determine the effects of opioid peptides on the turnover or in vivo release of GABA to directly test the hypothesis that GABA may mediate the excitability effect of opioid peptides in the hippocampus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90051-01 LBNT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Brainstem and Spinal Cord Modulation of Neurological 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:	J. S. Hong	Pharmacologist	LBNT	NIEHS
	K. Nanry	Psychologist	LBNT	NIEHS
	D. Herr	Biologist	LBNT	NIEHS
	C. Hamm	Electronics Tech.	LBNT	NIEHS
	C. Zimmerman	Biological Aid	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:
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.)*

The purpose of this research is to study the role of the brainstem and spinal cord in motor dysfunction, such as tremor and hyperreflexia, which are signs frequently associated with neurological disorders, such as Parkinson's and Alzheimer's Disease. Brainstem-mediated tremor was attenuated by blocking alpha-1 adrenergic receptors with phenoxybenzamine and prazosin, supporting the conclusion that the descending noradrenergic system facilitates motor outflow at the level of the spinal cord. Blockade of dopaminergic, beta-adrenergic and alpha-2 adrenergic receptors augment tremor, suggesting a reciprocal role for these different catecholaminergic receptor types in the mediation of tremor. In subsequent experiments, intrathecal application of noradrenergic receptor antagonists also attenuated tremor. We have also found that the intrathecal administration of a glutamate receptor antagonist blocks tremor, which is consistent with the accepted belief that motor outflow is mediated by an excitatory amino acid neurotransmitter. Our conclusions about the modulatory role of norepinephrine in tremor are further supported by recent studies on the acoustic startle reflex, which is also mediated in the brainstem and spinal cord. We report that pretreatment with alpha-adrenergic antagonists decreases the magnitude of the startle reflex and decreases the augmentation of the reflex produced by neurotoxic probes. Additional experiments have discovered that the activation of 5-HT1 receptors augments tremor. Other studies have found that augmentation of the startle reflex by 5-HT1 agonists, 8-OHDPAT and N,N-DMT, is attenuated by pretreatment with metergoline, a mixed 5HT1/5HT2 receptor antagonist, and is not affected by ketanserin, a 5-HT2 antagonist. Quipazine, a 5HT2 agonist, decreases the magnitude of the startle reflex, an effect blocked by pretreatment with ketanserin, but not by metergoline. We plan to study the role of descending monoaminergic pathways in the habituation and sensitization of the acoustic startle reflex, which are believed to be mediated within the brainstem and spinal cord.

LABORATORY OF GENETICS
Summary Statement

In general the Laboratory of Genetics is focused on 1) gene structure and the regulation of gene expression, 2) mechanisms of spontaneous and induced mutagenesis, mutation repair, DNA replication and recombination, 3) the amount and types of genetic variability found in populations or organisms and how that variability is maintained. There are two sections in the Laboratory, Eukaryotic Gene Structure and Mutagenesis.

Eukaryotic Gene Structure

To understand the nature and function of genes is important in defining them as targets for environmental mutagens. It is also important in this respect to have baseline data about the types and amounts of genetic variability that exist in natural populations of organisms and to understand the mechanisms that generate and maintain this variability. The Eukaryotic Gene Structure Section is investigating these fundamental problems using selected eukaryotic systems. The approach is to analyze specific genes genetically and biochemically to determine their molecular structure and examine the nature of mutational events that modify their expression and regulation. There are six senior research geneticists heading work groups in the section.

The Judd group studies gene expression in Drosophila. The focus is on a series of mutations at the white locus that upset normal regulation of the gene and its interaction with another locus, zeste. A majority of the mutations in this category are due to the insertion of transposable retrovirus-like elements in the region of the gene near the site of initiation of transcription. This region also has sequences known to bind to the zeste protein. The zeste-white interaction is sensitive to the relative positions of the two white alleles in the chromosomes, strongly suggesting that the three dimensional architecture of the chromatin is important in regulating gene expression. The mechanistic basis for this trans effect on regulation is being investigated.

The Voelker group is examining mechanisms of suppression of transposon-induced mutations in Drosophila. Mutations at the suppressor-of-sable, su(s), locus suppress specific mutant alleles associated with insertion of the transposon 412 at a number of loci. Voelker has molecularly cloned the suppressor gene and characterized it in detail. The protein product has been expressed and used to obtain antibodies to the protein. The basis for suppression of transposon-induced mutations is now under study.

The Langley group is studying several aspects of transposon-associated gene changes and is characterizing genetic variability at the molecular level in specific gene sequences of natural populations of Drosophila. A mutator system in D. ananassae generates a high frequency of abnormal eye phenotype, Om, changes. These map at more than 20 different loci and are associated with insertions of a retrovirus-like transposon, tom. One Om locus that has been examined molecularly shows that tom may integrate at any of several sites in a 25 kb stretch of DNA to produce the abnormal eye change. How this transposon produces the same syndrome of developmental changes when inserted at so many different loci is a central question being studied.

Langley has surveyed strains of Drosophila from natural populations at the molecular level to measure the frequency and types of genetic polymorphisms. Under investigation are the relative roles of mutation, migration and natural selection in determining levels of genetic variability. Langley is also investigating factors that control the evolution and numbers of transposons. Selection against mutant phenotypes caused by the insertions themselves cannot account for the copy numbers observed. Theoretical models involving the influence of asymmetrical recombination on transposon number and distribution are being tested experimentally, using a variety of chromosomes containing transposons at various locations.

The Li group is examining the genetic and molecular organization and expression of the mammalian lactate dehydrogenase genes. Full length cDNA clones of human LDH-B, mouse LDH-B and LDH-C have been sequenced and the genomic organization of those genes has been determined and mapped to specific chromosomes. The entire mouse LDH-A gene structure along with its promotor region has been determined. Recent studies show LDH-A binds single-stranded DNA. Other labs have shown that LDH proteins are components of centrosomes and that ε-crystallin of the duck eye lens is active LDH-B. The cancer-associated LDH has been shown by Li to be a tyrosylphosphorylated form of LDH-A. The many roles of LDH in the structure and metabolism of mammalian systems and the evolutionary significance of these relationships are under investigation.

Johnson studies systems for detecting spontaneous and induced mutations in mammals. He constructed a biochemical specific locus test that is used to detect mutations at more than 20 loci. Presently he has focused on analysis of skeletal abnormalities in offspring of crosses involving mutagen treated and control animals. This system is used to establish baseline data for skeletal variation and to correlate this variation with mutagen exposure and mortality data.

Malling is developing a system for detecting and characterizing mutation events at the DNA level in mammals. He has been able to integrate the virus ΦX174 with known mutations in its genome into mouse L cells. These cells are then grown and the ΦX174 DNA is recovered and characterized. This virus DNA is used as a mutation target system to measure directly the in vivo response of specific sequences of DNA in mammalian cells to various environmental conditions and agents.

Mutagenesis

A major component of the NIEHS mission is to understand and prevent environmentally caused diseases that appear only long after an exposure. Heritable birth defects and cancer, and probably heart disease as well, are such diseases. Because mutation plays an important role in their etiology, the aim of the Mutagenesis Section is to investigate how DNA damage causes mutations and lethality.

We use model systems (cultured mammalian cells, microbes such as yeasts, bacteria and viruses, and purely enzymatic systems) to investigate fundamental mechanisms of DNA replication, DNA damage, lethality and mutagenesis. The significance of these studies is twofold. First, a deep understanding of mechanisms may reveal ways in which mutagenesis can be prevented even when DNA damage does occur. Second, a study of the mutational consequences of different kinds of DNA damage will help in making regulatory decisions; on average, for instance, large deletions produce more serious consequences than do point mutations.

The research is conducted by six independent but strongly interactive lead scientists, together with their individual support staffs of technical and professional assistants. One lead scientist, Dr. James M. Clark, has just joined the Section; using cultured mammalian cells, he will investigate why and where DNA replication terminates when confronted by DNA damage.

Most mutations arise by a process called error-prone repair (EPR) in which damage-terminated DNA replication is induced to restart but in a poorly templated (and thus mutagenic) manner. The Drake group studies EPR in bacteriophage T4. They have recently discovered variants of the T4 DNA polymerase that change both the frequency and quality of EPR-dependent mutagenesis. These mutations directly implicate the polymerase in EPR and will be crucial in probing the enzymatic basis of mutagenic damage bypass. This group has also recently discovered a fundamentally new process of DNA repair, one that appears to circumvent DNA damage during DNA replication. Studies are underway to determine how many of the genes of DNA replication contribute to this repair system.

The Kunkel group studies the fidelity of DNA replication using DNA polymerases from sources as diverse as bacteriophages, mammalian cells and the AIDS virus. They have recently described the first known proofreading mechanism in a eukaryotic DNA polymerase, a result that helps to bridge the gap between the relatively high error rate of DNA synthesis and the much lower final mutation rate. They have also characterized the kinds and frequencies of mutations produced by a number of eukaryotic polymerases and will shortly begin a similar study using the polymerase from the apparently rapidly mutating AIDS virus.

The Schaaper group studies mutagenesis in DNA target molecules replicating in cell extracts, a useful system intermediate between enzymology and the intact cell. They have recently characterized the mechanism of action of a powerful and specific mutation-avoidance system, one that specifically protects against adenine:guanine mispairs during DNA replication. They have also described the difference, in frequency and quality, between mutations accumulating during DNA replication and those still present after the operation of another mutation-avoidance system, mismatch repair.

The Sugino group studies DNA replication, recombination and repair in yeasts. They have recently described several proteins that bind specifically to DNA and facilitate DNA synthesis and recombination. They have also discovered a novel recombinase, an enzyme that causes DNA molecules to exchange parts in a process that is crucial to both DNA repair and sexual reproduction.

The Tindall group studies mutagenesis in cultured mammalian cells. They have just completed an analysis of the abilities of mammalian mutation-screening systems to detect one of the most serious classes of mutations, deletions. They are now developing retroviral vectors that will allow DNA target sequences to be moved into cells defective in various components of DNA repair in order to analyze the roles of repair in preventing mutagenesis.

During the year, members of the Mutagenesis Section were represented, usually as invited speakers, at every meeting of note in the mutagenesis arena. John W. Drake received the 1987 Environmental Mutagen Society's Award of Excellence. Thomas A. Kunkel is patenting a major improvement in the technology of site-directed mutagenesis, a method coming into wide use and now commercially available.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60099-08 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	B. Yukihiro Hiraoka	Visiting Fellow	LG, NIEHS
	Tetsuo Takano	Visiting Fellow	LG, NIEHS
	Esther W. Hou	Biologist	LG, NIEHS
	Farida S. Sharief	Biologist	LG, NIEHS
	Jun M. Versola	Biological Aid	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:
3.8	3.6	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 full-length cDNAs for human LDH-B (heart), mouse LDH-B and LDH-C (testis) isoenzymes have been cloned and their nucleotide sequences determined. Human genomic clones containing LDH-B gene have been isolated and the exon-intron organization of the gene is being characterized. Human LDH-C gene has been mapped on chromosome no. 11 and human genomic clones containing LDH-C gene-related sequences are being isolated and characterized. The complete structure of mouse LDH-A gene has been determined, and its promoter region fused with gpt gene was shown to be functional in CHO cells. Further, the expression of mouse LDH-A promoter fused with cat gene was also shown to be induced by cyclic AMP and estrogen, and their regulatory elements were tentatively identified by sequence comparision with those of other mammalian genes. The nucleotide sequences of four mouse LDH-A processed pseudogenes were determined and molecular nature of spontaneous mutations present in these pseudogenes were analyzed. The information obtained from these studies will allow more accurate evaluation of genetic mutation events caused by environmental agents and eventually will be of value to improve human health care.

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, 1986 to September 30, 1987

(Discontinued)

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	Research Geneticist	LG, NIEHS
Others:	K. Bebenek	Visiting Fellow	LG, NIEHS
	M. P. Smith	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

Catherine M. Joyce, Yale University Medical School, New Haven, CT

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.)*

Certain aspects of this project are incorporated in Project Numbers Z01 ES 65038-02 LG and Z01 ES 65048-01 LG.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60147-04 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
Others:	R. L. Dunn	Biologist	LG, NIEHS
	R. Cornacchio	Stay-In-School Employee	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:
1.25	0.5	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.)*

The SOS system of Escherichia coli plays a crucial role in many aspects of mutagenesis in the organism. The system is not normally present in the cell but becomes induced upon blockage of DNA replication by DNA damage. Its induction entails the expression of a large number of new gene products, several of which are thought to interact with the process of DNA replication, rendering it error prone and producing mutations on both damaged and undamaged DNA. The evidence for the existence of these components rests largely on genetic experiments. However, the elucidation of the nature of these components and their mechanisms of action requires a more direct biochemical approach. We have designed an in vitro DNA replication system in which the existence of the error-prone replication components may be tested. The system uses the conversion of single-stranded bacteriophage M13 DNA into its double-stranded form (ss RF conversion) by crude extracts derived from either normal or SOS-induced cells. After replication, the product DNA is transfected to produce intact bacteriophage. The accuracy of the in vitro replication step is then assayed by measuring the frequency of mutant phage before and after replication. The accuracy of DNA replication in crude extracts is extremely high and resembles the in vivo accuracy. Our early efforts have already demonstrated that an understanding of the accuracy of DNA replication in SOS-induced cells requires a full understanding of the factors that are involved in maintaining normal accuracy. This aspect is therefore pursued simultaneously. E. coli mutator and antimutator strains with known (or presumed) defects in the process of DNA replication are essential tools in these studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60148-04 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Drake	Head	LG, NIEHS
Others:	D. C. Nguyen	Chemist	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:
0.7	0.1	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.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. Thus, the DNA polymerase is expected to play a crucial role in EPR. We have recently found that certain mutations of the bacteriophage T4 DNA polymerase gene can enhance or suppress EPR. The enhancing mutation, hm for hypermutable), also changes the specificity of EPR, preferentially increasing the frequency of base pair substitution mutations. Although the hm mutation is difficult to map by conventional crosses or by DNA sequencing, we now have data placing it roughly in the middle of the polymerase gene and are performing complementation tests to confirm its assignment. We plan next to ascertain the effect on EPR of some previously described mutator and antimutator DNA polymerase mutations and then to characterize the more interesting of these mutations by determining whether the mutant polymerases express altered fidelity in the insertional or proofreading steps of DNA synthesis.

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 61019-07 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Collaborative protein sequencing and peptide synthesis

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 Chemistry, University of North Carolina, Chapel Hill, North Carolina

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:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Several human prostatic acid phosphatase isozymes have been purified and partial amino-terminal sequences determined. The purified proteins will be subfragmented by chemical and enzymatic cleavage, and amino acid sequences of purified peptides will be determined. Some of antigenic peptides such as loop peptides of human LDH-A and LDH-B as well as mouse LDH-C proteins will be chemically synthesized, and they will be used to raise monoclonal antibodies in order to study the antigenic structure of LDH proteins. The collaborative protein sequencing and peptide synthesis established with the University of North Carolina, Chapel Hill will also provide research services to other scientists of the Division of Intramural Research at the NIEHS.

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 61022-06 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	E. A. Montgomery	Biologist	LG, NIEHS
	G. M. Simmons	Staff Fellow	LG, NIEHS
	W. H. Stephan	Visiting Associate	LG, NIEHS
	E. T. Moser	Biological Aid	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. N. Kaplan and R. Hudson, Biometry and Risk Assessment Program
Dr. Brian Charlesworth, Department of Biology, University of Chicago

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Laboratory of Genetics

SECTION

Eukaryotic Gene Structure 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.)*

Transposable elements are apparently parasitic DNA sequences that occupy a significant portion of the genome of most organisms. Using the Drosophila model system and theoretical population genetics analysis, the forces that control and shape the evolution of such elements are under study. By comparing the numbers of several retrovirus-like elements on the X and autosomes of individuals from a natural population it was possible to show that there is little role of natural selection against a mutant phenotype of the insertions themselves. Other experiments showed that inversions, which suppress crossing over, allow the accumulation of transposable elements. Theoretical models of the possible role of asymmetric crossing over between members of the same family of elements in the elimination of elements and thus the containment of copy number in the population were analyzed. Ongoing experiments are directed toward the relationship of the asymmetric crossing over to normal crossing over and the distribution of elements along the various chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61024-05 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	J. F. Sterling	Biologist	LG, NIEHS
	J. P. Graves	Biologist	LG, NIEHS
	W. Gibson	Research Chemist	LG, NIEHS
	S. S. Carpenter	Biological Aid	LG, NIEHS
	I. Oleksy	Biological Aid	LG, NIEHS
	T. J. Maness	Biological Aid	LG, NIEHS

COOPERATING UNITS *(if any)*

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Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	2	4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Recent findings in Drosophila have shown that 1) a significant proportion of spontaneous mutations are caused by insertions of mobile genetic elements, and 2) in certain genetic suppressor systems, suppressible alleles are caused by insertions of specific mobile elements. We are investigating the molecular mechanism of action 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. Current results suggest that this suppression occurs pretranslationally.

DNA sequences of su(s) have been cloned and are being characterized. An 8 kb segment of genomic DNA genes rise to a 5 kb poly A^+ RNA that consists of at least 5 exons. All of fourteen su(s) mutations that are caused by insertions of various mobile elements have these insertions in a 2.2 segment of largely intronic genomic DNA that gives rise to the 5' end of the message. Open reading frame segments from the two largest exons have been ligated into expression vectors to produce fusion proteins against which antibodies have been produced. Antibodies against the su(s) portion of the fusion protein are being recovered and are being used as probes to identify the location and function of the su(s) protein within the organism. Wild type su(s) sequences have been introduced into su(s) mutant flies by P element transformation and they confer the wild type phenotype on these flies. These P element constructs will also be used to determine the effect of the su(s) protein product on the biology of the mobile element 412. The DNA of the region is being sequenced to determine the structure and regulation of the su(s) locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61030-04 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular analysis of the Om mutation 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:	S. Tanda	Visiting Fellow, LG	LG, NIEHS

COOPERATING UNITS *(if any)*

Drs. C. W. Hinton and M. Matsuda, Wooster College, Wooster, Ohio; K. Saigo, Kyushu University, Fukuoka, Japan; Y. Yobari, Tokyo Metropolitan University, Tokyo, Japan; A. Shrimpton, Edinburgh University, Edinburgh, Scotland, UK

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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:
1.2	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.)*

Drosophila ananassae is a species characterized by several unusual genetic phenomena: hypermutability, meiotic male crossing over and a transposable element, tom, that often jumps causing a specific and unusual mutant phenotype, Om (dominant eye morphology). DNA sequences homologous to the tom element and the target sites of insertion have been analyzed. The tom element is a retrovirus-like transposon that is similar to the 297 element of D. melanogaster. The target site of insertion of the tom element is TATAT. The ATAT portion of the target is duplicated upon insertion. Many other features of typical retroviruses are found in the structure of the tom element. The analysis of a set of Om mutants at particular locus, Om(1D), indicates that the mutant phenotype is associated with the insertion of the tom elements(s) in a number of sites in a region greater than 25 kilobases. Spontaneous revertants are due to the excision of tom elements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61032-04 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	Ikuya Sakai	Visiting Fellow	LG, NIEHS
	B. Yukihiro Hiraoka	Visiting Fellow	LG, NIEHS
	Farida S. Sharief	Biologist	LG, NIEHS
	Jun M. Versola	Biological Aid	LG, NIEHS

COOPERATING UNITS *(if any)*

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Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.7	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 complete primary structure of human LDH-B (heart), mouse LDH-B and LDH-C (testis) isoenzymes have been determined by both protein and cDNA sequencing. The human LDH-A, human LDH-B and mouse LDH-C proteins have been/are being expressed in E. coli as well as in CHO cells. The cancer-associated lactate dehydrogenase was shown to be tyrosylphosphorylated form of human LDH-A, probably complexed with ras P21 protein. The mammalian LDH-A isozymes were shown by both liquid-binding assay and Western blotting to bind single-stranded DNA. The LDH proteins were also shown to be one of major protein components present in human and mouse centrosomes. Recently, the duck ε-crystallin (about 23% of lens proteins) was found by partial protein sequencing to be active LDH-B isozyme. These unexpected findings suggest that the LDH isozymes also function as structural proteins. Their physiological significance is being investigated by site-directed mutagenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61037-03 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	R. D. Gietz	Visiting Fellow	LG, NIEHS
	H. Hasegawa	Visiting Fellow	LG, NIEHS
	P. S. Alexander	Biologist	LG, NIEHS
	A. B. Clark	Biologist	LG, NIEHS
	P. Ponder	Summer Aid	LG, NIEHS

COOPERATING UNITS *(if any)*

Lucy M. S. Chang, Prof. and Chairperson, Dept. of Biochem., The Uniformed Service Univ. of Health Sci., Bethesda, MD; Dr. E. C. Friedberg, Pro. and Dir., Dept. of Path. and Cancer Biology Pro., Stanford Univ., Sch. of Med., Stanford, CA

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SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	4.0	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.)*

An in vitro DNA replication system of yeast 2-μm and ARS (autonomously-replicating-sequence) plasmid DNAs, developed in this laboratory several years ago, has been used to investigate the mechanism of DNA replication in eucaryotes. To identify and purify enzymes and components required for yeast DNA replication, the crude-extract system has been fractionated and reconstituted. Recently, two additional activities have been identified and purified: DNA topoisomerase I and ARS-binding protein(s). Independently, another group has shown that yeast DNA topoisomerase I is required for in vivo yeast DNA replication and for rRNA synthesis. This is consistent with our observation in vitro, further strengthening the usefulness of our in vitro system. We have also shown that the dna1-1 mutant is deficient in the initiation of chromosomal DNA replication. To aid in overproducing and purifying DNA1 protein, the DNA1 gene has been cloned, its nucleotide sequence determined and its regulation studied. Finally, two additional single-stranded DNA binding proteins (SSBs) (35KD and 45KD) have been purified from yeast crude extract, studied biochemically and their monospecific polyclonal antibodies raised from rabbits. The antibodies to these and to previously purified SSBs (14KD, 20KD, 26KD, and 38KD) have shown that the 38KD and 42KD and the 20KD and 26KD SSBs are immunologically related but the 14KD and 35KD SSBs are unique species. The antibodies have also been used to localize the 35KD, 38/42KD and 20KD/26KD SSBs to nuclei by immunofluoresence. The 35KD SSB is particularly interesting and important because it is recognized by an antiserum that reacts with mammalian PCNA/cyclin, a 36KD protein under cell-cycle control and thought to be involved in cellular DNA replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61039-03 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanisms 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:	H. Hasegawa	Visiting Fellow	LG, NIEHS
	C. C. Dykstra	Guest Worker (NRC Fellow)	LG, NIEHS
	A. B. Clark	Biologist	LG, NIEHS
	P. S. Alexander	Biologist	LG, NIEHS
	T. Sugino	Guest Worker	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. M. A. Resnick, Res. Geneticist, TRTP, NIEHS, Re. Tri. Pk., NC; Dr. F. E. Coleman-Wilson, Ass. Prof., Dept. of Microbio., Univ. of NC at Asheville, NC; Dr. K.-I. Arai, Dir., Dept. of Molecular Biology, DNAX, Res. Inst., Palo Alto, CA

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	1.9	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 replication, recombination and repair. Several single-stranded DNA binding proteins (SSBs) (14KD, 20KD, 26KD, 35KD, 38KD, 42KD and 45KD) have been purified from the yeast Saccharomyces cerevisiae and antibodies have been raised against them. Using the antibodies as probes, the SSB genes have been identified and sequenced. Deletions and/or disruptions of these genes have been constructed and the resulting phenotypes studied. At least the 20KD SSB gene is essential for cell viability.

An ATP-independent activity that catalyzes the transfer of one strand from a duplex linear molecule of DNA to a complementary circular single strand has been detected in crude extracts from mitotic and meiotic cells by adding yeast SSBs. This activity increases more than 15-fold during meiosis in MATα/MATα diploids, but not in neither MATα/MATα or MATα/MATα. The polypeptide responsible for this activity was purified to homogeneity from meiotic cells and characterized. Although this yeast strand-exchange protein (ySEP) has properties similar to those of prokaryotic SEPs and some eucaryotic SEPs, it is distinct because it requires no nucleotide cofactor, its reaction is efficient and rapid, and catalytic amounts of the protein are required for the reaction. This meiotic ySEP activity is controlled by the RAD50 and RAD52 genes which are required for meiotic recombination as well as sporulation. Both monoclonal and monospecific polyclonal antibodies have been raised against the homogeneous ySEP and identification and cloning of the gene are underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61041-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular genetic variation in natural populations

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

PI:	Charles H. Langley	Research Geneticist	LG, NIEHS
Others:	Naohiko Miyashita	Visiting Fellow	LG, NIEHS
	Gail M. Simmons	Staff Fellow	LG, NIEHS
	Barbara Lange	Guest Researcher	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Martin Kreitman, Department of Biology, Princeton University

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Laboratory of Genetics

SECTION

Eukaryotic Gene Structure Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.2	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.)*

A high proportion, perhaps 1%, of all nucleotides in most outbreeding organisms, including humans, mice and Drosophila, are heterozygous in an average individual. The relative roles of mutation, migration and natural selection in determining these polymorphisms is the fundamental issue in population genetics. The roles of migration versus selection in determining the geographic cline in allozyme frequencies at the alcohol dehydrogenase locus in Drosophila melanogaster are being tested by comparing the distributions of the DNA sequence haplotype variants in many populations with that predicted by several theoretical models of migration effects on gene frequency. The molecular genetic variation in the white locus in Drosophila has also been measured in great detail. The relationships of the amounts and types (base pair substitutions, deletions, etc.) of variation to the structure and function of the gene are the primary focus of investigation. Duplications of the metallothionein gene in Drosophila are known to afford resistance of heavy metal toxicity. The frequencies and properties of these duplication in relationship to environmental levels of heavy metals are being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61042-01 LG

PERIOD COVERED

May 6, 1987 to September 30, 1987

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

Gene Expression in Early Development of Drosophila

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

PI: Michael Abbott Staff Fellow LG, NIEHS

COOPERATING UNITS *(if any)*

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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:
.4	.4	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The head involution defective (hid) locus is a previously uncharacterized gene located on the left arm of chromosome III of Drosophila melanogaster. At present, eleven recessive lethal mutations of this gene have been recovered and studied. These investigations reveal that the hid gene must be expressed sometime during the first 12 hours of embryogenesis so that the head of the fly larva will form correctly. In addition to its embryonic role, hid must also be expressed during the pupal stage to facilitate the construction of the adult fly. The role of the hid gene during embryogenesis appears to be the same as its role later in development: the wild-type hid product is required in specific groups of cells to permit these cells to be rearranged during normal development.

The genetic control of cell movement and tissue reorganization, two fundamental developmental processes, has not been well studied in most organisms, including Drosophila. Further investigation of the hid gene may provide insight into a mechanism by which such processes are controlled; such a mechanism would then serve as a model for future studies. The hid DNA is being cloned, this will permit a more precise determination of when and where hid is expressed during development. In addition, the cloned DNA will be utilized to: (1) obtain monoclonal antibodies to the presumptive hid protein(s), and (2) identify other Drosophila genes that share homologous DNA sequences.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65021-15 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. S. E. Lewis, Research Triangle Institute, Life Sciences Group, Research Triangle Park, N.C.; Dr. D. P. Lovell, 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:
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 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. We have recently completed the description of expanded set of variable skeletal characteristics in the mouse. Examination of the skeleton reveals a wide range of objectively scoreable characteristics, effects, from subtle variation to gross abnormality. Animals that die in the course of an experiment can still be subjected to skeletal analysis (even when genetic analysis is not possible). This feature permits skeletal variation to be examined for correlation with mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65033-04 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1	1.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this research is to study chemical mutagenesis at the DNA level in mammals and to evaluate genetic and biochemical events in certain mutants as models of human genetic diseases. One major problem is that the level and specifity of the mutagenic response is very different between organisms. Some of this variation may be due to the genetic diversity of markers; a single (constant) sequence needs to be used as a target (indicator) in the various organisms and tissues. Our analysis is based on variance between single copies of the ϕX 174 virus containing am3 and cs70 mutations. The experimental approaches and accomplishments are as follows. 1) The mutability of the ϕX markers during replication in bacteria has been demonstrated. 2) Incorporated ϕX DNA has been recovered from nuclear DNA of cultured mouse L-cells and, most important, viable phages have been rescued in sufficient numbers for mutagenesis studies to be practical in cell cultures or tissues of transgenic mice. 3) The ϕX recovery technique does not appear to induce new mutations. 4) The conditions for mutagenic treatment of L-cell cultures have been determined and a test panel of mutagens selected. 5) The collection of mutation data from tissue cultures is in progress. An approach using an integrated viral vector in transgenic mice can combine a theoretical study of mechanisms of mutation across several model organisms with the applied need for assessing mutagenic hazard. This DNA sequence can be exposed and analyzed: 1) as naked DNA (single stranded and double stranded), 2) as a single stranded virus particle, 3) double stranded in bacteria, and 4) as vector DNA incorporated into the nuclear genome of cell cultures or transgenic mice (allowing tissue-specific study of mutagenic action).

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 65034-03 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
Others:	R. L. Dunn	Biologist	LG, NIEHS
	R. Cornacchio	Stay-In-School Employee	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:
1.25	0.5	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.)*

In this project the mechanisms of mutagenesis are investigated through a detailed study of its specificity. In this approach, DNA sequence information is gathered on all the classes of mutations that occur under a given condition, e.g., base substitutions, frameshifts, deletions, duplications, insertion elements, complex rearrangements, etc. These classes have their own dependence on the local DNA sequence and generally result from different mutational pathways. The specificity of mutation can thus be used to separate and analyze the various contributing components of mutation. Our system uses the *lacI* gene of the bacterium *E. coli*. This gene codes for the repressor of the *lac* operon. Forward mutations to *lacI*$^-$ are selected on the basis of the constitutive expression of the operon. The *lacI*$^-$ genes (typically several hundreds at a time) are transferred by *in vivo* recombination to a single-stranded recombinant-phage vector and sequenced, yielding the mutational spectrum of interest. The power of the system can be increased by the comparison of spectra in strains that are affected in DNA repair or replication pathways, such as mutator or antimutator strains. When the affected enzymatic pathways are known (e.g. *mutD* or *mutH,L,S* strains) the observed spectra can be used to directly correlate mutational classes with specific enzymatic pathways. In case of induced mutagenesis the specificity of mutation can, in addition, be used to identify the nature of the premutagenic lesions. Examples of current interest to our lab are mutagenesis by UV light and the chemical carcinogen N-acetoxyacetylaminofluorene (NAAAF). In these cases correlation of DNA damage spectra with mutational spectra, in a number of different repair or replication backgrounds, provides insights into the responsible mutagenic lesion(s) as well as the mechanisms by which they are converted into mutations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65036-03 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	Katherine M. Peterson	P 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:
2.25	0.25	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.)*

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 expressions 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 a mutation at the zeste locus, 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 zeste. Another aspect of the zeste-white interaction is a transvection effect that is dependent on the relative positions in the genome that the two white genes occupy. Two w^+ genes close together (as in paired homologs or a tandem duplication) are suppressed by z^1, but if separated by chromosomal rearrangements, z^1 has no effect. We are examining the basis for this effect of chromatin architecture on gene regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65037-03 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	Shu-Mei Huang	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Johng K. Lim, Professor of Biology
University of Wisconsin, Eau Claire

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:
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.)*

The study of mutations generated in a highly mutable strain of Drosophila melanogaster has shown that insertion of the retrovirus-like transposon gypsy into various chromosomal sites accounts for a high proportion of the mutation events. Historically a number of spontaneous mutations are known to be due to the insertion of gypsy. In general, these have been very stable mutations, thus it is of considerable interest to determine the conditions under which gypsy becomes highly amplified and mobilized. Genetic, cytological and molecular studies of stable strains show that the transposon is usually found in low copy number (3-4) per genome but in the unstable strain some sublines show fifty to sixty copies. High copy number cannot account entirely for the instability since some highly mutable sublines have become stable in a single generation, though they still harbor a number of copies of gypsy. In crosses of stable by unstable lines only the F_1 females exhibit amplification and mobilization of gypsy. This occurs early in embryogenesis, so that mutations occur as mosaics in both somatic and germinal tissues. Curiously both male and female offspring of these F_1 females continue for a number of generations to show high mutation rates, while F_1 males and their offspring show no mobilization of gypsy and are mutationally stable. We are attempting through various crosses and by molecular characterization of the transposons to discover the conditions that bring stability or that cause a stable strain to mobilize and amplify the transposons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65038-02 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Mechanisms of Mutagenesis by Animal Cell DNA Polymerases

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

PI:	T. A. Kunkel	Research Geneticist	LG, NIEHS
Others:	A. Soni	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

Robert A. Bambara, University of Rochester, Rochester, NY

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Low spontaneous mutation rates in animal cells are achieved by a variety of DNA metabolic processes, including DNA replication, recombination and several types of DNA repair. Each of these processes requires DNA synthesis; the central enzymes for this are the DNA polymerases. In animal cells there are (at least) four classes, designated α, β, γ and δ. We have characterized the accuracy and mutational specificity of three of these four polymerases (α, β and γ) for base substitution, frameshift and deletion errors produced during a single round of in vitro DNA synthesis, using the M13mp2 lacZ alpha complementation gene as a mutational target. Two major efforts in the past year have been to 1) test two specific molecular models proposed to explain the mutations produced by these enzymes and 2) establish the fidelity of DNA polymerase δ, the only mammalian DNA polymerase clearly established to contain a 3'exonuclease. The first objective has been partially accomplished; by the combined use of site directed mutagenesis, fidelity assays and DNA sequencing, the slippage model for frameshifts and the dislocation model for base substitutions have been confirmed. Regarding the second objective, we have measured the fidelity of Pol-δ. This enzyme, which functions in both replication and repair, is highly accurate for base substitution errors. Much of this accuracy is due to proofreading by an associated 3' exonuclease. This is the first demonstration that proofreading contributes to the fidelity of natural DNA synthesis by a normal mammalian DNA polymerase. We will continue a detailed analysis of each of these four classes of DNA polymerase, focusing on two aspects. First we will continue to test specific models that we have formulated to explain certain mutational events. Second, we will examine the fidelity of alternative (and in most cases, more complex) forms of these polymerases to identify additional fidelity components.

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, 1986 to September 30, 1987 (Discontinued)

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. H. Halderman	Biol. Lab. Tech.	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA

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.)*

Study continued under Project Numbers Z01 ES 65050-01 LG entitled "Molecular Analysis of Deletion Mutations in Chinese Hamster Ovary Cells", Z01 ES 65051-01 LG entitled "Molecular Analysis of Point Mutations in Chinese Hamster Ovary Cells", and Z01 ES 65052-01 LG entitled "Use of Retroviral Vectors in the Analysis of Mutations in Mammalian Cells".

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 65041-01 LG

PERIOD COVERED

July 5, 1987 to September 30, 1987

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

DNA Repair in Mammalian Cells

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

PI: J. M. Clark Senior Staff 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:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Mutagenic DNA damage usually consists of lesions that block DNA replication. Error-prone repair, the major mechanism of damage-induced mutagenesis, occurs when DNA replication bypasses such damaged bases in a poorly templated (and thus highly mutagenic) manner. Replication blocks have been studied in vitro but hardly at all in vivo. We are establishing systems to map, at the nucleotide level, those sites at which mammalian replication forks terminate synthesis when replicating damaged DNA and to analyze the relationship between DNA blocking lesions and mutation-prone sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65042-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Role of Gene uvsW in Error-Prone Repair by 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

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:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. uvsW is a crucial but mysterious gene in the bacteriophage T4 EPR system and is expressed in small amounts at an unusual time during phage development. Mutations in uvsW depress recombination, increase killing and abolish mutagenesis by agents acting through EPR. Temperature-sensitive mutations of uvsW have now been generated and characterized by mapping and complimentation tests and their effects on survival and mutagenesis are being determined. A plasmid bearing the uvsW region has been constructed and a deletion mutation thereof reintroduced into the T4 chromosome. This mutation will provide a rigorously defined null allele. It and other uvsW mutations will be used to determine the basis of the increased hydroxyurea sensitivity which is diagnostic of uvsW mutations. The wild-type uvsW gene will be placed on a plasmid under conditions suitable for overproduction of the gene product, whose properties will then be examined.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65043-01 LG

PERIOD COVERED
May 17, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Gene uvsX in Error-Prone Repair by 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:	M. O. Rosario	IRTA 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:
0.4	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.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. The bacteriophage T4 uvsX gene plays a central role in EPR and also in recombination. Its product is a recombinase, a protein that catalyzes homologous strand exchange between DNA molecules. The specific role of this protein in EPR remains mysterious. Two analyses are underway. First, although several severe mutations of uvsX are only semilethal, there are hints that an even more drastic disruption of uvsX may be fully lethal. Therefore, host-suppressible mutations will be introduced into several of the early codons of the gene and the resulting mutants will be tested for viability under nonsuppressing conditions. Where the mutants are inviable, the cause of death will be sought by analysing DNA metabolism. Second, a correlation between recombination and mutagenesis will be sought: in a cross employing outside markers, newly induced mutations will be screened for locally enhanced frequencies of recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65044-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Replication Repair in Bacteriophage T4

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

PI:	J. W. Drake	Head	LG, NIEHS
Others:	D. C. Nguyen	Chemist	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:
0.2	0.05	0.15

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most of the mutagenic and lethal effects of DNA-damaging agents are prevented by DNA repair systems. The classically described systems repair damage before DNA replication (by direct reversal or by excision repair), at the replication fork (by mutagenic bypass of damaged bases) or after replication (by a recombinational process). We have recently discovered a new mode of repair operating in bacteriophage T4. It is defined by several mutations that enhance killing by DNA-damaging agents. These mutations reside in vital genes encoding enzymes of DNA replication and repair appears to occur during replication itself. The repair system is accurate (nonmutagenic). To date, mutations defective in replication repair have been found in the genes encoding the DNA helicase and the SSB protein (which binds single-stranded DNA, holding it in an extended configuration suitable for DNA replication). Attempts are underway to find further mutations affecting this repair process by screening the genes encoding T4 DNA primase and DNA polymerase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65045-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Bacteriophage T4 rI Mutations

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

PI:	J. W. Drake	Head	LG, NIEHS
Others:	D. C. Nguyen	Chemist	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:
0.3	0.05	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.)*

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Bacteriophage T4 has been widely employed as a model system to analyze mechanisms of mutagenesis. One of the most common T4 mutation assays recognizes r (rapid lysis) mutants by their large, sharply edged plaques. Although the rII mutants are those most often subjected to further analysis, most mutagens produce more rI than rII mutants. Since little is known about rI mutants, we have investigated their general properties. Mutations that produce the characteristic rI phenotype fall into two loci, one the classically described locus at about 1:30 on the standard map and another a locus at about 8:30. Point mutations at the 1:30 locus recombine inter se at low frequencies, suggesting a small gene; several are suppressed by unlinked but as yet unmapped suppressor mutations. The "8:30" locus is being more closely mapped.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65046-01 LG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Accuracy of DNA Replication in vitro

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

PI:	T. A. Kunkel	Research Geneticist	LG, NIEHS
Others:	J. D. Roberts	Staff Fellow	LG, NIEHS
	A. Soni	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:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

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

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

We are interested in determining the mechanisms by which human cells control spontaneous and induced mutation rates. We have previously shown that the major replicative DNA polymerase in human cells, when purified as a relatively simple DNA polymerase of limited complexity, is not accurate enough during in vitro DNA synthesis reactions to account for low spontaneous mutation rates in vivo. However, it is well established that replication of DNA involves the concerted action of a number of proteins interacting as a complex "replisome". We therefore want to examine the fidelity of actual semiconservative bidirectional replication by this complex protein machine, in a way in which we can ultimately dissect the importance of individual proteins and their interactions to specific mutational pathways and molecular mechanisms. To do this we are using a mammalian viral model system of human DNA replication in combination with our recently developed, highly sensitive genetic assay to monitor mutagenesis. Our initial results suggest that this SV40 replication system is highly accurate, exhibiting more than 20-fold higher fidelity than the purified replicative DNA polymerase alone. The effect could be much greater, since our current measurements are limited by the background mutation frequency of the first assay we have employed. This higher fidelity could result from any of several steps in discrimination against or correction of errors. We intend to focus first on rigorous quantitation of the error rate of replication using more sensitive assays. We will then focus on mechanisms by fractionating and defining the contributions to fidelity of individual protein components and by using specifically engineered template DNA molecules as mutational targets.

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65047-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Fidelity of Retroviral Reverse Transcriptases

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

PI:	T. A. Kunkel	Research Geneticist	LG, NIEHS
Others:	J. D. Roberts	Staff Fellow	LG, NIEHS
	A. Soni	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:
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.)*

A critical feature of the life cycle of retroviruses, including the human immuno-deficiency virus (HIV), is their ability to generate diversity. Retroviruses have exceptionally high mutation rates, permitting rapid evolution of new forms of the virus that are better able to escape host defense mechanisms. A major source of this diversity may be the infidelity of the viral-encoded reverse transcriptase. Our objective is to elucidate the molecular mechanisms responsible for the genetic diversity of the HIV and other retroviral genomes. We have begun to examine the mutagenic potential of reverse transcriptase using our recently developed M13mp2 mutagenesis assay. This assay measures the error frequency of a single round of natural DNA synthesis in a system that permits analysis of a wide variety of mutational events, each precisely defined at the nucleotide sequence level. Our initial results with reverse transcriptases isolated from avian myeloblastosis virus and murine leukemia virus demonstrate a high error frequency for reverse transcriptase during copying of natural DNA. This supports the concept that the observed high mutation rate of retroviruses indeed reflects, at least in part, low reverse transcription fidelity. We plan to purify various forms of viral reverse transcriptase and examine their error frequency and the specificity of mutations produced during synthesis. We will then use reversion assays to monitor specific mutational pathways to test models of mutagenesis by reverse transcriptase. Ultimately we would like to focus on the reverse transcriptase encoded by the HIV genome, to elucidate the mechanisms by which diversity is generated by this enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65048-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Engineering DNA Polymerases to Probe Mutational Mechanisms

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

PI:	T. A. Kunkel	Research Geneticist	LG, NIEHS
Others:	K. Bebenek	Visiting Fellow	LG, NIEHS
	M. P. Smith	Biologist	LG, NIEHS

COOPERATING UNITS *(if any)*

Catherine M. Joyce, Yale University Medical School, New Haven, CT

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	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.)*

Our objective is to understand in precise molecular detail the mechanisms used by DNA polymerases to increase the accuracy of DNA synthesis. At least two mechanisms contribute to fidelity during DNA polymerization, discrimination against errors as the phosphodiester bond is formed (termed base-selection) and 3' exonuclease correction of rare misinsertions or misalignments after bond formation but before further polymerization (proofreading). Both mechanisms are known to operate with a variety of prokaryotic and eukaryotic DNA polymerases involved in the replication, recombination and repair of genetic information. Precise definition of the contributions to fidelity for various mutational pathways by these two mechanisms depends on the ability to probe each one independently at the molecular level. It is now possible to do this using as a model enzyme E. coli DNA polymerase I. This protein has been cloned, sequenced and overproduced (by Catherine M. Joyce at Yale) to provide detailed X-ray cystallographic and NMR structural information on the active sites for both its polymerization activity and its 3'exonucleolytic activity. This information has permitted a single base change to be introduced into the protein coding sequence for the exonuclease active site which inactivates the proofreading activity while not affecting polymerization. We are analyzing this mutant polymerase and contrasting its overall error rate and specificity to that of the wild type protein, using a series of highly sensitive in vitro mutagenesis assays. This approach has allowed us to dissect cleanly, for the first time, the contributions to fidelity of base selection and proofreading. Overall base selection by Pol I achieves an error site of one error for each 10,000 bases polymerized, and proofreading improves fidelity 5- to 10-fold. There are wide variations in these average values depending on the DNA sequence and the type of mutation. This is the first in a series of mechanistic experiments to be performed on the relationship between enzyme active site structures and mutational endpoints.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65049-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanisms of Mutagenesis With Yeast Replication and Repair Proteins

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

PI:	T. A. Kunkel	Research Geneticist	LG, NIEHS
Others:	A. Sugino	Visiting Scientist	LG, NIEHS
	R. K. Hamatake	Staff Fellow	LG, NIEHS
	M. P. 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:
0.9	0.2	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are using the simple eukaryote Saccharomyces cervisiae as a source of highly purified replication and repair proteins with which to study the factors that control mutation rates and the accuracy of DNA synthesis. Yeast offers the important advantage over higher eukaryotes of a good genetic system to dissect DNA metabolic processes. We have begun by determining, in the M13mp2 mutagenesis assay, the accuracy of in vitro DNA synthesis by yeast DNA polymerases I and II, without accessory proteins. DNA polymerase I, the replicative enzyme for the nuclear genome, has been purified free of DNA primase subunit(s) and the β sub-unit. This enzyme also lacks associated proofreading exonuclease activity. The accuracy of this form of Pol I is insufficient to account for the low spontaneous mutation rates observed in vivo. Pol I produces base substitution errors at a frequency that varies from 1 in 2000 to less than 1 in 60,000 nucleotides polymerized, depending on the composition and site of the mispair. Frameshift errors are also produced, at a frequency of 1 in 2000 to less than 1 in 75,000 nucleotides polymerized, again depending on the site. Pol I also produces deletion errors at high frequency. Most, but not all, of these mutants can be explained by either simple or complex variations of direct repeat mutagenesis. The fidelity of a more complex form of Pol I and the effects on fidelity of accessory proteins, including single-stranded DNA binding proteins, are currently being examined.

Yeast DNA polymerase II, a single polypeptide containing associated 3' exonuclease activity, is much more accurate than Pol I for base substitutions, frameshifts and deletion errors. Variations in reaction conditions which diminish 3' exonuclease activity also decrease fidelity, clearly demonstrating that proofreading by this enzyme normally functions to reduce errors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65050-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Analysis of Deletion Mutations in Chinese Hamster Ovary 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. H. Halderman	Biol. Lab. Tech.	LG, NIEHS

(This project incorporates certain aspects of FY 86 Project No. Zo1 ES65039-01)

COOPERATING UNITS *(if any)*

Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	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.)*

Deletion mutations and chromosome rearrangements occur in mammalian cells during specialized cellular processes such as immunoglobulin gene maturation as well as during aberrant cellular processes such as the generation of chromosome abberations or the activation of some oncogenes. To study the mechanisms by which such deletions and rearrangements occur, we have used a Chinese hamster ovary (CHO) cell line, AS52, that exhibit an increased mutational response at a transfected selectable locus (gpt) following treatment with agents that cause DNA strand breakage, e.g. x-rays, bleomycin, mitomycin C, etc. Such agents cause DNA deletions, rearrangements, and chromosomal abberations in a variety of systems. This increased mutational response in AS52 cells appears to reflect an ability to recover large deletions as viable mutants due to the unique site of integration of the transfected gpt gene. Other loci, notably hgprt, show a relatively weak mutagenic response to these deletion-inducing agents. We suspect that this is due to the hemizygous nature of the hgprt locus and the fact that large deletions affecting adjacent genes or essential DNA sequences (multilocus deletions) will be lethal and will affect mutant colony recovery. Multilocus deletions at the gpt locus, however, are suspected not to affect clonal viability either because the site of integration is autosomal and there are homologous chromosomal sequences that can complement function if an adjacent essential gene is deleted or because the gpt gene has integrated into a site that can withstand extremely large deletions without affecting adjacent essential sequences. We are in the process of characterizing the gpt site of integration. In addition, we plan to analyze the size of these deletions and to characterize deletion endpoints. We anticipate these studies will provide insights into the mechanisms by which both spontaneous and induced deletions occur in these mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65051-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Analysis of Point Mutations in Chinese Hamster Ovary 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. H. Halderman	Biol. Lab. Tech.	LG, NIEHS
	B. P. Holway	Guest Worker/Summer Grad	LG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA

LAB/BRANCH

Laboratory of Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.65	0.25	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.)*

Point mutations have been implicated as important etiological factors in a number of human genetic disorders including several of the haemopathies, osteogenesis imperfecta, as well as in the activation of some oncogenes. Little is known, however, about the mechanisms by which such mutations occur in mammalian cells. Determination of mechanistic pathways by generating mutational spectra using DNA sequence analysis has proven extremely powerful in bacterial systems. Similar studies in mammalian cells have been limited due to technical problems associated with the rapid isolation of target gene sequences from mammalian cells. We are in the process of analyzing both spontaneous and induced point mutations in a Chinese hamster ovary (CHO) cell line, AS52, that carries a single copy of the bacterial gpt gene transfected and functionally integrated into the CHO genome. The gpt gene is analogous to the mammalian hgprt gene and mutations at either locus can be isolated by selecting for resistance to the purine analog, 6-thioguanine (6TG). The small size of the gpt gene (456 base pairs) provides for the convenient rescue of mutant gpt sequences from the CHO genome for DNA sequence analyses. Our data suggest that genomic sequences at the site of the gpt integration may influence sequence stability in cloning experiments. We are in the process of performing cloning experiments utilizing alternative E. coli hosts known to stabilize genomic inserts. In addition, we have begun to amplify the gpt gene sequences using the polymerase chain reaction (PCR) technique. This approach bypasses the requirement of cloning each mutant gene and provides enough DNA to allow direct sequence analysis without further subcloning. Initial studies with the PCR technique indicate the entire 456 base pair gpt structural gene can be amplified from 1 g of genomic DNA. Using these amplified DNA sequences we will perform DNA sequence analysis and determine the utility of the PCR technique in generating point mutational spectra from mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65052-01 LG

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Use of Retroviral Vectors in the Analysis of Mutations 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. H. Halderman	Biol. Lab. Tech.	LG, NIEHS
	B. P. Holway	Guest Worker/Summer Grad	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:
0.65	0.25	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.)*

DNA sequence analysis of mutations induced in mammalian cells has been limited by the lack of systems available for the convenient analysis of isolated mutants. Our approach has been to construct cell lines designed to simplify the isolation and characterization of mutations induced in mammalian cells.

To generate mammalian cell lines useful for mutagenesis studies, we have constructed several retroviral vectors that carry gpt and other selectable markers. These vectors can be used to infect a variety of mammalian cell types. The integration of retroviral sequences occurs randomly and usually in single copy in the genome. Therefore, it is unlikely that any two isolated cell clones will carry the gpt gene inserted at precisely the same genomic location. We can, therefore, study the effect of genomic position on mutagenesis using the same target gene (gpt) integrated at several sites. We have data with the AS52 cell line that suggests deletion mutations are influenced by the genomic position of the target gene; however, there are no data available to assess the influence of genomic position on either spontaneous or induced point mutations. We will utilize retroviral vectors to isolate human and mouse repair proficient cell clones with single copy gpt integrations and will generate both spontaneous and induced point mutations at the gpt locus. Mutant DNAs will be isolated and sequenced to generate mutant spectra. Comparison of the spectra generated will allow an assessment of the influence of genomic position on the occurrence and type of point mutations generated in mammalian cells. These data should provide a foundation for the use of human repair deficient cell lines, e.g. Xeroderma pigmentosum, Ataxia telangiectasia, Bloom's Syndrome, etc., derived using retroviral vectors in future studies of mutational mechanisms in mammalian cells.

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 development 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 bioorganic 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: Research is concerned with understanding at the molecular level the interaction of chemical and physical agents with target biological systems including nucleic acids, proteins and membranes.

Mass Spectrometry: The work of this 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.

Nuclear Magnetic Resonance: The objective of the nuclear magnetic resonance (NMR) program is to elucidate the mechanisms by which chemicals and heavy metals in the environment cause cell injury with the aid of advanced NMR techniques including magnetic resonance imaging (MRI) and in vivo spectroscopy.

Prostaglandin Biochemistry: The Prostaglandin Workgroup investigates the metabolism of arachidonic acid to prostaglandins, 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.

Metabolism: The Metabolism Workgroup studies the manner in which environmental chemicals are absorbed into the body, accumulate in specific organs, are metabolically altered to other chemicals, and eventually are secreted and eliminated. The alteration products are identified, and studies are undertaken to determine whether they or the original environmental chemicals are actually responsible for toxic effects. In addition, the workgroup studies the manner in which environmental chemicals interfere with normal metabolic processes occurring (primarily) in the liver.

Recent Scientific Accomplishments

Molecular Biophysics: Free radicals are chemically reactive species that are thought to be responsible for the toxicity of many environmental agents. While evidence for the generation of free radicals is often easy to obtain in the test tube, it is much more difficult to prove the existence of these species in the intact animal, or in isolated organs, eg. liver. This has now been achieved by using specially designed molecules (spin traps) which react with the free radicals to form more stable species (spin adducts) which can be detected using an electron spin resonance (ESR) spectrometer. With the aid of this approach, evidence has been obtained for the metabolism of carbon tetrachloride, a known hepatotoxin, to the trichloromethyl radical ($^{\cdot}CCl_3$) and a novel oxygen-containing carbon dioxide anion radical ($^{\cdot}CO_2^-$), by the intact rat liver. The appearance of these radicals correlated well with liver damage as measured by the release of the enzyme lactate dehydrogenase. In other studies the ascorbic acid (Vitamin C) radical was detected in the skin of hairless mice which had been exposed to light following treatment with the photosensitizing drug chlorpromazine. This finding implicates free radicals as possible toxic species responsible for diseases, eg. phototoxicity and photoallergy, caused by simultaneous exposure to light and chemical agents in the skin or eyes.

Mass Spectrometry: Mass spectrometry is a technique which is capable of determining the molecular weight and structure of chemical substances. It is also capable of detecting the presence of very low levels of substances in complex mixtures coming from biological origins or from environmental samples. The Mass Spectrometry Workgroup is actively engaged in developing and applying these capabilities to problems related to the interaction of environmental toxins and biochemicals within the body.

A recent project has involved the analysis of blood from Taiwanese children whose mothers had been exposed to polychlorinated dibenzofurans (PCDF) during pregnancy. This project is important since it represents the first time that a study can be made of the fate of these compounds in human subjects. The results from this study will provide valuable information on persistence of PCDF in human tissues. PCDF are representative of a large group of chemicals, including polychlorinated biphenyls (PCB), which are a worldwide pollution problem.

Nuclear Magnetic Resonance: Nuclear magetic resonance spectroscopy is powerful tool of analytical chemistry which has been used for years to study chemical structure and reactivity in the test tube. It is based on the principle that the nuclei of atoms have magnetic properties which render them observable when placed in a large magnetic field. During the past decade, techniques have been developed which allow studies to be carried out on intact experimental animals which are anesthetized and placed within the bore of large magnets. The animals can then be dosed with various chemicals and metabolic response monitored in various organs of the intact animal. Such an approach can be extremely useful for providing an understanding of how chemicals affect living systems, since reliance on model systems is ultimately only as useful as the validity of the model itself. During the past year, these NMR studies have focused on the role which cellular calcium plays in the normal metabolism of various kinds of cells, and on the possibility that changes in the normal calcium level of cells may produce irreversible injury. Since calcium cannot be monitored directly using the NMR technique, it is monitored indirectly by introducing NMR sensitive

calcium indicators into cells. Studies carried out to date suggest that different types of stress or foreign chemicals do lead to increases in the calcium content of heart tissue which can produce irreversible injury after a sufficient period of time.

An important recent development has been the completion of a new building to house the NIEHS magnetic resonance imaging (MRI) facility. This important resource will permit the imaging of intact experimental animals. Since MRI is non-invasive, it can be used to follow the pathological changes occuring in animals exposed to environmental chemicals over long periods of time. This approach should permit the same amount of information on toxicity to be obtained using fewer animals than the conventional methodology which involves the periodic sacrifice of animals.

Prostaglandin Biochemistry: Many chemicals, which are themselves non-toxic, are chemically changed (metabolized) by enzymes in the body to more reactive forms which are highly toxic. One such important enzyme is prostaglandin H synthase (PHS) which is found in high concentrations in the bladder. Recent studies have shown that PHS metabolizes the bladder carcinogen 2-aminofluorene to a highly chemically reactive species which may be responsible for the toxicity of this compound. Other work has demonstrated that aromatic amines found in cooked food are also metabolized by PHS to free radicals. In the absence of foreign chemicals PHS converts arachidonic acid, an essential fatty acid, to prostaglandins and leukotrienes. These oxidized lipids have potent biological activities. An important recent discovery is that prostaglandins control chloride ion secretion. This finding may be important in uncovering the defect involved in cystic fibrosis, a hereditary disease of infants, children and young adults which is characterized by the excretion of high levels of chloride ions in the sweat.

Metabolism: A tumor promoter is a chemical which itself does not cause tumors but which enhances the cancer causing potential (carcinogenicity) of other chemical agents. Since tumor promoters have widely differing chemical structures, it is difficult to predict whether a given chemical will be a promoter or not. However, studies have now shown that a broad range of liver tumor promoters all bind to and activate an enzyme called protein kinase C. These and other observations suggest that many tumor promoters may act by changing the properties of the cell membrane, a protective envelope that surrounds animal cells. If this correlation holds, then it may be possible to design simple tests for the detection of potential tumor promoters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10004-08 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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
	Michael Perlman	Senior Staff Fellow	LMB	NIEHS
	B. Raju	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Charles Steenbergen, Department of Pathology, Duke University, Durham, NC.

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:
2.6	2.1	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In vivo NMR studies are carried out at the level of cell suspensions, perfused organs, and intact experimental animals in order to determine the mechanisms by which chemicals and other types of stress produce irreversible cell injury. Physiological, biochemical, and magnetic resonance measurements are carried out in parallel when possible, both to validate the techniques used, and more importantly, to determine differences which arise as a consequence of the selectivity of each type of measurement for different cellular pools. During the past year, this parallel appraoch has been used to demonstrate significant differences between the response of cytosolic and mitochondrial high energy phosphate pools resulting from ischemic stress. In order to establish cause/effect relationships, it is desirable to correlate measurements of many metabolic parameters, some of which are directly accessible to NMR observation, and some of which can be made accessible via the intorduction of NMR active indicators into the cells of interest. Most effort has been focused on the use of intracellular fluorinated indicators for cytosolic calcium which can be detected by F-19 NMR. Basal free calcium levels in beating and arrested perfused rat hearts have been measured, and the response to ischemia and hypoxia studied. Cytosolic calcium levels are initially unchanged in response to ischemia, but gradually increase to micromolar levels, consistent with a role for cytosolic calcium in the mediation of irreversible cell injury. More recent studies have shown that cardioplegic arrest, which is known to retard ischemic injury, also delays the increase in cytosolic calcium relative to simple ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30003-16 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Development of Biochemical 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:	Ram 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:
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.)*

Objectives: to develop improved methods for identification of metabolic products, monitoring biochemical events such as lipid peroxidation, enzyme translocation, and enzyme activation, and the application of quantitative analytical techniques to the measurement of biological damage. A new approach to the monitoring of lipid peroxidaiton occurring in vivo has been developed, to address the problem of (1) previously unsuspected peroxidation products and (2) repair of peroxidative damage. Using this approach, which involves "loading" the lipids of rat liver with ^{14}C-labeled linoleic acid, we have detected a previously unreported, major effect of carbon tetrachloride on turnover of diglycerides, and have obtained evidence that 2,3,7,8-tetrachlorodibenzo-p-dioxin has only a minor effect on lipid peroxidation in vivo, although it has major effects on other aspects of lipid metabolism. Other accomplishments include the development of a technically much simpler assay for diglyceride kinase than those previously available, an HPLC method for fractionation of cholesterol-singlet oxygen oxidation products, and the identification of several factors that tend to invalidate traditional methods for studying translocation of protein kinase C in cell cultures. We are currently attempting to develop a method for the chromatographic isolation of spin-trapped lipoxygenase products, and an approach to the use of membrane fragments as phospholipase substrates.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30015-13 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 cancelled.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-09 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	Anson S.W. Li	Staff Specialist	CSC	NIEHS
	Colin F. Chignell	Chief, LMB	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:
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 play a role in photosensitization. Electron spin resonance studies in conjunction with spin trapping techniques have shown that most halogenated aromatic photosensitizers, eg. amiodarone, bithionol, fentichlor, chlorpromazine, undergo dehalogenation upon UV irradiation to yield the corresponding aryl radicals and halogen atoms. These aryl radicals were capable of abstracting hydrogen atoms from suitable donors, suggesting that in vivo they could initiate peroxidation by reacting with unsaturated lipids. A comparison among chlorpromazine and related phenothiazines showed that there was a good correlation between phototoxicity, both in vivo and in vitro, and the yield of aryl radicals generated upon UV irradiation. The only exception was chlorpromazine sulfoxide, a known metabolite of chlorpromazine, which did not photodehalogenate but instead generated the highly reactive hydroxyl radical upon irradiation. The toxic effects of other photosensitizers, eg. anthracyclines, tetracycline, are enhanced in the presence of oxygen. These compounds were found to generate the superoxide anion radical upon irradiation. For the tetracyclines there was a good correlation between light-induced superoxide production and in vivo phototoxicity.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-05 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
	Christian Guenat	Visiting Associate	LMB	NIEHS
Other:	Jos de Wit	Chemist	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS

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:
1.15	1	.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.)*

One of the components of the mass spectrometry workgroup mission is to provide other groups within LMB and NIEHS access to mass spectrometric analyses on a service basis. The workgroup provides the following services on an ongoing basis: 1) low and high resolution electron impact (EI) mass spectra; 2) low and high resolution chemical ionization (CI) mass spectra; 3) negative ion chemical ionization (NICI) mass spectra; 4) gas chromatography/mass spectrometry (GC/MS) in conjunction with EI, CI and NICI MS; 5) thermospray (TSP) liquid-chromatography/mass spectrometry (LC/MS) in conjunction with CI and NICI MS; 6) fast atom bombardment (FAB) under both positive and negative ion conditions; and 9) tandem MS in combination with positive and negative ion FAB, EI and CI MS.

During the past year approximately 370 samples have been analyzed on a service basis (not including collaborative work).

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50082-04 LMB

PERIOD COVERED
October 1, 1987 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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 Pnncipal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Phillip W. Albro	Research Chemist	LMB	NIEHS
OTHER:	Ram 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:
2.0	1.5	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.)*

Current objectives include assessment of the effects of a diverse set of hepatic tumor promoters and chemically similar non-promoters on liver cell membranes. Present studies are emphasizing two membrane-related phenomena, protein kinase C (PKC) activation and lipid peroxidation. Among the classes of polychlorinated biphenyls, phthalic acid esters, and chlorinated dibenzo-p-dioxins, those individual compounds that have been reported to be hepatic tumor promoters were found to activate PKC in a purified, reconstituted system, and to bind strongly to this enzyme. Compounds from these classes that are reportedly negative in tumor promotion assays either did not affect PKC activity, or inhibited. In hepatocyte cultures, the promoters increased the binding of PKC to the plasma membrane. Most of the compounds tested significantly altered the rate of hydrolysis of phorbol esters (strong tumor promoters) in hepatocytes. 2,3,7,8-Tetrachlorodibenzo-p-dioxin was shown to have either a very limited ability to cause lipid peroxidation in rat liver, or to produce only easily repaired peroxidative damage. Future studies will emphasize PKC, its autophosphorylation, and its regulation by diglycerides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50086-02 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 professionel 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

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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 cancelled.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50087-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Mechanisms of Singlet Oxygen-Dependent Photosensitivity

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

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

COOPERATING UNITS *(if any)*

Albert W. Girotti, Medical College of Wisconsin, Milwaukee, WI; Ann G. Motten, Duke University, Durham, NC; Garry R. Buettner, Institut fur Strahlenbiologie, West Germany; K. Reszka, University of Alberta, Alberta, Canada.

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Photosensitization can result when light interacts with endogenous or exogenous chemical agents in the skin and other tissues. This process can produce undesirable clinical consequences, as in phototoxicity and photoallergy; or it can have beneficial effects, as in tumor photodynamic therapy (PDT) and coal-tar or psoralen (PUVA) therapy against psoriasis. Photosensitization results from the light-induced production of free radicals and/or singlet oxygen ($^1O_2$), the lowest electronic excited state of molecular oxygen. Because the latter species may be important in both phototoxic reactions and PDT, we have developed state-of-the-art instrumentation capable of detecting the characteristic phosphorescence of $^1O_2$ at 1268 nm. This has permitted us to delineate the photophysics of $^1O_2$ production in solution by a number of chemicals with photosensitizing activities in vivo, including phenothiazine derivatives (e.g., promazine [PZ] and chlorpromazine [CPZ]), benzoxazole derivatives (e.g., benoxaprofen [BP]), tetracyclines, anthrapyrrazoles, anthracene, and hematoporphyrin derivative. We have shown, for example, that, when they are excited with light, PZ and CPZ sensitize significant $^1O_2$ production in organic solvents (the quantum yield, ϕ varies between 0.1 and 0.4 depending on the solvent) but they do not sensitize detectable amounts of $^1O_2$ in water ($\phi < 0.01$). In contrast the sulfoxide metabolites of PZ and CPZ, do not sensitize $^1O_2$ production in any solvent when they are irradiated. Another drug, BP, also sensitizes very little $^1O_2$ production in water ($\phi < 0.01$); however, its decarboxylated photoproduct is lipid soluble and efficiently produces $^1O_2$ in organic solvents. We are extending our investigation to artifical and natural membranes in order to understand the biological importance of $^1O_2$ production. As part of this development, we have already demonstrated that anthracene, eosin Y and hematoporphyrin derivatives produce $^1O_2$ in erythrocyte ghosts when excited with light.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50088-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Relationship of Free Radicals to Halocarbon-Induced Toxicity in the Liver

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:	Lynn LaCagnin	NRC Staff Fellow	LMB	NIEHS
	Henry D. Connor	Research Chemist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

Dr. Ronald G. Thurman, Department of Pharmacology, UNC, Chapel Hill, NC; Kathy Knecht, graduate student, Curriculum in Toxicology, UNC, Chapel Hill, NC

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	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.)*

CCl_4 has been shown previously to be metabolized to the trichloromethyl radical ($\cdot CCl_3$) and to a novel oxygen-containing carbon dioxide anion radical ($\cdot CO_2^-$) in the perfused rat liver. These free radicals were detected by electron spin resonance using the spin-trapping technique. The $\cdot CO_2^-$ radical adduct also was observed in urine following the intragastric administration of CCl_4 or $CBrCl_3$ and spin trap. Detection of the $\cdot CO_2^-$ adduct in the effluent perfusate was decreased 3-4 fold by DIDS (0.2 mM), an inhibitor of the plasma membrane anion transport system. The rate of formation of $\cdot CO_2^-$ radical adduct was decreased 2-3 fold following inhibition of cytochrome P-450-dependent mono-oxygenases by metyrapone (0.5 mM) and was increased about two-fold by induction of cytochrome P-450 by phenobarbital pretreatment. Toxicity of halocarbons in the perfused liver was assessed by measuring the release of lactate dehydrogenase (LDH) into the effluent perfusate in livers from phenobarbital-treated rats under conditions identical to those employed to detect radical adducts (i.e., during the infusion of CCl_4 or $CBrCl_3$ into livers perfused with either nitrogen- or oxygen-saturated perfusate). Metabolism of halocarbons to $\cdot CO_2^-$ radical adduct was 6-8 fold faster during perfusion with nitrogen-saturated rather than with oxygen-saturated perfusate. Concomitantly, liver damage detected from LDH release occurred much sooner during halocarbon infusion in the presence of nitrogen-saturated perfusate. An excellent correlation (r= -0.80) between the rate of formation of PBN/$\cdot CO_2^-$ and the time to onset of LDH release following halocarbon infusion was observed. Therefore, it is concluded that PBN/$\cdot CO_2^-$ is a useful marker for oxygen-containing free radical intermediates which may be causally related to halocarbon-induced hepatotoxicity. Recently, the $\cdot CCl_3$ and .CO2- radical adducts also have been detected in the bile from anesthetized rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50089-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

The Reaction of Free Radical Metabolites with DNA

PRINCIPAL INVESTIGATOR *(List other professionel 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
	William D. Flitter	Visiting Fellow	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.2	0.2

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

☐ (a1) Minors

☐ (a2) Interviews

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

The interaction of free radical metabolites with DNA has been a major area of interest and speculation, but previous electron spin resonance (ESR) investigations of this area have been very limited. Our ESR studies have used free radical metabolites of intercalating redox-active compounds, such as adriamycin and chlorpromazine. The DNA is oriented by capillary flow, and the orientation of the free radical relative to the DNA helix is determined from the anisotropy of the ESR spectrum. In order to accomplish this, a capillary has been shaped like an ESR flat cell. Orientation of the intercalated chlorpromazine cation radical perpendicular or parallel to the magnetic field can be obtained by simply twisting the cell in the microwave cavity. In contrast to most intercalators, the chlorpromazine cation radical binds to single-stranded DNA. This gives an ESR signal characteristic of an immobilized free radical, but no orientation dependence could be found, indicating the DNA must be in the double helix configuration for orientation to occur. Actinomycin D intercalates into DNA, but no ESR spectra of an immobilized free radical could be obtained, possibly indicating that the free radical metabolite of actinomycin D does not intercalate. Hydroxyl radical-damaged DNA radical adducts have been obtained, and their orientation relative to the DNA helical axis will be investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50090-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Porphyrin Ion Radical Metabolites and Their Reactions

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
	Herbert Sipes	IPA	LMB	NIEHS
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

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

☐ (a1) Minors

☐ (a2) Interviews

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

Uroporphyrin I, which accumulates in body tissues of congenital erythropoietic porphyria patients, can undergo an enzymatic one-electron reduction to the porphyrin anion radical when a suitable reducing cofactor is present. We have demonstrated that anaerobic microsomal incubations containing NADPH and uroporphyrin I give an electron spin resonance spectrum of a porphyrin anion free radical. Inhibitor studies indicate that NADPH-cytochrome P-450 reductase is the electron donor. This radical undergoes a second-order decay due to nonenzymatic disproportionation of the radical. Aerobic microsomal incubations were also investigated for the reduction of oxygen to superoxide by monitoring oxygen consumption and the spin-trapping of superoxide. These experients demonstrated that electron transfer from the porphyrin radical to molecular oxygen does occur, but due to the slow formation of the radical anion, no oxgyen consumption above the basal level could be detected in the microsomal incubations. The photoreduction of uroporphyrin I in aerobic and anaerobic incubations was also investigated. Similar results have been obtained with photofrin II, a photo-activated antitumor agent. The oxidation of a variety of porphyrins to cation free radicals by peroxidases also has been investigated. Since the enzyme intermediate of horseradish peroxidase, compound I, is itself a porphyrin IX cation radical, this work will have implications for electron transfer as well as porphyrin metabolism.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50091-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Phenyl Radical Formation by Oxyhemoglobin from Phenylhydrazine In Vivo

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:	Kirk R. Maples	Staff Fellow	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

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

☐ (a1) Minors

☐ (a2) Interviews

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

The reaction of oxyhemoglobin with phenylhydrazine has received considerable attention for many decades. The basis for this interest stems from the ability of phenylhydrazine and hydrazine-based drugs to induce hemolytic anemia. Considerable evidence obtained from in vitro electron spin resonance (ESR) experiments implicates free radicals in the events leading to red blood cell hemolysis. However, until this report, no corroborating ESR evidence for in vivo free radical formation has been presented. We have successfully employed ESR to detect the formation of the phenyl radical adduct in the blood of rats which received an intragastric dose of phenylhydrazine followed by an intraperitoneal injection of the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). This spin adduct appears to be immobilized by the hemoglobin protein. It was detectable with ESR when phenylhydrazine was administered in a dosage comparable to that prescribed for currently-employed, hydrazine-based drugs. In addition, we have shown that pretreatment of the rat with ethanol prior to administration of phenylhydrazine and DMPO decreases the radical adduct level in the blood stream, presumably due to the scavenging of the phenyl radical by ethanol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50092-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

The Mechanisms of Reduction of Nitroheterocyclic Drugs

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

PI:	Ramakrishna Rao	Visiting Associate	LMB	NIEHS
OTHER:	Ronald P. Mason	Research Chemist	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programm/Analyst	CSC	NIEHS

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The nitroheterocyclic drugs such as derivatives of 2-nitrofurans, and 2-, and 5-nitroimidazoles are used as antimicrobial agents. Misonidazole, a 2-nitroimidazole derivative, was used as a radiation sensitizer. The mechanism of action of these drugs involves reduction of these compounds to form nitro anion radicals which can produce reductive metabolites in hypoxic cells or oxygen-derived radicals in aerobic cells. One-electron reduction of nitro compounds is catalyzed by cytochrome P-450 reductase and other reductase enzymes.

We have shown that nitro anion radicals were formed by intact rat hepatocytes and that these radicals are present inside hepatocytes.

We have also obtained electron spin resonance evidence for the generation of nitro anion radical by ascorbate reduction of nitro compounds. The generation of oxygen-derived radicals by the reoxidation of nitro anions has been observed by oxygen-consumption studies.

The nitroheterocyclic drugs also cause neuropathy and neurotoxicity. The mechanism of neurotoxicity is suggested to involve oxidation of neurotransmitters such as dopamine and norepinephrine by the nitro drug. The formation of semiquinone, and nitro anion and oxygen-derived radicals has been suggested to be important in the mechanism of neurotoxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50093-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

NMR Studies of the Metabolism of Leishmania Braziliensis

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:	Donald G. Davis	Expert	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor J.J. Blum, Department of Phyisology, Duke University, Durham, NC.

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.9	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.)*

Although Leishmania species are estimated to infect about 100 million people worldwide, little is known concerning the products of glucose catabolism under either aerobic or anaerobic conditions. We have carried out carbon-13 NMR studies of both intracellular and excreted metabolites of L. braziliensis incubated in a medium containing [1-^{13}C]glucose. NMR analyses indicate that the primary excreted metabolites are [2-^{13}C]acetate, [2-^{13}C]succinate, [3-^{13}C]pyruvate, [3-^{13}C]lacetate, and [3-^{13}C]alanine. A small amount of [1,2-$^{13}C_2$]succinate was also detected. Anaerobically cultured cells excreted most of the same labeled metabolites, however, the primary product was [3-^{13}C]glycerol. Although the results were in general agreement with the results of enzymatic assays and chemical analyses, a discrepancy was noted with respect to the presence of lactate. This issue was resolved by the use of an alternative assay sensitive to D- rather than L-lactate. Analyses of composition of the intracellular pools indicated a qualitatively similar pattern of labeled metabolites, the primary exception being that in the anaerobic case [3-^{13}C]glycerol-3-phosphate rather than glycerol was observed. Work to detect the enzyme responsible for D-lactate production in L. braziliensis is in progress. Detection of D-lactate might be useful in the diagnosis of leishmaniasis, especially in visceral leishmaniasis where large parasite burdens and anaerobic conditions may result in significant production of D-lactate.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50094-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

NMR Studies of Dihydrofolate Reductase

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:	Barry Selinsky	Staff Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Raymond L. Blakley, Chairman, Division of Biochemical and Clincial Pharmacology, St. Jude Children's Research Hospital, Memphis, TN.

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.6	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Interest in the enzyme dihydrofolate reductase (DHFR) derives in part from its clincial relevance as a target enzyme of anti-folate drug therapy. The reductase is inhibited by methotrexate, used in the treatment of various types of cancer, and the bacterial enzyme is strongly and specifically inhibited by trimethoprim, which is used in combination with sulfa drugs for treating a variety of infectious diseases. We have previously demonstrated through NMR studies of isotopically labeled anti-folates that enzyme inhibitors containing either the 2,4-diaminopterdine moiety (as methotrexate) or the 2,4-diaminopyrimidine moiety (as trimethoprim) are protonatd at N-1 when complexed to the enzyme, and remain protonated at all pH values which are accessible to measurement (up to pH ca. 11). This behavior has been interpreted to reflect a charge-charge interaction between the protonated N-1 of the inhibitor and the active site carboxyl group of Asp-27. During the past year, analogous studies have been carried out on DHFR analogs produced by site directed mutagenesis, in which the active site aspartic acid residue was replaced by either an asparagine or a serine residue. In general, such substitutions were found to invert the selectivity of the active site for the unprotonated form of the inhibitors. Thus, the enzyme complexed inhibitors remain unprotonated down to pH values near 4.0, despite the pK values of 5.7 and 7.6 for methotrexate and trimethoprim, respectively. One exception to this observation is the complex formed between trimethoprim and Ser-27 DHFR, in which separate resonances corresponding to both the protonated and unprotonated enzyme-complexed inhibitor can be observed. Studies currently in progress, using both carbon-13 and nitrogen-15 labeled dihydrofolate and analogs, are aimed at providing a more detailed understanding of the catalytic mechanism of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50095-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

In Vivo F-19 NMR Studies of the Metabolism of Fluorinated Anesthetics

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:	Barry S. Selinsky	Staff Fellow	LMB	NIEHS
	Michael Perlman	Senior Staff Fellow	LMB	NIEHS
	Louis A. Levy	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	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.)*

Several lines of evidence indicate that some post-surgical illnesses are caused by toxic metabolites of the anesthetics used during surgery. The presence of fluorine atoms in most of the general anesthetics in clinical use makes F-19 nuclear magnetic resonance (NMR) an ideal technique for analyzing the metabolism of these compounds, both in vitro and in vivo. Using this technique, studies of the metaoblism of halothane, methoxyflurane, and enflurane have been carried out. In vivo studies of hepatic metabolism in anesthetized rats utilized a surface coil tuned to the fluorine resonance and positioned directly above the liver of animals in which the intervening layer of muscle had been previously removed surgically. Methoxyflurane, perhaps the most toxic of the fluorinated anesthetics used and one now confined to veterinary use, has been postulated to undergo both oxidative, P450 dependent metabolism to methoxydifluoroacetic acid, and demethylation to yield dichloracetic acid plus inorganic fluoride. In vivo NMR studies have indicated significant levels of an organic fluorinated metabolite in addition to the methoxyflurane. This metabolite was identified as methoxydifluoroacetic acid by comparison of the NMR parameters with the directly synthesized metabolite. However, no resonance corresponding to inorganic fluoride is observed in vivo, due either to excessive broadening and/or rapid excretion. Since the metabolism of several anesthetics, particularly halothane, is postulated to involve free radical intermediates, strategies for observeration of such species by NMR are being evaluated. In particular, NMR active, fluorinated spin traps, analogous to those utilized to study free radical metabolism by ESR, are under development. The reducing environments of most in vivo systems will be sufficient to yield diamagnetic spin adducts, which can be monitoried by NMR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50096-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Changes in Tissue Non-Cyclic Phosphodiesters Produced by Toxins

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

PI:	C. Tyler Burt	Expert	LMB	NIEHS
OTHER:	Robert E. London	Research Physicist	LMB	NIEHS
	Bruce Fowler	Research Pharmacologist	TRTP	NIEHS

COOPERATING UNITS *(if any)*

Dr. Charles Hill, Department of Poultry Science, North Carolia State University, Raleigh, NC; Dr. Jay Levine, North Carolina Veterinary School, Raleigh, NC.

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.1	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.)*

In vivo 31-P nulcear magnetic resonance (NMR) techniques have been used to evaluate the effects of several toxic substances on tissue metabolism, with particular emphasis on the potential significance of changes in the levels of non-cyclic phosphodiesters. In mammalian systems, the phosphodiesters glycerophosphoryl choline (GPC) and glycerophosphoryl ethanolamine (GPE) are readily observed in many tissues and hence are present at near millimolar levels. Although most generally postulated to be lipid catabolites, it is difficult to reconcile this lack of function with the high tissue levels observed. Further, the presence of analogous phosphodiesters such as serine ethanolamine phsophodiester (SEP) which is found in the chicken, and threonine ethanolamine phosphodiester (TEP) which we have found to be the major phosphorus-containing metaboite in fish lens, also suggests functions beyond that of lipid breakdown product. Studies on the effects of acute arsenite poisoning using surface coil 31-P NMR spectroscopy on the liver of anesthetized rats indicate significant time-dependent increases in the levels of both GPC and GPE. These changes are consistent with an increase in lysophospholipase activity in response to the arsenite. Studies on the effects of chronic vanadium administration to chickens using surface coil observation of muscle tissue failed to indicate any significiant changes in the SEP levels, although pH changes consistent with the inhibition of the Na/K ATPase were noted. Further studies designed to unravel the potential role of non-cyclic phosphodiesters in the regulation of lipid metabolism are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50097-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Development and Application of an OTLC-MS Interface

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Jos de Wit	Chemist	LMB	NIEHS
Other:	Carol E. Parker	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor James Jorgenson, Department of Chemistry, UNC, Chapel Hill, NC.

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:
1.15	.30	.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.)*

An interface has been developed for the direct coupling of Open Tubular Liquid Chromatography (OTLC) and Mass Spectrometry. The interface has similarities to both direct liquid introduction (DLI) and thermospray (TSP) interface designs. The interface is introduced into the vacuum system via the solid probe inlet; thus, it does not compromise the use of other inlet systems, such as GC, DLI, or direct probe. In this interface design, the capillary column passes through a probe inserted into the solid probe inlet. The end of the capillary column is tapered, creating a submicron exit orifice. The end of the capillary column passes through a copper heating block, the temperature of which can be controlled and monitored. Tapering the capillary column tip led to significant improvements in both the peak shape and sensitivity. Probe temperature also had a significant effect on these parameters.

The system has been used with both glass and fused silica capillary columns, with inner diameters of 16 or 10 microns, respectively. With flow rates of nanoliters per minute, the entire effluent can be introduced into the mass spectrometer. When used with an electron-impact source, electron-impact mass spectra are produced. With a chemical ionization source, positive and negative CI spectra can be obtained when CI reagent gas is added through an inlet on the interface. The successful analysis of mixtures of organophosphorous pesticides, triazine herbicides, and polynuclear aromatic hydrocarbons has been demonstrated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50098-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Development of FAB/MS and FAB/MS-MS for Environmental Health Sciences

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Christian Guenat	Visiting Associate	LMB	NIEHS
	Sunita Chatterjee	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Carol Djerassi, Stanford University, CA, Professor Arno Spatola, University of Louisville, KY.

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:
.9	.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.)*

Fast atom bombardment (FAB) has become one of the most widely utilized techniques for obtaining mass spectra of thermally labile and/or very polar substances. In conjunction with tandem MS (MS-MS), the fragmentation information so vital for structure elucidation can be obtained from ions normally not observed to fragment and from mixtures where the parent ion-daughter ion relationships are not known.

This project involves three areas: 1) instrumental developments in MS-MS; 2) determination of underlying structural features which influence the spectra obtained so that data from unknowns can more easily be used for structure elucidation; and 3) application of FAB/MS and FAB/MS-MS for structure elucidation.

As part of the instrumental development we have extended our present instrument capabilities from MS-MS to MS-MS-MS-MS (MS4). With MS4, the consecutive fragmentations of a molecule can be followed.

In the determination of structural effects on MS-MS data, we have investigated the effect various N- and C-terminal amino acids have on the data and the utility of MS-MS spectra of (M+Na)+ ions of peptides for sequencing peptides.

We have applied FAB/MS and FAB/MS-MS to the structure elucidation of chemically modified analogs of Leu-enkephalin in which a CH2S moiety replaces an amide linkage, to the structure elucidation of phospholipids containing unusual fatty acids and head groups, to the differentiation of steroid glucuronide stereoisomers by MS-MS, and to the characterization of cross-linked peptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50099-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Application of Thermospray LC-MS to Structure Elucidation of Biomolecules

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
OTHER:	Jos de Wit	Chemist	LMB	NIEHS
	L.T. Burka	Research Chemist	DTRT/STB	NIEHS

COOPERATING UNITS *(if any)*

Professor Buhler, Oregon State University

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:
.7	.65	.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.)*

The majority of biochemical samples are complex mixtures. Separation and isolation of each component prior to analysis is often laborious and tedious. Therefore, considerable effort has been expended on the development and application of methodologies designed to separate and introduce each separated component of a mixture directly into a mass spectrometer. GC/MS, used for the separation and analysis of volatile and thermally stable substances is a relatively mature technique. LC/MS, which combines liquid chromatographic separation with MS analysis is still at a stage where several interfaces are in popular use and where the capabilities of the technique in its various manifestations have not been thoroughly explored.

We are currently exploring the applicability of thermospray (TSP) as an LC-MS interface to biochemical problems. One project involves the use of LC/MS for the detection of methylated nucleosides in order to relate these DNA modifications to altered gene expression. TSP LC/MS was used to determine the background level of the modified nucleosides in untreated calf thymus and salmon sperm DNA. In this study we have identified several methylated guanosines. A second major area of current interest is the development of TSP LC/MS for the determination of glutathione conjugates of exogenous chemicals and the mercapturic acid metabolites. Our results indicate that the mercapturic acid metabolites and derivatized glutathione conjugates can be successfully analyzed by this technique.

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 50100-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Structure Elucidation of Carcinogen-Nucleoside Adducts

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Christian Guent	Visiting Associate	LMB	NIEHS

COOPERATING UNITS *(if any)*

Andrea Dietrick, Guest Worker, UNC, Chapel Hill, NC; Dr. Louise M. Ball and Dr. Avram Gold, Dept. Env. Sci., UNC, Chapel Hill, NC; Drs. Steven Nesnell and S. Agarwal, USEPA, Research Triangle Park, NC

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:
.20	.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.)*

One of the major research concerns at NIEHS is to understand the interactions between exogenous chemicals and biomolecules within the body. For a complete understanding of this interaction, the structures of the products of covalent interactions must be elucidated. Of special concern are the products of reactions between environmental carcinogens and DNA.

As model systems, we are currently studying the in vitro interactions between 3-nitrofluorene and calf thymus DNA and between AZQ and quanosine.

3-Nitrofluorene must be reduced to the reactive hydroxylamine intermediate before it can react with DNA. This reductive interaction has been carried out in vitro using both chemical reduction and biologically mediated reduction (xanthine oxidase). The structure of the modified nucleoside isolated from the reaction has been elucidated by the use of a combination of FAB/MS and FAB/MS-MS and determined to be N-(deoxyguanosin-8-yl)-3-aminofluoranthene.

The analysis of the reaction of AZQ with quanosine has been initiated. An adduct has been isolated and tentatively identified. The final structure proof is currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50101-01 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Identification of Tetrachlorodibenzofuran Metabolites

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
	L.T. Burka	Chemist	DTRT/STB	NIEHS
Other:	Jos de Wit	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

DTRT/STB

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:
.27	.17	.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 2,3,7,8 isomer of tetrachlorodibenzofuran is a highly toxic contaminant found in polychlorinated biphenyls and in chlorinated phenol mixtures. In both structure and toxicity, 2,3,7,8-tetrachlorodibenzofuran (TCDF) is closely related to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In rats, TCDF, unlike TCDD, is readily excreted via the bile into the feces. Preliminary investigations of the biliary metabolites by other researchers has revealed, after methylation of glucuronidase/sulfatase-treated bile, tetrachloromethoxy-, trichloromethoxy-, and trichlorodimethoxy-dibenzofuran isomers, and a possible ring-opened product, a tetrachlorodimethoxybiophenyl.

In order to confirm the structure of the biliary TCDF metabolites, a program of rational synthesis of possible metabolites has been initiated. Three compounds are currently under investigation: 2,2'-dimethoxy-4,5,4'5'-tetrachlorobiphenyl (DMTCB), 1-methoxy-2,3,7,8-tetrachlorodibenzofuran (1-MTCDF), and 4-methoxy-2,3,7,8-tetrachlorodibenzofuran (4-MTCDF). Analysis by HRGC/NCI/MS of bile from rats treated with TCDF has confirmed the presence of 1-MTCDF. Although there is relatively little structural difference, the sensitivity of 1-MTCDF in the negative ion mode is several orders of magnitude greater than that of DMCTB. In positive ion chemical ionization, this sensitivity difference is not oberved. For 1-MTCDF, NCI is more sensitive than PCI; for DMCTB, PCI is more sensitive than NCI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50102-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Mass Spectral Analysis of the Benzo[a]Pyrene-DNA Reaction Product

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

PI:	John Dino, Jr.	Chemist	LMB	NIEHS
	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Christian Guenat	Visiting Associate	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor David G. Kaufman, Department of Pathology, UNC, Chapel Hill, NC

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:
1.25	.25	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project entails the mass spectral characterization of the previously uncharacterized products of interactions between the carcinogen benzo[a]pyrene (BP) and DNA in vivo or oligonucleotides in vitro.

The in vitro studies were aimed at elucidating the compound(s) comprising so-called "early-eluting" peaks which are observed in the C-18 chromatography of liver DNA digests from tritiated BP-treated A/HeJ mice. The two major techniques used in this work were HPLC and mass spectrometry. Three HPLC systems, employing Partisil 10 SAX, silica or C-18 were developed. Extensive mass spectral studies were done with electron impact or fast atom bombardment. FAB experiments were done in both positive and negative ion modes. The result of this project was that the "early-eluting" material was comprised of tritium-exchanged nucleosides of DNA and RNA, phosphoric acid and tris buffer from the DNA digestion.

The in vitro modeling studies examined the chromatographic and mass spectral behavior of unmodified oligonucleotides and those modified in vitro with benzo[a]pyrenediol-epoxide, the ultimate metabolite of BP. The "early-eluting" components identified in the in vivo system were not observed. The mass spectral characterization of the carcinogen modified oligonucleotides is currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50103-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

GC-MS Analysis of PCDF Blood Levels in Children Exposed In Vitro

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

PI:	Walter Rogan	Chief, Epidemiology Branch	DRA/EB	NIEHS
	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Linda Sheldon, RTI, Research Triangle Park, NC

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:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

There have been two outbreaks of human poisoning by polychlorinated biphenyls (PCBs) and their thermal breakdown products; the first, in Japan in 1968, the second in Taiwan in 1979. Because PCBs are a world wide pollution problem, these episodes have been studied carefully, since they have presented the only opportunity to observe directly the toxicity of PCBs in human beings outside the workplace. Laboratory methods for the evaluation of these outbreaks were relatively unsophisticated in 1968; there has been great progress in analytical methods since. In collaboration with Taiwanese scientists, the Epidemiology Branch, NIEHS, had the opportunity to examine over 100 children who had been in utero at the time of the 1979 poisoning or afterward. These children continued to be affected, since the chemicals cannot be excreted from the mother's body.

In a collaborative effort, the mass spectrometry workgroup will analyze approximately 120 serum samples for tetra-, penta- and hexachlorodibenzofurans at the part per trillion level. At the present time extraction procedures are being validated by RTI in preparation for the analysis of the samples.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50104-01 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

In Vivo NMR Studies of Cellular Magnesium

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
	B. Raju	Visiting Fellow	LMB	NIEHS
	Elizabeth Murphy	Senior Staff Fellow	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:
1.7	1.5	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.)*

Magnesium plays an essential role in cellular metabolism, functioning as a cofactor for kinase, phosphatases, and synthetases in all cellular compartments. Additionally, hypomagnesemia has been demonstrated to be associated iwth cardiovascular disease and diabetes. However, methodology for determinig cysotolic levels of free magnesium ionns has been limited. A number of recent sutdies have utilized in vivo phosphorus-31 nculear magnetic resonance (NMR), which provides information based on the equilibrium between free and magnesium complexed ATP. However, there are several important limitations to this technique, most significantly the relatively low dissociation constant of ATP for magnesium such that the cellular ATP pool is generally almost fully commplexed with magnesium. Fluorinated, intracellular chelators have recently been shown to be useful for the determination of cytosolic calcium levels using fluorine-19 NMR. During the past year we have designed, synthesized, and tested several fluorinated chelators which have dissociation constants suitable for the determination of basal cytosolic magnesium levels. The indicators developed are fluorinated analogs of o-aminophenyol-N,N,O-triaetic acid (APTRA). Magnesium dissociation constants determined for the 4-fluoro, 5-fluoro-, and 4-methyl, 5-fluoro derivatives of APTRA are 10, 3, and 1.5 mM, respectively. Preliminary measurements have yielded values of 0.3 mM free magnesium for erythrocytes, and 3.0 mM for perfused rat heart. Work is currently in progress to evaluate these indicators for measurements in different systems, and to develop analogous fluorescent magnesium indicators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80008-13 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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:	Roger Nolan	Visiting Fellow	LMB	NIEHS
	Roberta Danilowicz	Biologist	LMB	NIEHS
	Jeff Handler	IRTA	LMB	NIEHS
	Mike Luster	Research Microbiologist	TRTP	NIEHS
	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Paul Nettesheim	Chief, LPP	LPP	NIEHS

COOPERATING UNITS *(if any)*

Dr. David Henke, UNC, Department of Medicine, Chapel Hill, NC

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:
2.1	1.1	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.)*

Investigations are concerned with the oxidation of arachidonic acid to prostaglandins (PG), leukotrienes and hydroxy-fatty acids and the relationship of this metabolism to the regulation or modulation of biological processes. We have previously shown that formation of PGD_2 in canine tracheal epithelial cells correlates with Cl^- secretion. We have extended these studies to human airway cells and found that these cells do not biosynthesize PGs but make 15-HPETE and its metabolites. Studies are in progress to determine the possible relationship of 15-HPETE formation to ion movement in these cells. We have also investigated the role of arachidonic acid metabolism in mitogenesis. Mouse B lymphocytes, oxidized arachidonic acid to 12-HPETE and 12-HETE. No prostaglandins are formed. Inhibition of 12-lipoxgenase by nordihydroguaiaretic acid (NDGA) inhibits lipopolysaccharide (LPS) dependent mitogenesis. The addition of benzidine and other cofactors for peroxidase inhibited mitogenesis without altering the total oxidation of arachidonic acid. However, these chemicals altered the ratio of 12-HPETE to 12-HETE, suggesting a requirement of the peroxide or an involvement of the peroxidase in LPS induced mitogenesis. We have also studied arachidonic acid metabolism and EGF induced mitogenesis in BALBc 3T3 cells. EGF induces the formation of PGE_2 (the major arachidonic acid metabolite) and mitogenesis. Indomethacin, dexamethasone, and NDGA block PGE_2 formation and mitogensis. Prostaglandins are weakly mitogenic but the addition of PGs to indomethacin inhibited EGF-stimulated mitogenesis restored DNA synthesis. Moreover the addition of PGs greatly enhance EGF mitogenesis. The addition of arachidonic acid to those cells stimulated DNA synthesis. These studies suggest a possible important role for arachidonic acid metabolism in regulating cell growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-11 LMB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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

Cooxidation of Xenobiotics by the Prostaglandin Synthethase

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/Julie Angerman-Stewart	Chemist	LMB	NIEHS
	Yolanda Van der Zee	Visiting Fellow	LMB	NIEHS
	Kenneth B. Tomer	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Michael Hughes, UNC Post-doctoral Fellow, Chapel Hill, NC

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:
5.0	3.2	1.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The long range goal of this project is to study the oxidation of chemicals to toxic or carcinogenic metabolites by prostaglandin H synthase (PHS) and to demonstrate the importance of this enzyme system in chemical-induced toxicity or carcinogensis. We have shown that aromatic amine carcinogens, for example 2-aminofluorene (2-AF), are metabolized to mutagens by PHS. PHS dependent oxidation occurred by a free radical mechanism and resulted in the formation of 2-AF-DNA adducts which can be used as in vivo markers for PHS-dependent oxidation. A dog was fed a single dose of ^{3}H-2-AF and 24 hrs. later the 2-AF-DNA adducts isolated in extra-hepatic tissue. The liver was devoid of peroxidase-catalyzed adducts but the kidney cortex and medulla contain significant amounts of these adducts. The peroxidase adducts were exclusively the same 2-AF-DNA adducts isolated from bladder urothelium. We have also investigated the metabolism of amino acid pyrrolysates and the thyroid carcinogen amitrole by PHS. These aromatic amines were oxidized by PHS to free radical metabolites that covalently bound to DNA and other macromolecules. We have also discovered a new mechanism for the formation of glutathione adducts that occurs in the absence of epoxide formation and conjugation by glutathione transferase. By this mechanism the model compound, styrene reacts with a thiyl radical formed enzymatically by PHS. The resulting styrene-glutathione carbon-centered radical then reacts with molecular oxygen to form a peroxyl radical which eventually yields the conjugate. Our data suggest that PHS is a versatile enzyme system that can catalyze a variety of reactions and appears to be important in conversion of chemicals to carcinogenic metabolites in extra hepatic tissue.

LABORATORY OF PHARMACOLOGY
Summary Statement

The Laboratory of Pharmacology at NIEHS is interested in the mechanisms that govern toxic reactions brought about by exposure to harmful chemicals present in the environment. The approach to research in this laboratory is based on the premise that many biological systems are not sufficiently understood to allow for mechanistic conclusions to be drawn from results of experiments involving the direct use of toxic agents. Therefore, our emphasis is placed upon basic research aimed at defining certain biological systems at the molecular level, with particular attention being paid to regulatory mechanisms.

One element in common to all of the research programs in The Laboratory of Pharmacology is membrane function. This is an integral aspect of our studies on the metabolism of environmental chemicals by membrane-bound enzymes, the transport of solutes across epithelial membranes and the modification of cellular responses by surface membrane receptors. With all three areas of investigation, the goal is to first understand the operation of the systems at the molecular level. Once this has been accomplished in sufficient detail, it should be possible to determine the mechanisms by which various toxicants affect these systems, and to enumerate the implications of such interactions on human health.

The majority of the harmful substances present in the environment exhibit little or no biological activity unless they first undergo some chemical modification. For the most part, this is accomplished by oxidative reactions that are catalyzed by enzymes present in most mammalian tissues. Although it is clear that this biotransformation is a prerequisite to most chemically induced carcinogenic, mutagenic, and other manifestations of toxicity, the role metabolism plays in directing the tissue- and cell-specificities of these events is not understood. This particular problem is being assessed by investigating drug-metabolizing enzymes in liver and lung, two tissues that respond quite differently to a number of toxic chemicals. The primary enzymes involved with the metabolism of toxic chemicals are various isozymes of cytochrome P-450, and the distribution, regulation, and concentrations of these isozymes in liver and lung have been found to be markedly different. In some instances these differences appear to be important factors in site-directed toxicity. For example, the pulmonary toxicity of certain substituted furans, which exert their effects on a specific population of pneumocytes, appears to result from the relatively high concentrations of two isozymes of cytochrome P-450 present in the target cell type. These two isozymes are also present in liver but at much lower concentrations. In other studies significant sex-dependent differences in the expression of cytochrome P-450 isozymes have been discovered. These isozymes, which are under hormonal regulation, also exhibit some remarkable tissue specificities. One isozyme is expressed in male kidney, but not in male liver, while the opposite is true in females. These findings of highly regulated systems that can be modulated by both endogenous and exogenous chemicals indicate that individual variation within the human populations could be significant. Such variation may play a major role in the determination of individual susceptibility to various toxic substances. The interrelationships among pituitary hormones, sex hormones, and exogenous

chemicals, all of which effect the expression of these enzymes, is presently under investigation. Unique patterns of distribution have also been discovered in the case of another drug-metabolizing enzyme called the flavin-containing monooxygenase. With this enzyme it has been found that the forms present in lung and liver are immunochemically distinct. In addition, the substrate specificities of the pulmonary and hepatic enzymes differ substantially. Since this enzyme is active in the formation of mutagenic metabolites from a number of aromatic amines, such tissue differences may be an important factor in determining the tissue-specific effects of these compounds.

Transport of chemicals across membranes is another controlling factor in the biological disposition of environmental chemicals. This process is associated with uptake into the organism, distribution within the organism, and excretion. In addition, the transport of biological molecules across membranes, which is part of the normal metabolic scheme, can be drastically altered by a number of foreign compounds. Because of their complex organization, functional importance, and exposed locations, epithelial membranes are particularly susceptible to the toxic effects of foreign chemicals. This has important implications with respect to the transport functions of the kidney, which controls, via excretion, the time of exposure to toxic chemicals. It is now known that this process requires the action of two carrier proteins. One of these proteins exchanges internal glutaric acid for the molecule to be excreted and the second utilizes the sodium gradient to maintain a high intracellular concentration of glutarate. A second process, which is involved with the transport of organic cations, has also been found to be carrier-mediated and strongly potential dependent. In addition, the major driving force responsible for the intracellular accumulation of organic cations via this carrier is the electrical potential gradient across the basolateral membrane.

Surface-membrane receptors are central to the processes by which cells receive the information required for the regulation of their functions. In order to maintain proper biochemical balances, these receptors, and the intracellular reactions they control, must operate with a high degree of precision. Because these systems are highly complex, they offer numerous opportunities for foreign compounds to modulate their actions. The implications of this are just beginning to be understood, but should become clearer when cellular signal transduction is better understood. One transduction system being studied in The Laboratory of Pharmacology involves the mobilization of cellular calcium, which is controlled, at least in part, by messenger molecules called inositol phosphates. The pathways involved in the metabolism of inositol phosphates are being investigated, and the elucidation of several novel reactions has uncovered new metabolites that may play a major role in the control of calcium. Recent findings indicate that an early event in the action of certain receptors is the hydrolysis of a membrane phospholipid with the subsequent formation of two putative second messenger molecules, diacylglyerol and inositol 1,4,5-triphosphate. The first of these is thought to activate a specific kinase, the second is thought to be involved in the release of calcium. The triphosphate compound is further metabolized by two distinct pathways resulting in a complex mixture of inositol phosphates all of which may have significant biological functions. In turn, the formation of inositol phosphates is controlled by peptide receptors located on the cell surface. One of these receptors,

which appears to be regulated by desensitization, is being studied in detail. In addition to receptors on the cell surface, recent findings indicate that there are also intracellular receptors for some compounds. Marked increases in both protein and RNA synthesis have been observed by direct injection into oocytes of insulin at concentrations below those required to initiate processes at the cell surface. The unique aspect of this work is the development of techniques that allow for changes to be monitored in single isolated cells. The microinjection system enables the researcher to bypass the cell membrane and observe the direct effects of substances in the cell. Results to date indicate that insulin, which is known to mediate cell function by interacting with a surface receptor, has a direct effect that does not require binding to molecules on the outside of the plasma membrane.

While the study of the metabolism and membrane transport of foreign chemicals in The Laboratory of Pharmacology represents a continuation of investigations that were initiated some time ago, the work on signal transduction represents a new initiative. This important area of research, which has not been a part of the effort at NIEHS in the past, will be expanded in the future. The importance of receptor mechanisms to environmental health may not as yet be understood, but the central role these systems play in the maintainence of biological functions indicates that they are a likely target for toxic insult. Also, the direct interaction of some toxicants, such as TCDD, with receptors has been clearly established. However, our lack of understanding of the events that occur subsequent to receptor-ligand interaction, has blocked all efforts to define the mechanisms by which chemicals like TCDD exert their effects. Clearly, the molecular interrelationships that transmit messages between the cell surface and the nucleus must first be unraveled before we will be in a position to define how signal transduction can be affected by xenobiotics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80001-15 LP

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	R. Gasser	Visiting Fellow	LP	NIEHS
	R. Tynes	Guest Worker	LP	NIEHS

COOPERATING UNITS *(if any)*
University of California, Davis, CA; Scripps Institute and Research Foundation

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:
5.0	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.)*

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. However, treatment of hamsters with phenobarbital does not increase the concentration of this isozyme in liver. Rabbit liver expresses three forms of isozyme 2, two of which are also present in rabbit lung. These forms have been defined on the basis of restriction mapping and sequencing of cDNAs derived from mRNA isolated from liver and lung. A second form present in both tissues differs by 6 out of 491 amino acid residues. A third varient, which is present only in liver, differs at 11 and 15 positions from the other two. The expression of the three forms of isozyme 2 in liver appears to be under independent control with respect to induction by phenobarbital. Results with oligo-specific probes indicate that one form is not induced, one is induced initially and is then repressed, and the third undergoes induction only following prolonged treatment. Although the total mRNA for isozyme 2 in lung is not affected by phenobarbital, it is not clear whether or not the relative proportions of the two forms present are altered. Partial sequence analysis of cDNAs for isozyme 5 indicate that the pulmonary and hepatic forms are the same. Contrary to reports in the literature, we find that pulmonary mRNAs for isozymes 2 and 5 are confined entirely to the fraction that binds to oligo-dT. The reason for this difference appears to reside in the methods used for RNA isolation. We have found that great care must be exercised in order to isolate intact, poly-A mRNA from lung. A reproducible method, which involves centrifugation in cesium chloride followed by precipitation, has been developed for the purification of pulmonary mRNA in high yield.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80007-16 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 professionel personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

PI: Cosette J. Serabjit-Singh Research Chemist LP NIEHS

Others:

COOPERATING UNITS *(if any)*

J.R. Bend, University of Western Ontario, London, Ontario, Canada

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:
1.0	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 spece provided.)*

The cytosolic glutathione S-transferase (GST) of the rabbit lung is composed primarily of two equally abundant isozymes. These isozymes, GST 25kd and GST 24kd, are homodimers that are distinguishable by 1) the mobilities of their subunits 25 and 27kdaltons, respectively, in SDS polyacrylamide electrophoretic gels; 2) their isoelectric points, pI 7.4 and 9.1, respectively, by 2-dimensional gel electrophoresis; 3) by distinct immunoreactivity with polyclonal antisera elicited against each isozyme and; 4) differences in the N-terminus (GST 27kd appears to have a blocked N-terminus similar to the alpha class of GST, and GST 25kd has 15 of 17 residues in common with the pi class isozymes of other species). The specific activity of GST 27kd is half that of GST 25kd with chloro-2,4-dinitrobenzene as substrate, but is twice that of GST 25kd with pyrene 4,5-oxide (PO) as substrate. The activity of both isozymes toward PO is almost non-stereoselective in contrast to the stereoselectivity of the cytosol for the S-configured prochiral carbon. The total PO activity of the purified isozymes represents at best 10% of the cytosolic activity. The subunits of GST 27kd and GST 25kd are present in heptic cytosol, which is stereospecific for the S-configured prochiral carbon of PO, but were not purified as homogeneous isozymes by the methods used for the pulmonary cytosol. However, similar to the pulmonary isozymes, the stereospecificity and the specific activity towards PO was not maintained during purification of the hepatic GST. The specific activity toward PO of the rabbit hepatic and pulmonary preparations were comparable to those of hepatic GST preparations from other species, except for the rat and human GST μ, which were 10 times more active. Whether there is an endogenous cytosolic factor that modulates the PO activity of some GST isozymes remains to be determined, in addition to studying the cellular distribution and genetic regulation of GST and the relationship, if any, to the susceptibility of the lung to toxicants/carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80031-11 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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; 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:
6.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The ability to transport solutes across epithelial membranes is a vital function of many organs, e.g., kidney. In turn, epithelial transport depends upon individual transport systems located in apical (BBM) and basolateral (BLM) membranes. Because of their complex organization, functional importance, and exposed location, epithelial membranes are particularly susceptible to toxic effects of foreign chemicals. Recent work has focussed on the renal organic anion transport system, since this system determines the extent of elimination of toxic xenobiotics. Using p-aminohippuric acid (PAH), a model substrate for this system, it was shown that transport requires the coordinated action of two distinct carrier proteins. One mediates exchange of external PAH for internal glutaric acid (or α-ketoglutarate). The second taps energy stored in the Na gradient to drive glutarate back into the cell, maintaining the steep (in>out) glutarate gradient needed to drive PAH uptake. Together the two systems indirectly couple BLM PAH transport to the sodium gradient and metabolic energy stores. BBM are unable to couple the two processes. Thus, PAH secretion requires BLM uptake and intracellular accumulation, followed by exit of PAH down its electrochemical gradient at the BBM. Studies using electrophysiological and radiochemical techniques to examine organic cation transport (the second major xenobiotic excretory pathway) show that the basolateral step in secretion of the model organic cation, tetraethylammonium (TEA), is carrier-mediated and strongly potential dependent. To focus on intracellular events, including binding, subcellular compartmentalization, and information transfer between the surface membrane and intracellular organelles, cryomicrodissection and microinjection techniques were developed in amphibian oocytes. Particularly striking was the observation that intracellular insulin, at doses (0.5-5 pmoles) too low to alter surface membrane transport, caused marked changes in both protein and RNA synthesis rates. These preliminary results argue strongly for a physiological role of intracellular insulin receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80040-04 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	R. Masaki	Visiting Associate	LP	NIEHS
	J. Squires	Visiting Fellow	LP	NIEHS
	T. Ichikawa	Visiting Fellow	LP	NIEHS
	R. Lindber	Visiting Fellow	LP	NIEHS
	B. Burkhart	Biologist	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:
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 mechanisms of sex-dependent gene regulation of steroid hydroxylases in liver and kidney. Full-length cDNAs encoding for three sex-specific hepatic testosterone hydroxylases in mice were isolated and sequenced. With the use of the cDNAs, the following have been uncovered. I-P-450$_{16\alpha}$ (female-specific 16α-hydroxylase): The specific expression in females is regulated by a single locus named Rip, and its repression in males is under the control of the locus named Ripr. Both loci are closely localized on chromosome 7, along with the I-P-450$_{16\alpha}$ structural gene which is greater than 35kb in size and has 9 exons. It was found that growth hormone and estrogen are repressors of I-P-450$_{16\alpha}$ in males. Genetic evidence suggests that estrogen acts on Ripr locus. C-P-450$_{16\alpha}$ (male-specific 16α-hydroxylase): The comparisons of mRNA and activity levels in livers from androgen-treated 129/J mice, which had been castrated at day one or at adulthood, provided the evidence that there are at least two differentially regulated C-P-450$_{16\alpha}$'s in livers from adult males; one is neonatally imprinted and the other is reversibly regulated by androgen. The expression of C-P-450$_{16\alpha}$ in males is also regulated by growth hormone. It is, therefore, interesting to see how the pituitary and sex hormones are cooperating to regulate this hydroxylase gene. The cDNA of C-P-450$_{16\alpha}$ was ligated to pcD vector and transfected in COS cells, resulting in expression of 16α-hydroxylase activity. The gene for reversibly regulated C-P-450$_{16\alpha}$ was characterized to be about 5kb with nine exons. P-450$_{15\alpha}$ (female-specific 15α-hydroxylase): Two Two highly homologous, but differentially regulated genes (Types I and II) were identified and isolated. The type I gene is predominantly expressed in female but not male livers, but expressed only in male but not female kidney. The repression of P-450$_{15\alpha}$ in male liver and the expression in male kidney are reciprocally regulated by androgen. Type II genes are female-specific in both liver and kidney, although the level of expression in kidney is minimal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80042-01 LP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Calcium Regulation and Signal Transduction Mechanisms

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

PI:	James W. Putney, Jr.	Pharmacologist	LP	NIEHS
Others:	Arlene Hughes	Staff Fellow	LP	NIEHS
	Hiroshi Sugiya	Visiting Associate	LP	NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Calcium Regulation 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.)*

The broad aim of this project is to understand at the cellular and molecular level, the mechanisms by which surface membrane receptors for hormones, neurotransmitters and growth factors modify cellular responses through mobilization of cellular calcium. Recent findings indicate that an early event in the action of receptors of this class is the hydrolysis of a membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, to yield two putative second messenger molecules, diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP3). DG is believed to activate a specific kinase in cells (protein kinase C) and IP3 is believed to act by releasing stored calcium. IP3 is subsequently metabolized by two distinct pathways, one involving dephosphorylation, and the other phosphorylation and dephosphorylation; the result is a complex mixture of different inositol phosphates which might have significant biological actions. The formation and metabolism of these compounds is being investigated in intact and broken cell systems to determine the quantities formed of each of these substances, and the kinetics of their formation and degradation. Some of the studies involve the use of tissues from euthanized laboratory rats, while others use immortal cell lines (rat pancreatoma). These techniques involve the use of tritium-labeled precursors, and separation of compounds by a combination of anion exchange chromatography and HPLC. Cellular calcium is being investigated in the same model cell systems with fluorescent indicators to determine correlations between calcium and the various inositol phosphates. These studies should provide insights into mechanisms of action of calcium mobilizing agents, and may indicate vulnerable sites of interaction with environmental toxins such as heavy metals.

THE LABORATORY OF PULMONARY PATHOBIOLOGY
Summary Statement

Two broad research topics are being pursued in the Laboratory of Pulmonary Pathobiology (LPP). One is concerned with pulmonary cell biology and mechanisms of pulmonary disease, the other with mechanisms of carcinogenesis.

I. Pulmonary Studies

A major part of LPP's research efforts is concerned with the exploration of mechanisms of particle and fiber toxicity for the respiratory tract. Upon deposition in the distal lung, particles such as silica and asbestos cause a multitude of cellular and biochemical reactions which ultimately result in pulmonary fibrosis (silicosis and asbestosis). Our studies have shown that within hours after particle exposure, macrophages are attracted to the sites of deposition and a wave of cell proliferation is triggered in the epithelium of the bronchioles and the alveoli, as well as in the pulmonary interstitium. It is not clear which of these cellular reactions are important in the pathogenesis of pulmonary fibrosis. Since the recruitment of macrophages to the sites of particle deposition is such an early and prominent event, the hypothesis has been advanced, that many of the cellular changes, particularly the proliferation of interstitial fibroblasts are mediated by factors elaborated and released from macrophages during phagocytosis of the particles.

The studies carried out to date on the role of macrophages have produced the following major findings. 1) Within a few hours of exposure to a variety of inhaled particles, a complement-dependent chemotactic factor for macrophages is activated in the alveolar lining layer. 2) Consequently, macrophages are attracted to the sites of deposition and phagocytize the particles. 3) During particle binding and phagocytosis, macrophages release a number of arachidonic acid metabolites of both the cyclooxygenase and lipoxygenase pathways. Several of these are known to be powerful mediators of inflammation. 4) In addition, the macrophages produce growth factor(s) which stimulate proliferation of pulmonary fibroblasts. This growth factor is similar to platelet-derived growth factor (PDGF) which is known to be encoded by the c-cis proto-oncogene. Current studies are aimed at quantifying this growth factor and establishing its identity and biological activity in vivo.

Another series of studies is concerned with the response of alveolar epithelial type II cells to toxic particles, namely silica which is known to induce silicosis. Type II cells are the producers of pulmonary surfactant, a mixture of lipids and proteins which prevents the collapse of distal airspaces. Silica exposure causes a massive accumulation of surfactant in the lungs of rabbits, producing a disease state reminiscent of alveolar proteinosis. Both the intra-and extra-cellular surfactant pools are increased and we found that the normal equilibrium between synthesis, secretion and clearance of surfactant from the alveolar spaces is disrupted. Hypertrophic type II cells have been isolated from silica exposed lungs to identify precisely which steps in the biosynthesis and secretion of surfactant are affected. Whether the type II cell response (hyperplasia, hypertrophy and increased surfactant production) is the result of a direct interaction of silica particles with the type II cell membrane or whether it is mediated by some factor released from other cells, is the subject of future studies.

Another major research effort of the Laboratory is to study specific pulmonary cell types, in order to elucidate biochemical and molecular mechanisms regulating their differentiation as well as various other functions. Under intensive investigation is the pulmonary Clara cell, a cell type located preferentially (but not exclusively) in the bronchioles. Clara cells are known to be rich in drug metabolizing enzymes and are suspected of secreting proteinaceous products of an undefined nature. This cell also is believed to play a major role in the maintenance and repair of the bronchiolar epithelium and to have stem-cell functions. Using highly purified Clara cell suspensions it was shown that their major secretory product is a 12 kDa protein which reacts with Clara cell antisera and is localized in osmiophilic granules. The same protein is also found in pulmonary lavage fluid supporting the notion that the 12 kDa protein is a secretory product. Messenger RNA was isolated from purified Clara cells and it was demonstrated that the primary translation product encodes a protein with a molecular weight about 1 kDa larger than the secreted 12 kDa protein and is therefore believed to be a signal peptide. Studies are currently underway to determine the function(s) of this low molecular weight protein.

In vivo culture studies with highly purified Clara cells have shown that these cells have a high proliferative potential, considerable self-renewal capacity and can give rise to terminally differentiated ciliated cells. Current studies are designed to examine the relationship between Clara cells and other secretory cells in the conducting airways.

To study the biochemical and molecular mechanisms of differentiation of epithelial cells from the conducting airways, an epithelial cell culture system has been developed over the last several years. Using this in vitro model of tracheo-bronchial cell differentiation, it was shown that these cells have a dual differentiation potential. When grown on collagen gels in the presence of retinoids they differentiate as secretory cells. Analysis of the ^{3}H-glucosamine-labeled secretory products revealed that the cells secrete mucous glycoproteins under these conditions. In the absence of retinoids the cells instead undergo squamous differentiation and acquire many features typical of epidermal keratinocytes. The pathway of keratinocyte differentiation occurs in atleast three discrete steps: a) terminal cell division with loss of clongenic potential which is induced by removal of EGF from the medium or by the addition of TGF_{β}; b) expression of the keratinocyte phenotype (which is measured by increase in epidermal transglutaminase, increase in cholesterol sulfate, and increase in specific, large molecular weight keratins) and c) the formation of cross-linked envelopes. Expression of the keratinocyte can be enhanced by addition of Ca^{++} to the media and inhibited by retinoids. cDNA clones from squamous differentiated cells were obtained for the purpose of isolating the genes controlling keratinocyte differentiation in airway epithelial cells.

II. Carcinogenesis Studies

The development of cancer is believed to occur in multiple steps. According to the most widely accepted model of carcinogenesis, the process is initiated by the interaction of a carcinogen with normal stem cells, converting them to preneoplastic cells. These heritably altered cells have an increased probability of generating neoplastic offspring. Through a series of secondary changes which may either be spontaneous or induced (e.g. by carcinogens or tumor promoters), preneoplastic cells are converted to neoplastic and malignant cells.

Our carcinogenesis studies have focused on the following major problems: 1) the importance of chromosome mutations in neoplastic transformation; 2) the role of proto-oncogenes and tumor suppressor genes in different stages of neoplastic transformation and 3) the mechanism by which the c-src gene product transforms Syrian hamster embryo (SHE) cells.

A large number of carcinogens induce DNA damage and gene mutations at specific loci and their carcinogenicity has often been linked to their mutagenic activity. However, a number of carcinogens have been found, which have little or no demonstrable mutagenic activity. One of these is the synthetic estrogen diethylstilbestrol (DES), which we showed is able to transform SHE cells without causing any detectable point mutations. But DES does disrupt microtubule organization and causes numerical chromosome changes i.e. aneuploidy. Other agents which are inactive as gene mutagens but active as chromosomal mutagens and induce neoplastic transformation are sodium arsenite, sodium arsenate and asbestos fibers. Benzene, a weak mutagen, also causes aneuploidy and cell transformation. These results indicate that some agents are carcinogenic because they cause chromosome mutations rather than gene mutations. This finding has not only considerable mechanistic significance but also important practical implications in the development of bioassays for screening potential human carcinogens.

The multistep hypothesis of carcinogenesis implies that multiple genes are involved in the phenotypic cellular changes occuring at various stages of neoplastic transformation. We showed that neither v-Ha-ras, nor v-myc, when transfected alone, are able to neoplastically transform normal SHE cells. However, transfection with a combination of these oncogenes results in neoplastic transformation. Cytogenetic studies of the ras/myc tumors revealed the nonrandom loss of chromosome 15 in 6 out of 6 tumors suggesting that in addition to the activation of the two cooperating oncogenes a third step is required for tumorigenic conversion of normal SHE cells. The importance of monosomy of chromosome 15 in these tumor cells is supported by studies involving cell-cell hybridization between ras/myc tumor cells and normal SHE cells. The tumorigenicity of these hybrids is initially suppressed but is re-expressed at later passages concomitant with the loss of one copy of chromosome 15, suggesting that this chromosome plays a role in suppressing the tumorigenic phenotype. These findings indicate that activation of proto-oncogenes and inactivation of tumor suppressor genes are crucial steps in neoplastic transformation.

Studies on the role of oncogenes in late stages of neoplastic transformation of airway epithelial cells suggest the involvement of a gene related to the oncogene fms, whose normal cellular cognate is the receptor for the hematopoietic growth factor CSF-1. This fms related gene is expressed in the tumorigenic epithelial cells as a 9.5 kb transcript. In contrast the rodent as well as the human fms transcript is 4 kb in length. All studies carried out to date indicate that the gene expressed in these neoplastic epithelial cells while related to fms is probably a new gene encoding a receptor for a related hematopoietic growth factor. Our findings raise the possibility that inappropriate expression of a hematopoietic growth factor receptor related gene may be an important mechanism underlying the neoplastic behavior of these transformed cells.

While the evidence has been mounting over the last 10 years that activation or overexpression of oncogenes play an important role in neoplastic transformation, the biochemical mechanisms by which oncogene products effect the transformed phenotype are only poorly understood. In our studies concerning oncogene expression in chemically induced preneoplastic and neoplastic SHE cells, it was found that neoplastic cells have a 4-20-fold increased $pp60^{c\text{-}src}$ protein kinase activity compared to preneoplastic and normal SHE cells. This is not due to increased levels of c-src protein in the neoplastic cells but rather to an increase in the specific activity of the protein kinase. Currently studies are underway to determine a) whether activation of $pp60^{c\text{-}src}$ in neoplastic SHE cells is causal in the conversion of preneoplastic to neoplastic cells b) which modifications of the $pp60^{c\text{-}src}$ protein in neoplastic cells are responsible for its increased protein kinase activity and c) which intracellular targets are being modified by the activated $pp60^{c\text{-}src}$.

In summary, the Laboratory of Pulmonary Pathobiology is attempting to understand the molecular, biochemical and cellular basis of normal cell growth and differentiation and the mechanisms underlying the disease processes of pulmonary fibrosis and carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25001-10 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

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Others:	P. Lamb	Biological Lab Tech	LPP, NIEHS
	T. Nicotera	Postdoctoral Fellow	LPP, NIEHS
	W. Fletcher	Graduate Student	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:
1.5	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.)*

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 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. DES has colcemid-like activity in that it disrupts microtubule organization. DES also can be metabolically activated to mutagenic intermediates which is another mechanism for DES carcinogenicity. Currently, DES-induced DNA adducts are being examined in the treated cells. 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 in vivo for mesothelioma induction. We have proposed that asbestos induces cell transformation due to its ability to induce chromosomal changes in the treated cells. Arsenicals were effective inducers of cell transformation. Sodium arsenite and sodium arsenate were inactive as gene mutagens but were potent inducers of chromosome aberrations and also gene amplification. Benzene induced cell transformation and was a weak gene mutagen. This chemical was a very effective inducer of aneuploidy in this system. These results further support our hypothesis that cell transformation involves a chromosmal 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-25020-05 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	G. E. R. Hook	Research Chemist	LPP, NIEHS
Others:	L. B. Gilmore	Biologist	LPP, NIEHS
	B. E. Miller	Graduate Student	LPP, NIEHS
	G. J. Baker	Visiting Fellow	LPP, NIEHS

COOPERATING UNITS *(if any)*

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:
1.75	0.75 1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Pulmonary surfactant is a complex mixture of lipids and proteins that prevents collapse of the alveoli and distal airways at low lung volumes. Silica dust causes a massive increase in the surfactant content of the lungs but the mechanisms through which this occurs are not known. Examination of the rates at which surfactant phospholipids are synthesized, secreted, and cleared from the lungs of silica-treated rats revealed that expansion of the intra and extracellular pools of surfactant could be accounted for by imbalances between the rates at which surfactant phospholipids are synthesized by alveolar Type II cells, secreted, and then cleared from the alveoli.

Under normal circumstances these rates of synthesis, secretion, and clearance are the same and the intra- and extra-cellular pools of surfactant do not change in size. Our studies further indicated that some, but not all Type II cells, were substantially increased in size. We hypothesize that the expanded surfactant pools are related to the appearance of these hypertrophic Type II cells. By using protease digestion and centrifugal elutriation procedures we have now succeeded in isolating hypertrophic Type II cells from the lungs of silica-treated rats and preliminary evidence indicates that these cells are hyperactive in their ability to synthesize surfactant. In future studies we will further characterize the changes that have occurred in the silica-exposed Type II cells and identify which steps in the biosynthetic pathway are responsible for the increased rate of biosynthesis of surfactant phospholipids. We will also investigate phospholipid biosynthesis in Type II cells under conditions of in vitro cultivation as a means of elucidating the mechanisms through which surfactant biosynthesis is affected by silica.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 25021-04 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Regulation of Differentiation of Tracheobronchial Epithelial Cells

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:	E. E. Floyd	Staff Fellow	LPP, NIEHS
	C. Nervi	Visiting Fellow	LPP, NIEHS
	J. Rearick	Senior Staff Fellow	LPP, NIEHS
	M. George	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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

To study the regulation of differentiation of tracheobronchial epithelial cells we have developed an in vitro cell system using rabbit tracheal epithelial cells. We have shown that these cells, under different conditions, can express either a mucous-secretory or a squamous phenotype. Cells grown on a collagen type I gel matrix in the presence of retinoids express the mucous-secretory phenotype. Biochemical analysis of ^{3}H-glucosamine-labeled secretory products shows the release of mucous glycoproteins. Cells grown in the absence of retinoids, either on a collagen Type I gel matrix or on collagen-fibronectin coated dishes, undergo squamous differentiation. This pathway of differentiation is a multistep process. First, cells undergo terminal cell division which is characterized by the accumulation of cells in the Go/G1 phase of the cell cycle and reduction in colony forming efficiency. Terminal cell division normally occurs at high cell density but can be induced at low density either by the removal of epidermal growth factor or by the addition of transforming growth factor β. Terminal cell division is followed by the expression of a squamous phenotype which is characterized by the appearance of a squamous morphology and changes in several biochemical markers. The formation of cross-linked envelopes is the last stage in this terminal differentiation. The commitment to terminal cell division is insensitive to retinoids whereas the expression of the squamous phenotype is under the control of retinoids. The differential effects of retinoids on the commitment to terminal cell division and expression of the squamous phenotype indicate that the control of the two events are separable; however, other evidence shows that they are regulated in a coordinate manner. We developed a cDNA library from poly(A)$^{+}$ RNA isolated from squamous differentiated cells and obtained cDNA clones that hybridize with mRNAs that are expressed in squamous differentiated cells but not in undifferentiated or retinoic acid-treated cells. Study of the regulation of these genes are in progress.

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-25023-04 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cellular and Molecular Mechanisms of Progression of Transformed RTE cells

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

PI:	Paul Nettesheim	Chief	LPP, NIEHS
Others:	C. Walker	Senior Staff Fellow	LPP, NIEHS
	S. Endo	Visiting Fellow	LPP, NIEHS
	T. Gray	Biologist	LPP, NIEHS
	M. Joyce	Technician	LPP, NIEHS

COOPERATING UNITS *(if any)*

Environmental Carcinogenesis Group
J.C. Barrett and T.M. 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:

CHECK APPROPRIATE BOX(ES)

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

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

The overall objective of our studies is to elucidate cellular and molecular mechanisms important in multistage neoplastic transformation of epithelial cells using rat tracheal epithelial (RTE) cells in culture as an experimental model. Our hypothesis is that some early steps in neoplastic transformation of RTE cells may result from loss in responsiveness of the cells to negative growth regulators. We found that retinoic acid (RA) acts as a negative growth regulator of normal RTE cells, in culture. We also found that induction of early RTE cell transformants by carcinogens was effectively inhibited with nanomolar concentrations of RA. However, once preneoplastic RTE cell clones developed, their cells became rapidly resistant to RA. At 3 weeks after exposure to carcinogen, the 50% growth inhibitory concentration of RA for these transformants was 0.1 nM while at 12 weeks it was >100 fold greater. These data suggest that loss of growth inhibitory mechanisms may be important in early stages of neoplastic RTE cell transformation. The role of oncogenes in late stages of RTE cell transformation was investigated. We found that RNA homologous to the cellular oncogene fms was expressed 5-19 times higher than in normal cells in three of five neoplastic RTE cell lines examined. This increased expression of fms-related mRNA was not due to gene amplification or gene rearrangment. The fms probe detected a 9.5 kilobase mRNA in the neoplastic RTE cells which contrasts to normal rat alveolar macrophages and the human choriocarcinoma line BeWo which expressed a fms transcript of ~4 kilobases. We tentatively conclude that the 9.5 kilobase transcript is related to but distinct from the c-fms gene that encodes the macrophage colony-stimulating factor (CSF-1) receptor. Conceivably these neoplastic cells express another growth factor receptor gene. Studies aimed at identifying this gene using cDNA cloning stratagies are currently underway. To identify the ligand of this putative growth factor receptor, binding studies with hematopoietic growth factors related to CSF-1 are being performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES-25027-04 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	R. P. Gupta	Visiting Fellow	LPP, NIEHS
	Y. Inayama	Visiting Fellow	LPP, NIEHS
	L. B. Gilmore	Biologist	LPP, NIEHS

COOPERATING UNITS *(if any)*

Cell Biology Group (A. M. 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:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The functions of the bronchiolar Clara cell are not known although it is generally believed that the cell is secretory. Using a model system, developed in this laboratory, we have identified a low molecular weight protein (Mr 12,500) as the major protein secreted by Clara cells. We have also identified the major secretory protein in pulmonary lavage effluents from the lungs of rabbits and developed a simple procedure for its isolation. Antiserum developed in goats against highly purified Clara cells (purity greater than 90%) has been used to localize Clara cell secretory proteins within the osmiophilic cytoplasmic granules of bronchiolar Clara cells indicating the storage nature of the granules and that secretion of the low molecular weight protein probably occurs via a regulated pathway. We have also isolated mRNA from rabbit lungs and demonstrated that the primary translation product has a molecular weight about 1 kDa larger than the secreted form of the protein. The additional peptide was a signal peptide as indicated by its loss from the molecule when translation of the messenger was performed in the presence of microsomes. These studies identify the major secretory protein of Clara cells as a low molecular weight protein that appears to be stored within the cytoplasmic osmiophilic granules. We have also investigated the differentiative potential of Clara cells by inoculating purified cells into rat trachea denuded of their own epithelia. These trachea were then grafted onto the backs of nude mice and examined periodically for regeneration of epithelium. We have determined that Clara cells can multiply and generate an epithelium which consists of Clara cells and ciliated cells thus establishing the stem cell nature of this bronchiolar cell. Future studies will explore the ability of Clara cells to differentiate into mucous cells using the tracheal graft model and focus on the extracellular function of the low molecular weight secretory protein.

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-25029-03 LPP

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

COOPERATING UNITS *(if any)*

Cell Biology Group, LPP

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.)*

A large number of retroviral oncogenes and their cellular homologues have been identified that play a role in cellular transformation. The product of the Rous sarcoma virus (RSV) oncogene, v-src, is a 60-kDa phosphoprotein, $pp60^{v\text{-}src}$. The cellular oncogene c-src encodes a similar phosphoprotein, $pp60^{c\text{-}src}$. Both proteins possess an intrinsic protein kinase activity that is specific for tyrosine. In addition, both proteins are myristylated at the amino terminus and are associated with the plasma membrane. However, the specific activity of $pp60^{c\text{-}src}$ protein kinase is much lower than that of $pp60^{v\text{-}src}$. We are studying the mechanisms leading to the activation of $pp60^{c\text{-}src}$ protein kinase and its importance in chemical carcinogen-induced neoplastic transformation of Syrian hamster embryo (SHE) cells. As an inital attempt to study the role of $pp60^{c\text{-}src}$, we compared the level of $pp60^{c\text{-}src}$ kinase activity in several chemically induced tumor-dervied SHE cell lines and their related preneoplastic cell lines to the level present in normal SHE cells. The $pp60^{c\text{-}src}$ activity was measured by autophosphorylation and enolase phosphorylation in immunoprecipitations of cellular extracts containing equal protein formed with a src-specific monoclonal antibody. The fmole ^{32}P incorporated into c-src/min/mg extract protein was determined for each cell line. Tumor-derived cell lines incorporated 4-20 fold more ^{32}P than preneoplastic or normal SHE cells. The increased level of activity was not the result of increased levels of c-src protein as demonstrated by quantitating the amount of [^{35}S] methionine-labelled $pp60^{c\text{-}src}$. These results indicate that the specific activity of $pp60^{c\text{-}src}$ is increased in the tumor-derived chemical transformed SHE cell lines. Current research is focused on the mechanism of activation and the effects of the altered c-src on the growth properties of these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25030-01 LPP

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Cellular and Biochemical Mechanisms of Particle-Induced Lung Disease

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

PI:	Arnold R. Brody	Research Biologist	LPP, NIEHS
Others:	R. Bennett,	Staff Fellow	LPP, NIEHS
	R. Kumar,	Fogarty Fellow	LPP, NIEHS
	T. Bertram,	IPA	LPP, NIEHS
	L. Overby,	Chemist	LPP, NIEHS
	C. Butterick	Biologist	LPP, NIEHS

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Pulmonary of Pathobiology

SECTION: Pulmonary Pathology

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 standerd unreduced type. Do not exceed the space provided.)*

Our laboratory has focused on the mechanisms through which toxic inhaled particles such as asbestos and silica cause progressive interstitial fibrosing lung disease. Using rats and mice, we have shown that inhaled particles are deposited initially at the bifurcations of alveolar ducts and that macrophages are attracted to these sites of deposition through activation of chemotactic complement proteins on alveolar surfaces. A clear interstitial lesion develops at the bifurcations within 48 hr. after a one-hr. exposure to chrysotile asbestos and progresses for at least one month post-exposure. We showed that as a result of exposure to particulates, lung macrophages produce arachidonic acid (AA) metabolites and growth factors for lung fibroblasts. We carried out a series of experiments using high performance liquid chromatography to show that AA metabolites of both the cyclooxygenase and lipoxygenase pathways are released by alveolar and intravascular macrophages. The metabolites include several prostaglandins, HETEs and leukotriene B_4. The biological role of potential inflammatory and vasoactive mediators currently is being investigated. Another potential mediator of pulmonary fibrosis was found to be secreted by macrophages. This factor is similar to platelet-derived growth factor (PDGF) in several ways: molecular weight(~25 KD), acid and heat stability, ability to induce quiescent fibroblasts into a "competent" state to complete the cell cycle, and inhibition of growth promoting activity by an antibody against the c-sis oncogene which codes for PDGF. We now have developed a quantitative immuno-enzyme competitive inhibition assay which detects as little as 1 ng of PDGF in serum and platelet poor plasma. Ongoing studies are directed toward quantifying the amounts of PDGF-like activity in macrophage-conditioned medium and cell-free fluids recovered from the lungs of asbestos-exposed animals. We postulate that interstitial lung macrophages synthesize and secrete PDGF-like factors which cause fibroblasts to proliferate as a consequence of exposure to fibrogenic particles. Future work is directed toward testing this hypothesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-25031-01 LPP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Tumor Suppressor Genes and Oncogenes 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:	O. Sugawara	Visiting Fellow	LPP, NIEHS
	R. Wiseman	NRC Fellow	LPP, NIEHS
	N. Ozawa	Visiting Associate	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:
4.0	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.)*

Cancer development in humans and animals is a multistep process involving multiple genes. We have shown that the neoplastic transformation of Syrian hamster embryo cells (SHE) in culture is a multistep process involving both activation of proto-oncogenes and inactivation of a tumor suppressor gene. Normal, early passage SHE cells were not neoplastically transformed by either the v-Ha-ras or v-myc oncogenes alone; however, transfection of SHE cells by the two oncogenes in combination resulted in tumorigenic conversion. In order to determine whether additional changes occurred in the ras plus myc induced tumors, cytogenetic analyses of the tumors were performed. A nonrandom chromosome loss (monosomy of chromosome 15) was observed in the ras/myc tumors. The biological role of this chromosome loss was studied in hybrids between ras/myc tumor cells and normal SHE cells. Tumorigenicity of the ras/myc tumor cells was suppressed following hybridization with normal cells; re-expression of tumorigenicity at later passages correlated with the loss of chromosome 15 suggesting that this chromosome plays a role in suppressing tumorigenicity. The hybrid cells which were suppressed for tumorigenicity still expressed the ras and myc oncogenes. An early change in carcinogen-induced neoplastic progression of SHE cells was induction of immortality. Carcinogen-induced immortal cells at early passages still retained the ability to suppress tumorigenicity in cell hybrids. This ability decreased with passaging of immortal cell lines and subclones are heterogeneous in their ability to suppress tumorigenicity. The susceptibility of immortal cell lines to neoplastic transformation by DNA transfection with v-Ha-ras oncogene or tumor DNA inversely correlated with the tumor suppressive ability of the cells in cell hybrids. Taken together these observations indicate that neoplastic transformation of Syrian hamster embryo cells involves at least three steps: (1) induction of immortality; (2) activation of transforming gene or oncogene and (3) loss of or inactivation of a tumor suppressor gene.

PHS 6040 (Rev. 1/84)

GPO 914-918

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 contribute to public health efforts and clinical investigations. The Laboratory is organized in five research groups. The research emphases in these groups are as follows.

Research in Developmental Endocrinology and Pharmacology focuses on three major topics: (1) Pharmacology of estrogens, including target organ specific metabolism 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. (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 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.

The group in Experimental Teratogenesis 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. These studies utilize cell and whole embryo culture systems and especially emphasize the role of growth factors in embryonic development.

Research in Gamete Biology 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 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.

Receptor Biology research involves two primary areas of investigation: (1) the structure-activity relationships (SAR) of estrogenic chemicals which are directed towards the elucidation of the structural basis of estrogenicity and (2) receptor biology in which efforts are made to purify and characterize the estrogen receptor and study the receptor mediated cell biology of estrogen action primarily at the biochemical level.

Recent research in Reproductive Neuroendocrinology has focused on the mechanisms and interrelationships mediating neuroendocrine responses within the hypothalamic-putuitary-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.

In the current reporting period, several significant research accomplishments can be noted. For example, work in Reproductive Neuroendocrinology has demonstrated some of the fundamental mechanisms underlying the pulsatile nature of neuropeptide secretion. These efforts have utilized a computer controlled perifusion system for the in vitro determination of the mechanisms underlying the intermittent rhythmic or pulsatile secretion of neurohormones. These studies have been elegantly supported by in vivo work in which the micro-vasculature between the brain and the pituitary was cannulated and studied to confirm and validate the in vitro observations. This research is a pioneering effort in the understanding of the regulation of peptide hormone secretion. In addition, the same group has studied the chemical factors involved in the release of gonadotrophic hormones from the pituitary gland and has made significant strides in determining the role of gonadotropin-releasing factor (GnRH) and the non-GnRH portion of its prohormone. These studies have provided fundamental new information on the endogenous chemical regulation of peptide hormone secretion. Together these studies provide a crucial basis for elucidation of environmental influences on the neural control of reproductive function. The implications to public health and clinical medicine are enormous and continue to unfold.

Studies from Experimental Teratogenesis have pointed up the fundamental mechanisms by which the human teratogen, Accutane®, when taken during pregnancy, causes birth defects such as cleft palate. Accutane® (isotretinoin or 13-cis-retinoic acid) is a drug used in the U.S.A. since 1982 for treatment of various skin disorders such as cystic acne. Using the mouse embryo in culture or in vivo, it was demonstrated that several cell types which give rise to the affected craniofacial tissues in the early embryo are extremely sensitive to alteration by Accutane®. These include the migrating cranial neural crest cells, proliferating palatal mesenchymal cells and differentiating palatal epithelial cells. A similar mechanism is proposed for human malformation. These studies have already had significant public health and regulatory

relevance and have been used in regulatory decisions for the use of cis-retinoic acid in the human population.

Studies in Developmental Endocrinology and Pharmacology and Receptor Biology have shown that the developing mouse uterine epithelium is relatively deficient in estrogen receptors at the time when these cells are most susceptible to estrogen-induced neoplastic transformation. These studies have led to additional research to elucidate complementary mechanisms for estrogen-induced cell proliferation in immature tissues and have pointed out an important role for growth factors in mediating estrogen action. They further suggest that estrogen receptor deficient cells may be those most susceptible to cancer induction and, thus, ultimately will provide important new insights for determination of susceptible individuals and cell populations for endometrial and breast cancer.

Other research from Developmental Endocrinology and Pharmacology has been involved in the cloning and chromosomal mapping of a lactotransferrin gene obtained from the mouse uterus. This estrogen sensitive gene may be clinically useful as a probe for endometriosis and other gynecologic dysfunction. It also provides a fundamental tool for the study of sex differentiation at the molecular level.

Finally, the recent elucidation of a testis specific heat-shock protein in Gamete Biology has provided a tool for cell-specific and stage-specific expression of gene products during spermatogenesis. The further characterization of this protein will develop new understanding in testicular function, provide a potential biomarker for male reproductive toxicology and a unique target for contraceptive development.

In the coming year, the Laboratory will put increasing emphasis on molecular mechanisms in reproductive and developmental biology. This will be accomplished by the enhanced use of in vitro systems, the further production of protein and DNA probes, including the purification of important reproductive proteins and genes, and research on the localization of genes and their products within cells and tissues. This emphasis on the cellular biology and localization of genes is crucial to understanding the normal and abnormal processes seen in heterogenous cell populations and in tissues in which the target cells of interest comprise a small fraction. This research will provide a firm basis on which to continue to study the fundamental mechanisms underlying reproduction and development and the interaction of these systems with our environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70010-11 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	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

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	5.0	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.)*

The objective of this research project is to understand the morphological, cellular, and molecular aspects of normal and abnormal rodent and human embryonic craniofacial development. Retinoic acid (RA) is known to be essential for normal development, but non-physiological levels are teratogenic in both the rodent and the human. Our studies have revealed that there are several cell types in the developing craniofacial region which are sensitive to retinoid-induced alterations: 1) RA inhibits the migration of cranial neural crest cells in whole embryo culture; 2) RA decreases the proliferation of primary and secondary palatal mesenchymal cells in vivo and in vitro; 3) RA decreases proliferation and also increases the synthesis of a 97 Kd protein in an established cell line derived from the human embryonic palatal mesenchyme; and 4) RA alters the growth and differentiation of rodent and human embryonic palatal medial epithelial cells in vitro. Epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) appear to play an important role in development. Our cDNA hybridization studies have demonstrated that the implanting rodent embryo initiates the de nova expression of the TGF-α gene in the maternal endometrium. TGF-α may serve as an autocrine factor for the maternal decidual cells as well as a paracrine factor for the embryo. EGF influences the proliferation and differentiation of rodent and human palatal medial epithelial cells in cell and organ culture. In normal development, EGF receptors are present in medial, nasal, and oral epithelial cells in the early mouse palatal shelf (day 12); a dramatic reduction in the number of these receptors takes place in the medial epithelium on days 13 and 14. RA and EGF prevent this decline in medial EGF receptors and also inhibit medial epithelial programmed cell death. Additional studies have indicated that palatal mesenchymal cells can express the chondrogenic phenotype and the extracellular matrix (ECM) protein laminin under certain conditions in culture. Our results indicate that retinoids, ECM, and EGF-related growth factors play a key role in mammalian craniofacial development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70060-14 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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, Develop. Endocrinol. and Pharmacol.	LRDT NIEHS
Others:	R. R. Newbold	Biologist	LRDT NIEHS
	Y. Tomooka	Visiting Associate	LRDT NIEHS
	C. Bunyagidj	Visiting Fellow	LRDT NIEHS
	H. Fukamachi	Visiting Fellow	LRDT NIEHS
	C. T. Teng	Expert	LRDT NEIHS
	K. S. Korach	Senior Research Endocrinologist	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 Würzburg

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	4.5	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.)*

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 has continued to be used to understand both the development of the mammalian genital tract as well as the mechanisms underlying hormonally associated cancers. Ninety percent of mice treated neonatally with diethylstilbestrol (DES) later express uterine adenocarcinoma, which is hormonally dependent and requires a second exposure to estrogen at puberty for expression. It was determined that the immature mouse uterus, which is an especially sensitive tissue for estrogen-induced cancers, had abundant estrogen receptors (ER) in the underlying stroma, while the epithelium was relatively deficient in detectable ER. This raises the possibility that ER deficient cells may be those which are most susceptible to neoplastic transformation, leading us to explore complementary mechanisms for cell proliferation, including estrogen associated growth factors. We demonstrated, in this reporting period, that estrogen induced proliferation of the mouse uterine epithelium in vitro could be blocked by addition of antibodies to epidermal growth factor (EGF) suggesting a role for growth factor mediation of estrogen action. We also recently demonstrated the expression of the mRNA for a uterine secretory protein in the seminal vesicle of developmentally estrogenized male mice, the first example of pseudohermaphroditism at the molecular level. Studies on the metabolism of estrogens to reactive forms have shown that the mouse uterus forms catechol estrogens and that the enzyme responsible for their inactivation is relatively low. This provides a mechanism for generation of reactive estrogenic metabolites close to the target cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT ZO1 ES 70065-11 LRDT

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Head, Receptor Biology	LRDT NIEHS
Others:	K. Chae	Research Chemist	LRDT NIEHS
	F. Ogata	Visiting Fellow	LRDT NIEHS
	S. Yamashita	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	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 Medical School

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Receptor Biology

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.)*

Estrogen action in reproductive tract tissue involves a mechanism which includes interaction with a receptor protein with subsequent activation and localization in the nucleus. After hormone stimulation, nuclear receptor occupancy followed a bimodal temporal pattern consistent with stimulation of certain tissue responses such as DNA synthesis or enzyme induction. The bimodal receptor pattern was evident in other responsive target tissues such as human MCF-7 cell tumors. DNA synthesis in tumors or uteri can be produced with discontinuous stimulation with a weak agonist and occurs at similar times as the two nuclear receptor events. We have purified (~11000-fold) the mouse uterine estrogen receptor by steroid affinity chromatography and have characterized it by ligand affinity labeling as well as immunoblot analysis with a monoclonal antibody. Studies indicate multiple forms which are proteolytic fragments. Proteolytic processing of the receptor involves a two-step reaction. The first involving a nuclear cysteine protease and the second a soluble enzyme preliminarily characterized as a cathepsin protease. Additional biochemical characterization of the estrogen receptor protein with monoclonal antibodies demonstrated a doublet form differing by 1500 MW. This receptor form was specific to the nucleus and not found in the soluble fraction. The proportion of the doublet varied depending on the biological activity of the compound with potent agonists producing a greater amount of the upper band form. The profile of the nuclear doublet changed during estrogen stimulation in which the two components were of equal proportion until the time of the second nuclear peak at which time the proportion changed to be primarily the lower band form. The mechanism of this receptor modification is presently unknown and preliminary data indicate both bands are phosphorylated. Experiments to investigate other processes such as acylation or glycosylation are also under study. Receptor modification may be a signal for cellular processing of the protein or programming for its interaction with specific nuclear responsive sites involved in hormone stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70067-04 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	C. T. Teng	Expert	LRDT NIEHS
Others:	B. T. Pentecost	Visiting Associate	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Developmental Endocrinology and Pharmacology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.2	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 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. The protein was found to be a ~70 kDa peptide existing as a single chain polypeptide under native conditions with a pI ≥9.5. Further analysis of the protein revealed that it is glycosylated with a single N-asparagyl linkage and the N-terminus blocked to Edman degradation. The protein was not induced by testosterone or progesterone in mouse uterus. Antibody to this estrogen inducible mouse uterine protein has been used to isolate cDNA to the messenger RNA. Analysis of the deduced primary structure and additional biochemical characterization indicates that the protein is lactotransferrin. An increase in the level of lactotransferrin mRNA of at least 300-fold can be induced in the mouse uterus by estrogen. In contrast, the mRNA is virtually undetectable in rat uterine tissue following estrogen administration. The estrogenic stimulation in mouse uterus contrasts with the known prolactin dependence in mammary gland. We have mapped lactotransferrin gene to human chromosome 3 (q21-q23) and mouse chromosome 9. In future work we hope to gain some understanding of the factors leading to lactotransferrin secretion by two tissues, uterus and mammary gland, in response to different hormonal signals and, in addition, compare the structure and function of lactotransferrin gene to that of related transferrin genes that have been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 ES 70076-03 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	LRDT NIEHS
Others:	M. P. Hedger	Visiting Fellow	LRDT NIEHS
	D. R. Joseph	IPA	LRDT NIEHS

COOPERATING UNITS *(if any)*

U. of Tennessee, Knoxville
U. of Washington School of Medicine
U. of North Carolina, Chapel Hill
The Johns Hopkins University, Baltimore
Fred Hutchison Cancer Res. Center, Seattle
The Hospital for Sick Children, Toronto

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology

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.)*

Production of the mature gamete in the male involves intrinsic processes of spermatogenesis to produce the sperm and subsequent modifications by extrinsic processes in the epididymis to make the sperm able to fertilize. One area of study is spermatogenic cell gene expression and the identification of lineage-specific and stage-specific germ cell proteins. The hypothesis being tested is that these gene products are responsible for the unique structural and functional characteristics of germ cells. The flagellum is a unique structure of sperm and the hypothesis predicts that it contains germ cell-specific proteins. Although germ cells have been reported to lack intermediate filament (IF) proteins, we have identified cytoskeletal proteins in the flagellum that appear to be germ cell-specific IF proteins. Monoclonal antibodies have been used to characterize 67kD and 78kD proteins in the fibrous sheath of the mouse sperm flagellum that are synthesized during spermatogenesis and share antigenic determinants with IF proteins. However, they also contain unique epitopes and have molecular weights and other characteristics unlike known IF proteins. The fibrous sheath is important in determining effectiveness of the flagellar beat, and men with abnormalities in this structure are infertile. The genes for these proteins will be cloned to determine their structure and homology with known IF proteins. The other area of study is germ cell-somatic cell interaction and the identification of somatic cell products required for sperm maturation. A factor produced by Sertoli cells stabilizes binding of epididymal secretory products to the sperm surface. Dilution of this factor in the female reproductive tract allows the surface components to be shed during capacitation. The factor is present in Sertoli cell-conditioned medium and an assay has been developed to monitor its purification. Isolation of the factor and preparation of monoclonal antibodies will allow studies of the synthesis of the factor and the nature of its interaction with sperm surface molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70077-01 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

The Expression of Heat-Shock Genes in Mouse Spermatogenic Cells

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

PI:	R. Allen	Staff Fellow	LRDT NIEHS
Others:	E. M. Eddy	Head, Gamete Biology	LRDT NIEHS

COOPERATING UNITS *(if any)*

The Hospital for Sick Children, Toronto

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology

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 synthesis of heat-shock proteins (hsp) in cells exposed to stress is one of the most highly conserved genetic regulatory systems known and has been observed in organisms as diverse as bacteria and man. The major hsp are nearly 45% homologous over this evolutionary range. Although hsp have been studied intensively, little is known about their functions. We have shown that one of the most abundant proteins (P70) in mouse spermatogenic cells is related closely to hsp70, the major heat-inducible protein. When hsp70 is heat-induced in spermatogenic cells, it migrates to the same location as P70 on two-dimensional polyacrylamide gels. P70 shares antigenic and ATP-binding properties with products of the hsp70 gene family, but the peptide map of P70 differs from that of hsp70. This suggests that P70 is a novel hsp of spermatogenic cells which is synthesized in association with germ cell differentiation. We have also shown that P70 is synthesized normally in spermatogenic cells, along with the other members of this protein family. This is consistent with the recent observations that the expression of heat-shock genes may be regulated developmentally. Future studies will examine the stage-specific synthesis of hsp in germ cells. Information gained from these studies should be useful in determining the physiological role of hsp and in better understanding the effects of environmental agents on male reproduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70078-04 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Characterization of Stage-Specific 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	LRDT NIEHS
	M. Maekawa	Visiting Fellow	LRDT NIEHS

COOPERATING UNITS *(if any)*

University of Pennsylvania School of Medicine
Columbia University, College of Physicians and Surgeons

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Gamete Biology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

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

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

Spermatogenesis is a complex process of cell differentiation involving interactions between germ cells, Sertoli cells, and other somatic cells within the testis. One feature of this differentiative process is the appearance of several germ cell-specific constituents in a precise temporal sequence. Immunological and biochemical techniques have been used to characterize such constituents, both in the acrosome and on the cell surface. Central to these studies are methods for the purification of germ cells at defined stages of spermatogenesis by unit gravity sedimentation. Three areas of research have been pursued: (a) Monoclonal antibodies have been used to characterize germ cell constituents expressed during restricted periods of spermatogenesis. Antibody 1D4 recognizes a set of glycoconjugates that appear in the acrosome of early spermatids but are modified during the late haploid stages so that the determinant no longer is detected. Additional monoclonal antibodies have been prepared against presumptive surface constituents excised from two-dimensional polyacrylamide gels. (b) Conditions for the short term culture of spermatogenic cells in serum-free medium have been refined to facilitate metabolic studies and the development of *in vitro* functional assays. Both pachytene spermatocytes and round spermatids cultured for 1-2 days with Sertoli cell-conditioned medium (SCM) maintain elevated viabilities and ATP levels. The active fraction of SCM that improves germ cell viability is relatively heat-stable, is eliminated by trypsin proteolysis, and appears to be larger than most growth factors. Culture studies also have shown that round spermatids are more sensitive than pachytene spermatocytes to the toxic effects of retinoids. (c) Isolated spermatogenic cells in short-term cultures have been used to monitor changes in protein synthesis and glycosylation during germ cell differentiation. Germ cell constituents exhibiting both tissue and stage specificity are candidates for further studies exploring gene regulation and cell-cell interactions during spermatogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 ES 70090-04 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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, Reproductive Neuroendocrinology	LRDT NIEHS
Others:	C. A. Johnston	Senior Staff Fellow	LRDT NIEHS
	M. D. Culler	Senior Staff Fellow	LRDT NIEHS
	W. D. Wetsel	Senior Staff Fellow	LRDT NIEHS
	M. M. Valenca	Visiting Fellow	LRDT NIEHS
	C. Masotto	Visiting Fellow	LRDT NIEHS
	M. Ching	Expert	LRDT NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.0	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies are 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 were conducted 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 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 were 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 were 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 were directed to evaluate the mechanisms underlying the effects of neonatal neurotoxin treatment on the reproductive sphere, 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.

NOTICE OF INTRAMURAL RESEARCH PROJECT ZO1 ES 70092-04 LRD

PERIOD COVERED

October 1, 1986 to September 30, 1987

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, Reproductive Neuroendocrinology	LRDT NIEHS
Others:	M. D. Culler	Senior Staff Fellow	LRDT NIEHS
	C. A. Johnston	Senior Staff Fellow	LRDT NIEHS
	M. Ching	Expert	LRDT NIEHS
	M. M. Valenca	Visiting Fellow	LRDT NIEHS
	C. Masotto	Visiting Fellow	LRDT NIEHS
	F. Romanelli	Guest Researcher	LRDT NIEHS
	J. R. Dominquez/F. Lopez	Guest Researchers	LRDT NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina, Department of Anatomy
Yale University Medical School, Department of Obstetrics and Gynecology

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.0	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.)*

Analysis of the cellular and molecular mechanisms mediating peptide hormone action constitutes an important component of our research efforts. 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 were 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 has been to analyze the role of hormonal input signal in modifying cellular responses, using a computerized perifusion system that can exquisitely regulate the delivery of appropriately-designed hormone signals to cells or tissues (in vitro). Other protocols were 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.

In order to understand the role of pulsatile hormone secretion in providing coded messages for the activation of endocrine target tissues, experiments were designed and carried out to analyze the pulsatile pattern of secretion of different peptide hormones (LHRH, LH, ACTH, Prolactin) intimately involved in the regulation of endocrine and reproductive functions. The characteristic pattern of secretion of each hormone was defined as well as the role of certain neural or gonadal factors in modulating the pulsatile pattern. The information obtained provides a more clear understanding of the mechanisms regulating episodic hormone secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT ZO1 ES 70094-03 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. A. Johnston	Senior Staff Fellow	LRDT NIEHS
Others:	A. Negro-Vilar	Head, Reproductive Neuroendocrinology	LRDT NIEHS

COOPERATING UNITS *(if any)*

AmGen, Thousands Oaks, California
Centro de Referencia de Radioimmunoensayo, La Plata, Argentina

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

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

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

The abnormal secretion of prolactin (PRL), adrenocorticotropin (ACTH) and/or beta-endorphin (β-END) has been shown to dramatically affect reproductive function. Elucidating the cellular and subcellular neurochemical mechanisms governing the secretion of these hormones should provide insight into possible therapeutic regimens to alleviate reproductive dysfunctions associated with the aberrant secretion of those hormones. We have utilized HPLC with electrochemical detection for the concurrent evaluation of neurotransmission in four major monoamine neurotransmitter systems, combined with sensitive RIAs for the quantitation of neuropeptides to evaluate changes in these neuronal systems associated with experimental paradigms where dynamic changes in the secretion of PRL, ACTH and β-END are occurring. Specific pharmacological antagonists and specialized surgical procedures have been used in vivo as well as in vitro to evaluate the physiological significance, site(s) of action and interaction, and functional neuronal connectivity of individual neurotransmitters in governing the release of these hormones. Our recent results have demonstrated (1) a differential control of PRL secretion where oxytocin plays a major role in the preovulatory surge of PRL in the normal cycling female rat but not in the increases associated with 5-hydroxytryptophan injection, acute suckling or ether vapor stress, (2) strong evidence for a tonic stimulatory role of central epinephrine neurons in regulating corticotropin releasing factor levels in the hypothalamus as well as a vital role in the ether vapor stress-induced increase in plasma ACTH, and (3) an important role for the neurointermediate pituitary (NIL) in (a) the presentation of stress-related increases of these hormones, (b) maintaining hypothalamic levels of some neuropeptide transmitters, and (c) regulating the responsiveness of the anterior pituitary to some known physiological releasing factors. Future experiments will attempt to clarify further the neurochemical mechanisms and the role of the NIL in the physiological regulation of PRL, ACTH and β-END secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 ES 70096-03 LRDT

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Regulation of Pulsatile Gonadotropin Secretion*

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

PI:	M. D. Culler	Senior Staff Fellow	LRDT NIEHS
Others:	A. Negro-Vilar	Head, Reproductive Neuroendocrinology	LRDT NIEHS

*Title changed from Regulation of Pulsatile Pituitary Hormone Secretion

COOPERATING UNITS *(if any)*

Department of Anatomy, University of North Carolina, Chapel Hill, NC
Department of Anatomy, University of Pécs Medical School, Pécs, Hungary

LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

SECTION

Reproductive Neuroendocrinology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

CHECK APPROPRIATE BOX(ES)

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

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

In order to elucidate the mechanisms regulating the pulsatile secretion of the gonadotropins, LH and FSH, we have utilized a model in which endogenous gonadotropin affecting factors are selectively immunoneutralized and then replaced in a pulsatile manner using structurally modified agonists. Our results have demonstrated that pulsatile FSH secretion is independent of the gonadotropin releasing factor (GnRH) of the brain that controls LH secretion, and the involvement of, as of yet, unidentified, slow and fast acting FSH-affecting factors. Two candidates for these FSH regulating components, discovered by recombinant DNA methodology, are a peptide that may represent the non-GnRH portion of the GnRH prohormone (GAP) and a slow acting FSH inhibitor, inhibin. We have developed specific, high titer antisera and radioimmunoassays for these factors. Using our anti-GAP serum, we have demonstrated, by both immunocytochemistry and by radioimmunoassay quantitation, that the GAP peptide does indeed represent a portion of the GnRH prohormone. Screening of GAP fragments for biological activity, using cultured anterior pituitary cells, has revealed gonadotropin releasing activity in the N-terminal region of the GAP sequence. We are now generating antiserum against this region for immunoneutralization studies. Using the anti-inhibin serum we have been able to demonstrate the presence of significant quantities of inhibin in human and porcine ovarian follicular fluid, rat plasma, and rat Sertoli cell cultures. In addition, we have initiated immunoneutralization studies in the rat and have demonstrated that FSH secretion is selectively increased following injection of the anti-inhibin serum. In related studies, we have also used a computer assisted perifusion system to reproduce in vitro the pulsatile gonadotropin patterns observed in vivo and are studying the intracellular messengers involved in pulsatile gonadotropin secretion. These studies have already and will continue to greatly advance our understanding of the mechanisms regulating pulsatile gonadotropin secretion.

COMPARATIVE MEDICINE BRANCH
Summary statement

The Comparative Medicine Branch (CMB), administratively located within the Division of Intramural Research, extends a broad range of services to all three of the intramural research programs through four functional units: Animal Husbandry, Quality Assurance and Diagnostic Laboratory, Glassware and Media, and Office of the Chief. Major research animal responsibilities include program and facility management, health surveillance and diagnosis, procurement, clinical veterinary services, rodent breeding, technical assistance, quality assurance for food, bedding and water, and advice to the Institute on important animal issues. CMB also assists the scientific staff by providing glassware and media services, training for investigators and technicians, quality assurance support for research projects, consultation with investigators planning animal research projects, and administrative support to the Institute Animal Care and Use Committee. Independent and collaborative research studies relative to the major missions of the Branch are also conducted.

Adventitious infections in the research animal colonies can complicate and disrupt the experimental results of many intramural studies. Consequently CMB concentrates a great deal of time and effort on elimination and prevention of colony infections. Sendai virus had been resisting eradication efforts in Building 15 rats for the past year. In April a program was initiated to eradicate the infection in Building 15 without interfering with ongoing research. This program involved substantial changes in standard animal room procedures and traffic flow patterns. With the cooperation of the Laboratory of Behavioral and Neurological Toxicology, the program appears to be successful. Even with intensive monitoring techniques, no Sendai infections have been detected in sentinel rats for 12 weeks. In addition, monitoring in the only animal room on the North Campus which has had endemic Mouse Hepatitis Virus infections has also been negative for the past four months. The South Campus continues its pathogen free status at this time for the ninth consecutive month.

The Animal Husbandry group has seen the departure of its former head Kevin Denny and the recent appointment of the new Head, Barbara Sawyer. Ms. Sawyer brings an excellent academic background to this position with a BS in Animal Science and an MS in Microbiology. Animal Husbandry has focused on improvement and expansion of technical assistance, more formalized technical training for employees, and continued expansion of computer supported projects such as Autowei. In addition to contracting the service areas of the animal facilities, CMB plans to contract for animal care for the North Campus. The desire is to utilize the government employees from the North Campus to expand animal care and technical services on the South Campus into the E module. Ideally this will result in better utilization and expanded support of NIEHS research projects and eliminate initiation delays caused by lack of space.

The Glassware section is undergoing a transition into contractor operation. Bids are in and the contract is expected to be awarded momentarily. With the assistance of Industrial Engineering students on special assignment from North Carolina State University and their academic counselors, Professor Jerry Isley and Robert Fullenwider, areas of improvement in Glassware operations have been

identified. These changes will be integrated into the contract operation of the Glassware service. The fixed fee contract concept has been modified to allow an award fee incentive provision which should provide a basis for encouraging efficient operation of the service and closer coordination with the contractor.

The Media unit continues to perform important service functions for the scientific effort of the Institute. 2,613 batches of media were prepared in the past calendar year, 1,902 batches of plate media, and 711 batches of tissue culture media. The quality assurance program initiated several years ago has shown an overall improvement in the quality of the tissue culture media. Present efforts are concentrating on bringing similar improvements to the plate media.

The Quality Assurance Lab (QAL) has performed 11,988 total tests on 2,564 cases, collecting 7,986 specimens for the first six months of Fiscal Year 1987. In addition to the major functions of animal health and media monitoring, the QAL has worked closely with individual intramural projects, supporting the arsine gas and methylene chloride studies and recently assisting with water testing for the Chemical Mixtures study. An aggressive pest and vermin control program has headed off several potential problems in storage areas and is providing NIEHS with well controlled facilities.

The Diagnostic Laboratory had 232 accessions from 24 investigators and the CMB staff through the first six months of Fiscal Year 1987. The work up of individual accessions ranged from a routine necropsy to extensive pathologic and microbiologic examination with numerous tissues examined and tests performed. Oversight of animal vendors has resulted in correction of problems with housing and transport of aged F344 rats and deficiencies in surgically altered animals. An investigator was alerted to a chemical method for hypophysectomizing hamsters, thus eliminating the stress of surgery. This method also reduces the cost and allows the use of hamsters from a number of sources if necessary.

The Diagnostic Laboratory identified a source of non-drug induced rabbit uterine fluid from which uteroglobins can be isolated in large quantities. Fluid has been supplied to an investigator for his studies.

So far in Fiscal Year 1987 controlled substances have been issued 48 times. A North Carolina Drug Control Inspector oversaw the destruction of returned, spoiled, and outdated controlled substances. The Institute's policies and procedures for controlled substances are being reviewed and recommendations are being made for their revision.

Training of investigators, technicians and Animal Husbandry employees has been receiving greater emphasis. A workshop designed specifically to orient and train summer students and aides in animal technical procedures was held in mid-June. This summer workshop will become an annual part of the Institute training program. This student-oriented session emphasized hands-on demonstrations and direct training in the animal facility. In response to changes in legislation and regulations, workshops will be held more frequently and will emphasize alternative methods and techniques which reduce pain and distress in the laboratory animal host. The NIEHS Handbook has been reorganized and revised to incorporate newer ideas and to serve as an easy reference for investigators and technicians on animal handling and use techniques.

Research studies relative to the major missions of NIEHS have uncovered interesting and important findings. Corticomedullary intratubular nephrocalcinosis has been identified in female F344 rats fed NIH-31 or NIH-07 open formula diets. These lesions occur in a severe form never previously described with these diets and can potentially complicate toxicology studies. Experiments are underway to follow the progress and development of the lesion and to examine the role of phytoestrogenic substances in feed stuffs.

Research on rabbit coronavirus has focused on purification of the virus, serum neutralization studies with a human coronavirus, and studies of tissue tropisms. 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. Dr. Ralph Baric, a collaborator in this work from the University of North Carolina has received grants from the American Heart Association and UNC to study the virus and its relationship to other coronaviruses and human heart disease. Basic understanding of the biology of the coronaviruses is important in laboratory animal medicine due to widespread complications caused by representatives like Mouse Hepatitis Virus and Rat Coronavirus.

Increasing concern over estrogenic compounds in foods has focused interest from the scientific community on the mouse bioassay, modified and standardized by the QAL, to detect estrogenic activity in rodent diets. This bioassay has the ability to detect a wider variety of estrogenic substances than specific chemical methodology and is much less expensive. Future studies will include adaptation of the bioassay to detect estrogenic inhibitors in diets.

Basic studies on the modes of transmission of Pneumonia Virus of Mice, pathogenesis in host mouse strains, and post-infection antibody patterns are underway. Stock viral pools nave ben prepared for transmission studies focusing on the mode and length of shedding of virus by nude mice. Aerosol studies using a modified Horsfall isolator to separate direct contact from aerosol transmission are also being conducted. These studies should determine which strains of mice would serve as the most sensitive sentinel animal and which present the greatest hazard by their ability to shed virus and serve as a source of colony contamination.

In the last several years, animal research and support activities throughout the United States have been challenged by changing cultural and scientific views concerning the use of animals in research. The role of CMB to advise and assist the Institute in the management of these issues has become increasingly important. By maintaining a progressive and balanced view of national events, NIEHS can be responsive to public and congressional concerns, comply with complex legislative and regulatory demands and still provide an environment of free scientific inquiry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 22102-06 CMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	J. Clark	Bio. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS *(if any)*

CPB, TRTP, NIEHS, NIH (Dr. M. Thompson); Div. of Comp. Med., Johns Hopkins School of Medicine (Drs. J. Strandberg and L. Aurelian); NADC, ARS, USDA, Ames, IA (Dr. R. D. Woods); School of Public Health, UNC-Chapel Hill (Dr. R. S. Baric)

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 were extended using antiserum to human coronavirus (229-E). Two of 3 rabbits given RbCV treated with 229-E antiserum survived. All 3 exhibited clinical signs of disease but the 2 surviving rabbits had an accelerated recovery based on diminished eyes lesions and the body temperature returning to normal on post inoculation day 5 or 6. RbCV was purified on a sucrose density gradient and the bouyant density determined to be between 1.195 and 1.210. The bouyant density of 229-E determined at the same time was between 1.195 and 1.200. The RbCV recov-ered from the density gradient was infectious for rabbits and coronavirus like particles were seen by electron microscopy. Homology of RbCV and 229-E to transmissible gastroenteritis virus (TGEV) nucleic acid was demonstrated. This new data provides further proof for RbCV being a coronavirus of Group II related to 229-E. Purified RbCV is being used to make antibody in guinea pigs and rats for further studies of tissue tropisms. Assessment of myocardial damage by measurement of creatine kinase isozymes will be done. Attempts to adapt RbCV to tissue culture continue. Production of monoclonal antibodies and cloning of the RbCV genome are planned. 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. Further, it has been estimated that approximately 10-30% of human "colds" are caused by coronaviruses.

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 22103-04 CMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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.:	J. E. Thigpen	Head, Quality Assurance Lab	CMB, NIEHS
Others:	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

Quality Assurance Laboratory

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.)*

MHV is probably the most widely distributed infectious disease of mice used for experimental purposes. The virus is lethal to the athymic mouse and can alter the immune system in other strains of mice. Many important gaps remain in our knowledge of the epidemiology of MHV and the biology of the virus. The objectives of our studies were to study the natural history and transmission of mouse hepatitis virus (MHV) in the animal facility setting and to gain insight into methods of control of the infection.

In the past the transmission of mouse hepatitis virus (MHV) was studied in animal rooms where mice were naturally infected, using euthymic and athymic mice; and in unnatural settings where experimentally infected cage mates served as time-controlled donors.

This past year we planned to repeat these studies using CD-1 and nu/nu mice. Time nor space for isolation of infected and/or exposed mice have not permitted us to repeat these transmission studies with mice experimentally infected with MHV. In addition, MHV was endemic in only one mouse room during the past year. Therefore, we had only one room for comparative transmission studies.

Currently we plan to continue to collect murine viral (MHV) transmission data on a monthly schedule from sentinel CD-1 mice housed in the MHV endemic room and from other mouse rooms. These procedures are employed as a part of our sentinel animal program.

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 22107-02 CMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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.:	J. E. Thigpen	Head, Quality Assurance Lab	CMB, NIEHS
Others:	E. H. Lebetkin	Biol. Lab. Tech., QAL	CMB, NIEHS
	M. L. Dawes	Biol. Lab. Tech., QAL	CMB, NIEHS

COOPERATING UNITS *(if any)*

Div. of Lab Animal Med., Duke Univ. (C. B. Richter); Center for Electron Microscopy, N. C. State University (J. MacKenzie, C. S. Richter, D. Flynn)

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:
.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.)*

Pneumonia virus of mice (PVM) infects mice, rats, hamsters and guinea pigs. 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. The objective of this project is to study the natural history and transmission of PVM in euthymic and athymic mice. Little is known about the pathology, patent period, epidemiology, and target cell population of this virus.

We have observed, and reported 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. In addition we have used the electron microscope to confirm the presence, location and structure of the virus in infected mice.

Studies are underway to produce a supply of stock PVM virus (TC virus; lung suspension virus from nu/nu mice) for future studies. The stock virus will be assayed using hemadsorption, Infectious Dose 50 and Lethal Dose 50 methods to determine virus concentration. Studies will be undertaken soon to determine means and duration of virus transmission from experimentally infected CD-1 and nu/nu mice to susceptible cage mates (added at specified intervals post infection) and to other mice housed in nearby cages with or without filter bonnets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 22108-01 CMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Nephrocalcinosis in F344 Rats Fed NIH-31 Diet

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:	J. Clark	Biol. Lab. Tech.	CMB, NIEHS

COOPERATING UNITS *(if any)*

CPB, TRTP, NIEHS, NIH (Dr. G. Rao); Chemical Industry Institute for Toxicology (Dr. J. Everitt)

LAB/BRANCH

Comparative Medicine Branch

SECTION

Diagnostic Laboratory

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 objective of this project is to study the development of and to identify the cause of corticomedullary intratubular nephrocalcinosis (NC) observed in female F344 rats fed NIH-31 or NIH-07 open formula diets. In early 1986 in an intramural study severe NC was observed histologically in the cortex of 42 and 56 day old control and treated female F344/CrlBR rats which had been fed NIH-31 diet for 15 and 28 days respectively. Prior to 28 days of age they had access to a commercial rodent diet. Female F344 rats are recognized as being prone to NC; however, the lesions observed were more severe than had been seen previously. Analysis of the NIH-31 diet was consistent with the prescribed nutrient values, including Ca, P, Mg, and Vit. D. No estrogenic activity was demonstrated by bioassay. NC was observed in rats given NIH-31 and deionized or tap water. Parallel studies done in a second facility also revealed NC at 42 and 56 days of age. Two colonies of F344/N rats from the same supplier but maintained on NIH-31 from 2 different feed manufacturers were compared. Both colonies developed NC by 42 days of age. Kidneys from F344/N rats fed NIH-07 during long term studies in the National Toxicology Program were examined. Minimal evidence of NC was found in rats more than 20 weeks of age. A study of F344/N rats is underway to follow the progress of this lesion over a period of one year. Further, studies are being planned to examine the possible role of phytoestrogenic substances which may be present in certain feed stuffs depending on their origin and crop year.

PHS 6040 (Rev. 1/84)

GPO 914-918

DIVISION OF BIOMETRY AND RISK ASSESSMENT

DIVISION OF BIOMETRY AND RISK ASSESSMENT
Summary Statement

The Division of Biometry and Risk Assessment (DBRA) plans and conducts basic and applied research in the areas of quantitative and biochemical risk assessment, statistics, biomathematics, epidemiology, and molecular modeling. A major focus of this research effort is the qualitative and quantitative estimation of adverse health effects resulting from exposure to hazardous environmental agents, with particular emphasis on 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 collaborative basis, combining the scientific expertise found in DBRA's different organizational units.

In addition to conducting its own research effort, the DBRA 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 Division of Biometry and Risk Assessment is organized into 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). This unique combination of biomathematicians, statisticians, epidemiologists, biochemists, and molecular toxicologists in a single, unified research program permits the multidisciplinary approach that is required to resolve the many complex issues involved in the identification, evaluation, and characterization of environmentally related human health risks.

During the past fiscal year DBRA scientists have pursued a number of research issues that have important implications for the characterization of potential human health risks resulting from adverse environmental exposures. These research efforts included the continued investigation and evaluation of dose-response models for environmental toxins, which are the foundation of the quantitative risk assessment process; the further study and clarification of the role of oncogenes in the identification of carcinogenic mechanisms and the determination of human cancer risk; the development of epidemiologic methods for measuring reproductive outcomes and the application of these methods to the study of environmental hazards; the critical assessment of the relationship between genotoxicity, chemically-induced toxicity and subsequent carcinogenicity, and the importance of these types of data for cancer risk estimation and management; and the initiation of a new program in molecular computing.

Results from the analysis of nearly 200 NCI/NTP lifetime rodent carcinogenicity experiments indicate that the observed dose-response patterns in animal cancer studies may often be non-linear, and that the estimated cancer risk may increase more rapidly than would be expected if effect were proportional to dose. In such cases, the linear model might well overestimate

the cancer risk at the low doses typical of human exposure levels. While these results do not necessarily imply that linear low-dose extrapolation is not the best policy from a public health standpoint, they do provide evidence in support of cancer risk estimation procedures that incorporate all biologically pertinent and measurable parameters into the assessment process. Additional support for such procedures was also produced by DBRA investigations of mechanisms of carcinogen-induced DNA damage and cell transformation. Studies with 4-(N-Methyl-N-Nitrosamino)1-(3-Pyridyl) -1-Butanone (NNK), a major nitrosamine found in tobacco smoke, demonstrated that extrapolation from high to low doses in cancer risk estimation may be significantly improved by data on DNA adduct formation in the target cell.

Studies have been initiated to investigate oncogene activation and expression in spontaneous and chemically-induced rodent tumors. Results to date indicate that, in some cases, oncogene activation in spontaneous tumors may be different from that observed in chemically-induced tumors. For example, novel mutations in Ha-ras oncogenes were detected in mouse liver tumors induced by two important industrial solvents, furan and furfural, even though neither of these chemicals produced mutations in Salmonella assays. These experimental findings suggest that the B6C3F1 mouse liver may provide an especially sensitive assay system for the detection of carcinogenic insult. Further development and refinement of these oncogene assays may enable us to more accurately estimate the risk of cancer in humans exposed to specific classes of chemicals and, perhaps, to identify the probable causal agent(s) in cancer patients with complex histories of exposures.

In order to estimate how much pregnancy loss occurs before pregnancy is recognized, daily urine samples were collected from 230 women who were trying to become pregnant and analyzed for the presence of human chorionic gonadotropin, a pregnancy hormone produced about eight days after conception. It was determined that approximately 20% of the pregnancies detected by the assay were lost, and the loss went unrecognized by the woman under study. When this loss is combined with recognized spontaneous abortions, a total of about one-third of pregnancies are estimated to be lost by the twentieth week of pregnancy. Use of this assay method, or some modification of it, may enable epidemiologists to identify the hazardous effects of environmental or occupational exposures that damage early pregnancy. Future research efforts are likely to be concerned with modifying the methodology so as to require fewer urine samples for analysis, simplifying the assay itself in order to make it more useful for large scale field studies, and initiating studies of well defined cohorts of women in the reproductive age range who are exposed to industrial agents known to or suspected of adversely effecting reproduction.

A three-year, 73-chemical project to assess the prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays was completed. This study demonstrated that there were no significant differences among the the four primary National Toxicology Program in vitro assays for genetic toxicity with respect to individual agreement or concordance with rodent carcinogenicity results. Furthermore, the assays under study did not appear to be complimentary, i.e., there was no indication that two assays considered jointly would exhibit a greater concordance with rodent carcinogenicity than either would on an individual basis. Finally, the results of this study failed to support the battery concept or belief that a set of in vitro assays is needed to predict rodent carcinogenicity adequately.

The conclusions of this study may have profound implications for chemical and pharmaceutical regulation, since many of the existing genetic toxicology regulations for registration are build on scientific assumptions that are now open to serious question. In a related effort, the relationship between toxicity, genotoxicity and chemically induced carcinogenesis in laboratory rodents is being investigated. This work was undertaken in response to suggestions that agents that are both mutagenic and carcinogenic may, potentially, pose a greater risk than those chemicals that induce carcinogenesis through some apparent "secondary" process such as cytotoxicity or induced cell killing and resultant compensatory cell proliferation. Preliminary results indicate that there is no difference between "high dose only" carcinogens and the entire set of carcinogens in terms of genotoxicity as measured by short term in vitro test results. Moreover, few of the chemicals under investigation appeared to exhibit target organ toxicity that could be adjudged as the likely cause of the observed carcinogenicity.

Due to the nature of the experimental systems employed and the limitations of current methodology, it is not possible at present to isolate experimentally the factors primarily responsible for the observed site specificity of mutagenesis or the level (i.e., reaction, repair, replication or selection) at which this specificity emerges. Therefore, a molecular modeling effort has been initiated that uses 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. The development and use of these modeling techniques may well explain or even predict experimental results at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43010-02 DBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DBRA/OD	NIEHS
Others:	Marshall W. Anderson	Research Chemist	DBRA/LBRA	NIEHS
	Tom Darden	Sr. Computer Specialist	DBRA/OD	NIEHS
	Lee G. Pedersen	Research Chemist	DBRA/OD	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Division of Biometry and Risk Assessment

SECTION

Office of the Division Director

INSTITUTE AND LOCATION

NIH, NIEHS, 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 are using 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.

We are employing ab initio quantum chemical techniques to obtain charge distributions and geometric parameters on small systems, molecular mechanics and dynamics on larger systems (10 to 20-mer oligonucleotides) and computer graphics for analysis of predicted structures.

Computing equipment used includes: the dual DEC 8650's at NIEHS, an office microvax-II, a Silicon Graphics 2400 IRIS workstation and the National Cancer Institute CRAY-XMP at Frederick, MD.

Future emphases will be to characterize local structures of DNA sequences (native and chemically modified) that contain known hot spots from mammalian oncogenes and model bacterial systems.

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; 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. 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.

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 improving the access speed for users by replacing more than 200 1200 BPS modems with either 2400 BPS modems or 9600 BPS hard-wired access. A pilot project is underway to provide NIEHS scientists improved access to BITNET. Major activities underway include improving (i.e., higher speed and better reliability) access to remote computer systems, including NIH/DCRT and establishing access from the TDMS laboratories to the VAX.

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 was completed, following identification of a need for this function among several Institute laboratories. Custom circuitry has also been developed, for example to interface lab balances and terminals for on-line weighing of animals. 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 Group has responsibility for information systems project development for the Institute. The Information Systems Group manages 35 projects, assists users in developing their own systems, develops automated systems for the various Institute Divisions, develops automated systems for the Office of Administrative Management and for the Office of Facilities Engineering, and provides Institute support for various information systems software. The Information Systems Group is also chartered to evaluate and implement software engineering tools. These tools include both data management software as well as computer aided systems analysis and design software. The Information Systems Group is further required to provide analysis and planning for computer system integration with NIH and DHHS. In the past year, the Information Systems Group also acquired responsibility for the Institute wide Data Dictionary and will be coordinating its use for development and production. Current Information Systems Group plans for FY88 include the delivery of a new Travel System, an Equipment Tracking and Maintenance system, automation of the Contracts Management Office, and the standardization of project Management and System documentation for all projects. Also for FY88, the Group intends to establish centralized databases of commonly used and shared data within the Institute.

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). CBDS accomplishments include continued support of the NTP carcinogenicity and toxicity bioassays with the generation of 60 chemical package reports consisting of tumor and non-tumor pathology tables, survival curves and weight gain tables for the NTP Technical Reports. More than one hundred special reports were also generated, and 250 requests for information from approximately 600 bioassays were processed. TDMS is being converted to the ADABAS Data Base Management System and also being converted to run on a VAX 8600 at NIEHS. Nearly 5000 modules consisting of more than 500,000 lines of code are being converted or rewritten. In FY87, TDMS was used to process data for 118 chemical compounds during in-life and/or pathology stages. 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.

In FY87, a comprehensive study of office automation user requirements was completed by CTB. Several options for further study were considered before final recommendations were made. The recommendations included a pilot study of the All-In-1 office automation software; further investigation of additional integration software; continuing the policy of a standard tool for Institute secretaries; establishment of a personal computer resource center; and implementation of a "lead user" concept.

The workstation group organized and expanded its support of two DEC personal computers, the Rainbow and the Professional series. In support of the DEC personal computers, the group offered scheduled training classes in Rainbow WPS+, Lotus 1-2-3, MS-DOS and WPS for the Professional computer. Training in

LOTUS was extensive, ranging from the very basic, to advanced topics in graphics, macros, and data management. In addition to scheduled training, support was offered in communications, file transfer, and printing.

In FY87, the VAX User support functions were organized and presented to the CTB Computer Users' Committee. One number can be called by NIEHS users for training, problem resolution, new software testing, information and document distribution, and development of utilities and small software projects. A number of classroom training sessions were held on VAX topics, and several new Computer Aided Instruction courses were installed and tested. Quarterly User Group meetings were started. The group assisted the CTB Computer Engineering group in testing a new version of the VAX VMS operating system and began supporting VAX software for communications, printing, programming, data management/reporting, graphics, and statistics through telephone support, on-site visits, and electronic mail. The newsletter, CONNECTIONS, was continued and a special edition called INTERRUPT was also published. Version 1.0 of the New VAX Users Manual was published and work continued on the NIEHS Computing Manual. Several utility procedures were written or improved (WHO, WHAT, UNTAB, and FIX), and a limited number small software applications were developed, such as an on-line Library loan request system.

The Computer Technology Branch utilizes an interagency agreement with the General Services Administration to acquire computer and related expertise. Directed and monitored by five CTB personnel, sixty-five onsite contract personnel provided computer service to NIEHS in computer operations, systems analysis, programming, office automation, and data entry.

Substantial improvements have been made in the use of the GSA contract. An improved funds management system was implemented to provide the budget office and divisions and offices of NIEHS being served by the contract with monthly reports of contract cost and remaining funding. In addition, development and implementation of new project management and documentation standards are well underway. When completed, they will reduce the overhead associated with communication between scientists/administrators and contract employees; Government project management; and contractor resource management.

An Agency Procurement Request has been prepared and forwarded to NIH for approval. The objective is to award an NIEHS data processing support contract through competition to try to improve service at an affordable and justifiable cost. Award of the NIEHS contract is expected in mid FY88.

EPIDEMIOLOGY BRANCH
Summary Statement

Overview and Objectives

The Epidemiology Branch carries out research with human subjects and human data. We emphasize innovative methods, interdisciplinary collaboration, and laboratory methods. These studies are only feasible within a research institution like NIEHS, since the variety of disciplines available for collaboration coupled with the shared interest in environmental toxicology are not found elsewhere. There are three general areas of research: reproduction and early development, chronic disease, and human genetic damage. Reproduction, broadly defined, is both a plausible target for environmental toxins as well as a process of basic science and public health interest in its own right. Chronic diseases such as cancer, heart disease, and renal disease are among the major killers as well as causes of major morbidity in all developed countries. Environmental causes of cancer have been fruitfully studied for decades, and we have done and will continue to do cancer studies, but relatively little attention has been paid to the possible environmental causes of other kinds of chronic diseases. Finally, genetic damage, whether caused by radiation or chemical agents, is an extremely well studied phenomenon in the laboratory, but the extent of induced genetic damage that actually occurs in humans and the consequences of such damage are not understood. New laboratory methods and field application of developed methods may help to quantify the degree, severity, and consequences of human mutation.

Reproduction and Early Development

Early pregnancy loss, that is, loss that occurs before a woman knows she is pregnant, is undetectable in humans without special techniques. This year we are completing a prospective study of very early pregnancy loss, in which we have measured human chorionic gonadotropin in daily urines from women who are trying to become pregnant. This new assay allows the risk of early loss to be measured accurately in humans for the first time. Our preliminary estimate is that between 20% and 25% of pregnancies in this study end in unrecognized loss. Further work will be done to determine if the risk of such loss is related to common exposures such as use of alcohol, tobacco, caffeine beverages or medications. We have also explored the mechanism by which IUDs work as a contraceptive. We looked for evidence of unrecognized pregnancy loss in 40 women with IUDs. We found only one such loss in more than 100 cycles. This provides the most conclusive evidence to date that the IUD's contraceptive action occurs before the time of implantation. This method may be applicable in prospective studies of women exposed to suspected hazards.

A second approach to reproductive studies has been to study populations that have been accidentally exposed to high levels of a toxin, or to use large numbers of observations and sensitive methods in studies of relatively low exposures. An accidental poisoning over 2000 people in Taiwan presented an opportunity to study the offspring of poisoned mothers. One-hundred and seventeen exposed children and 108 controls were examined in the field. The exposed children have higher rates of respiratory disease, dental, skin, and pigment abnormalities, and developmental delay. In the US, we have studied almost 900 children exposed to PCBs and DDE, a form of DDT, through contaminated breast milk. We found that mothers with higher levels of DDE did not breast feed as long as mothers with lower levels, but there

was no increase in the rates of illness nor changes in rates of growth. Since exposure to PCBs and DDE is very common, and since both have been studied extensively in the laboratory, understanding the results of exposure has both public health significance and aids in the interpretation of laboratory data.

Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides and a variety of chemicals in plants. As a first step in exploring this question biological effects of plant estrogens will be measured in postmenopausal women. Postmenopausal women produce little or no estrogen of their own, and are thus a model for studying the effects of exogenous estrogens. Seventy volunteers will be recruited for the study. They will eat a diet high in soybeans, a food rich in plant estrogens. Urine, blood, and vaginal cells will be collected before and after the diet for measurement of several biological factors expected to change with low doses of estrogens. Planning and recruitment is underway.

Chronic Disease

Our approach to the study of chronic diseases includes both the study of disease endpoints and study of environmental risk factors. In both approaches we try to emphasize understudied diseases and new methods. For example, there are currently more than 50,000 individuals in the United States on maintenance dialysis for end stage renal disease. There are also an unknown, but likely substantial, number of additional persons with renal dysfunction requiring some medical attention. Two case-control studies are intended to evaluate the importance of several exposures that are suspected as causes of chronic renal disease. Data collection for a large study of patients with newly diagnosed chronic renal failure has been completed. Preliminary results suggest that both analgesic use and solvent exposure may increase risk for certain forms of chronic renal disease. In a related study, patients with IgA nephropathy were, as hypothesized, more likely to report frequent colds and more frequent use of antibiotics. A striking but unexpected finding is that cases appear to be much more likely than controls to report use of artificial sweeteners.

Cigarette smoking is the best studied environmental hazard, both epidemiologically and in the laboratory. The health consequences to the smoker are well known, but what hazards result from "passive" exposure to other persons' smoking is a new question. Using data from an ongoing study in Maryland, we have examined all-cause and heart disease mortality by levels of smoke exposure, and found that persons who live with smokers are more likely to die from heart disease, and that the greater the exposure, the greater the risk. In contrast, women who live with smokers have a lower risk for colon cancer, perhaps because cigarette smoke components antagonize the tumor-promoting effects of estrogens. Cigarette smoking and passive smoking per se are of substantial public health interest; in addition, however, smoking and passive smoking serve as model exposures with which to develop new epidemiologic techniques and also to compare the effects in humans with the results from pure exposures to the components of smoke as is done in the laboratory.

Exposure to radon-222 has been associated with excess risk of lung cancer in underground miners and the general population. Although the lung is the primary target organ for radon, other parts of the body may be exposed to circulating radon and decay by-products. We studied cancer mortality and measurements of groundwater radon concentration in two states, North Carolina and Maine. Preliminary results from this study suggest that there is a need to consider health effects other than lung cancer in relation to radon exposure.

Immunologic and cytogenetic advances suggest that acute nonlymphocytic leukemia (ANLL) and acute lymphocytic leukemia (ALL) are each comprised of many different diseases with similar appearance, and other studies suggest that risk factors may differ among them. Using a case-control design, adults with ANLL and ALL who enroll for treatment will be studied to determine leukemia risk associated with a variety of environmental and occupational exposures. Cytogenetic studies being done by those treating the patients will be used to classify patients according to the presence or absence of particular chromosomal abnormalities, and risk factors for specific subgroups of patients will be evaluated. Over 200 patients have been enrolled to date and control selection has been started. Preliminary analysis suggests that exposures vary with cytogenetic abnormality.

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.

Frequencies of sister chromatid exchanges were elevated in lymphocytes obtained at parturition from smokers but were not elevated in cord blood specimens from the offspring of these same women. Another study found that prostaglandin synthetases may be involved in the metabolic pathway by which diethylstilbestrol causes increased frequencies of sister chromatid exchanges. A third study investigated a novel approach to measuring chromosomal damage in humans: scoring frequencies of micronucleated erythrocytes in the peripheral blood of splenectomized humans. In a pilot study using this method highly elevated frequencies were found for one of the subjects, which was shown to be due to folate deficiency. In addition to these studies, longitudinal studies of the effects of precisely defined doses of cytotoxic chemicals on genetic material in somatic cells have been initiated. This latter study has the best data to date on precisely defined exposures and cytogenetic changes, includes observations over time, and should allow much better evaluation of the use of these measures in epidemiologic studies than has been possible in the past.

DNA adducts were observed in studies of human placenta. At least three of the adducts are associated with cigarette smoking during pregnancy. One adduct is more strongly associated with birthweight than questionnaire or biochemical measures of smoking exposure. The smoking associated adducts found in human placenta were also observed in respiratory tract tissues of smokers and in tissues of experimental animals treated with cigarette smoke condensate. Preliminary data suggest that certain of the other four adducts are associated with exposures other than smoking. This study provided direct evidence of DNA damage in humans by cigarette smoke, and also demonstrated the utility of this assay for endpoints other than cancer.

Oncogenes are a class of abnormal genes capable of inducing cancer. They are derived from normal genes, called proto-oncogenes, which are present in all cells. In animals, chemical carcinogens can change proto-oncogenes into oncogenes through mutation. Smoking and environmental exposure information is being collected on 30 patients about to undergo resection of bladder tumors. The patient's exposure will

be correlated and compared with the results of oncogene assays done on portions of their resected tumor. In a second study, oncogene assays will be done on bladder tumor tissues resected from 100 individuals with occupational exposures to the potent bladder carcinogens benzidine and b-napthylamine; and compared to oncogene assays done on bladder tumor tissues resected from 100 individuals without such exposures. If chemically induced tumors differ from "background" tumors in terms of their oncogene activation, then much more precise etiologic studies will be possible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43002-11 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Chief	EB	NIEHS
Others:	Beth C. Gladen	Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)* Statistics and Biomathematics 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

LAB/BRANCH

Epidemiology Branch

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.)*

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 a study 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. We had seen that women with higher levels of DDE breast feed for shorter lengths of time, and were able to show that there was not increased morbidity due to chemical contamination of milk. This makes it more likely that lactation is suppressed in the mother, perhaps by the estrogenic properties of DDE.

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 117 children who were born to mothers who were poisoned, 40 of their older siblings, and 107 controls. All children received a physical examination and the mothers answered a questionnaire about their children's health. Children exposed in utero are lighter, shorter, and have smaller head circumference. They are more frequently pigmented, with dysplastic nails, have abnormal teeth, and are developmentally delayed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43004-09 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Environmental Exposures and Chronic Renal and Other Disease

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

PI:	Dale P. Sandler	Epidemiologist	EB	NIEHS
Others:	Walter J. Rogan	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

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.35	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.)*

There are currently more than 50,000 individuals in the United States on Maintenance dialysis for end stage renal disease at a cost of more than $1.5 billion per year. There are also an unknown, but probably substantial, number of additional persons with renal dysfunction requiring some medical attention. Despite the magnitude of this health problem, chronic renal disease has received little attention in epidemiologic studies. The physiology and function of the kidneys are such that they are a likely target for detrimental effects of exposure to environmental hazards. Reports from diverse sources suggest that the kidney is sensitive to a wide variety of environmental exposures including lead, cadmium, chlorinated hydrocarbons, other solvents and metals, and analgesic medications.

Two case-control studies are intended to evaluate the importance of several exposures that are suspected as causes of chronic renal disease. Data collection for a multi-center study of over 500 patients with newly diagnosed chronic renal failure and over 500 population controls has been completed. Exposure histories were obtained by telephone interview. Preliminary results suggest that both analgesic use and solvent exposure may increase risk for certain forms of chronic renal disease. These findings support other reports, but the detailed nature of this study will allow for more careful evaluation of exposure histories and confounding factors. Data collection has also been completed for an interview study involving approximately 50 patients with biopsy diagnosed IgA nephropathy and 90 controls. Preliminary results suggest that while patients with IgA disease were not more likely to report allergic conditions, they were, as hypothesized, more likely to report frequent colds and more frequent use of antibiotics. A striking but unexpected finding is that cases appear to be much more likely than controls to report use of artificial sweeteners.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 43008-08 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

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

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(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 and individuals exposed to active and passive smoking. 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 44003-10 EB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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, University of Bergen, Norway.

LAB/BRANCH
Epidemiology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

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

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

The 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. This year we are completing a prospective study of very early pregnancy loss. By analyzing daily urine specimens from women who are trying to become pregnant, we are able to detect pregnancies that end before women know they are pregnant. Our preliminary estimate is that between 20% and 25% of pregnancies in this study end in unrecognized loss. Further work will be done to determine if the risk of such loss is related to common exposures such as use of alcohol, tobacco, caffeine beverages or medications. We used the same methods of urine analysis to explore the mechanism by which IUDs work as a contraceptive. There has been some evidence to suggest that the IUD does not actually prevent conception, but instead interferes with implantation. This possibility that IUDs prevent clinical pregnancies by causing early abortions was studied in a group of 40 women with IUDs. We looked for evidence of unrecognized pregnancy loss by testing daily urine specimens. We found only one such loss in more than 100 cycles. This provides the most conclusive evidence to date that the IUD's contraceptive action occurs before the time of implantation. In a separate analysis of the early pregnancy study data, we have examined the number of cycles that women required to become clinically pregnant. (Number of cycles to pregnancy is a function of the couple's monthly probability of conception). We found that a couple's probability of conception decreased for women over 30, and for women who smoked. We also found an association between decreased fertility and a woman's exposure *in utero* to her mother's smoking. This association is being explored further in other data sets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 46002-03 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Epidemiologist	EB	NIEHS
Others:	Gwen W. Collman	Staff Fellow	EB	NIEHS

COOPERATING UNITS *(if any)*

University of Minnesota, Harvard University, Cancer and Leukemia Group B member institutions

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

Despite extensive research, the etiology of a substantial proportion of the leukemias remains unexplained. Studies suggest, however, that a significant proportion of the leukemias, particularly acute nonlymphocytic leukemia (ANLL) might be due to environmental or occupational exposures. Immunologic and cytogenetic advances suggest that ANLL and acute lymphocytic leukemia (ALL) are each comprised of many different diseases with similar appearance, and other studies suggest that risk factor identification might depend upon identification of precise subgroups of either disease. A study being done in the epidemiology branch is designed to address the potential for enhanced risk factor detection in well-characterized subgroups of leukemia patients. The study was motivated, in part, by clinical reports suggesting that leukemia patients with specific chromosome changes in bone marrow were more likely than other leukemia patients to have had prior chemotherapy or occupational exposure to chemicals. Using a case-control design, as many as 550 adults with ANLL and 150 adults with ALL who enroll for treatment in first line treatment protocols sponsored by a cooperative cancer treatment group will be studied to determine leukemia risk associated with a variety of environmental and occupational exposures. Patients with leukemia are identified at diagnosis and interviewed by telephone while they are still hospitalized regarding exposure to solvents and chemicals, active and passive smoking, use of hair dyes, irradiation, and family history of certain diseases. Healthy comparison subjects chosen by random telephone screening are also interviewed. Cytogenetic studies being done by the cooperative group will be used to classify patients according to the presence or absence of particular chromosomal abnormalities, and risk factors for specific subgroups of patients will be evaluated. Risk factors will also be correlated with other clinical and laboratory parameters such as FAB classification and immunologic phenotype.

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-47001-01 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Exposure to Radon-222 and Cancer Mortality

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

PI:	Gwen W. Collman	Senior Staff Fellow	EB	NIEHS
Others:	Dale P. Sandler	Epidemiologist	EB	NIEHS
	Dana P. Loomis	Student	EB	NIEHS

COOPERATING UNITS *(if any)*

University of Maine, Orono, Maine

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Exposure to radon-222 has been associated with excess risk of lung cancer in underground miners and the general population. Radon enters the body primarily through inhalation and ingestion. Although the lung is the primary target organ for radon, other parts of the body may be exposed to circulating radon and decay by-products. The role of radon in the development of cancers other than lung cancer is not well studied, especially in children. The effects of alpha radiation exposure during pregnancy and early childhood are not known.

We studied the risk of various selected types of cancer using mortality data collected between 1978-1982 and measurements of groundwater radon concentration previously collected in two states, North Carolina and Maine. In adults, cancer sites of interest were cancers of the upper respiratory tract, cancers of the gastrointestinal tract, the leukemias, bone cancer and female breast cancer. In children aged less than 15 years, selected cancers included leukemia, brain cancer, soft tissue sarcomas, and bone cancer.

County mean radon values in North Carolina were calculated and ranged from 0 to 10,692 pCi/l. These values were ranked and divided into tertiles. Age-sex-adjusted mortality rates were calculated for each cancer site in each radon group. Studies are nearing completion in North Carolina and are ongoing in Maine. Initial results from this study suggest the need to consider cancer sites other than lung cancer in relation to radon exposure.

PHS 6040 (Rev. 1/84) GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-47002-01 EB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Biological Effects of Plant Estrogens in Postmenopausal Women

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

PI:	Donna D. Baird	Staff Fellow	EB	NIEHS
Others:	Allen J. Wilcox	Medical Officer	EB	NIEHS
	Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS
	John McLachlan	Chief	LRDT	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Reproductive and Development Toxicology; Statistics and Biomathematics Branch, NIEHS

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

Exposure to highly estrogenic substances can disrupt reproduction and increase cancer risk. Effects on bone metabolism and cardiovascular health also have been documented. Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides and a variety of chemicals in plants. Health effects of these environmental estrogens are not known. As a first step in exploring this question biological effects of plant estrogens will be measured in postmenopausal women. Seventy volunteers will be recruited for the study. They will eat a diet high in soybeans, a food rich in plant estrogens. Urine, blood, and vaginal cells will be collected before and after the diet for measurement of several biological factors expected to change with low doses of estrogens. These include pituitary hormones, liver-produced plasma proteins, urinary calcium, and vaginal cell cytology. Planning and recruitment is underway. The dietary experiment will begin in the fall of 1987. If plant estrogens are found to be biologically active in postmenopausal women, further questions arise: (1) What effects do these chemicals have on other segments of the population: males, reproductively active women, and babies on soy formula? (2) Do effects of plant estrogens explain some of the differences in morbidity and mortality seen in vegetarians compared with nonvegetarians? (3) Can dietary changes be used in prevention or treatment of estrogen-related conditions? (4) Do other environmental estrogens (like pesticides) affect human health through estrogenic changes in human biology?

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-48003-01 EB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Etiologic Significance of Oncogene Activation in Human Bladder Cancer

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

PI:	Jack A. Taylor	Staff Fellow	EB	NIEHS
Others:	Marshall W. Anderson	Research Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)* National Cancer Institute, Johns Hopkins University, University of North Carolina, University of Wisconsin, Roswell Park Memorial Institute, Salem County Memorial Hospital

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	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.)*

Oncogenes are a class of abnormal genes capable of inducing cancer. They are derived from normal genes, called proto-oncogenes, which are present in all cells. In animals, chemical carcinogens can change proto-oncogenes into oncogenes through mutation. The mutations are often chemical specific, so that a particular chemical carcinogen will induce a characteristic activating mutation within a specific proto-oncogene. The pattern of these mutations in a tumor may be an accurate marker of etiologic exposure.

Oncogenes are frequently found in a variety of human cancers. Studies in the Branch have been established to investigate the role of oncogenes in the etiology of carcinogen-induced human tumors. Bladder cancer was chosen as a model tumor for this investigation, since it is perhaps the best prototype of a chemically induced human tumor. In one study, smoking and environmental exposure information is being collected on 30 patients about to undergo resection of bladder tumors. The patient's exposure will be correlated and compared with the results of oncogene assays done on portions of their resected tumor.

In a second study, oncogene assays will be done on bladder tumor tissues resected from 100 individuals with occupational exposures to the potent bladder carcinogens benzidine and b-napthylamine; and compared to oncogene assays done on bladder tumor tissues resected from 100 individuals without such exposures. Individuals with exposure to these chemicals are rare, but samples of bladder tumor tissue from them are available in the form of paraffin-embedded tissue blocks made and stored following surgery. Recently, oncogene assays have been developed for use with these blocks, allowing us to determine the oncogene status of tumors resected many years ago from individuals with exposures to these powerful chemical carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-48004-01 EB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Health Effects of Passive Exposure to Cigarette Smoke

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

PI:	Dale P. Sandler	Epidemiologist	EB	NIEHS
Others:	Gwen W. Collman	Staff Fellow	EB	NIEHS

COOPERATING UNITS *(if any)*
The Johns Hopkins University, Training Center for Public Health Research, Hagerstown, MD, Laboratory of Biochemical Risk Analysis, NIEHS

LAB/BRANCH
Epidemiology Branch

SECTION

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

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

Studies in the Epidemiology 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 were intended to provide additional evidence in support of some of these early findings. A prospective study of over 48,000 adults has recently been completed. In this study, the mortality and cancer experience of nonsmokers who lived with smokers in 1963 was compared with that of nonsmokers who did not live with smokers and with smokers. Study subjects were residents of western Maryland who had participated in a special county-wide census in 1963. At that time, demographic data and housing characteristics were obtained for 98% of the households in the county, and smoking histories were obtained for all residents who were at least age 16 in 1963. These data were used to derive a measure of exposure to smoke at home for each participant in the study. The cohort was followed through 1975 using existing records. Underlying and contributing causes of death were obtained for census participants who died in the county between 1963 and 1975. Cancer incidence was ascertained through a county-wide cancer register. Preliminary findings suggest that nonsmokers who live with smokers are at increased risk for death from all causes, at increased risk for death from arteriosclerotic heart disease, and at increased risk for developing certain cancers when compared with nonsmokers who are not exposed to smoke at home. Other early findings include a dose-response relationship for heart disease mortality and degree of exposure to cigarette smoke with the relative risk for heart disease death among nonsmokers living with smokers similar to that of active smokers of fewer than 10 cigarettes a day, and an inverse relationship between colorectal cancer risk and degree of smoke exposure. Other work in this area includes continued exploitation of the data collected in the study of childhood smoke exposure and adult cancer risk, and an additional study of sister chromatid exchanges and urinary mutagens in active and passive smokers which demonstrated small differences related to cigarette smoke exposure.

LABORATORY OF BIOCHEMICAL RISK ANALYSIS
Summary Statement

The Laboratory of Biochemical Risk Analysis (LBRA) (1) conducts laboratory studies to determine the biochemical mechanisms responsible for epidemiological observations, (2) designs and conducts laboratory studies to enhance the scientific basis for extrapolating risk from animal experiments to human populations, (3) collaborates with epidemiologists and mathematical statisticians to design, implement and interpret research studies involving biochemical applications to risk analysis and biochemical epidemiology, and (4) establishes laboratory research contracts and collaborations outside of DBRA in support of LBRA's overall goals. Some of the ongoing research projects are summarized in the following paragraphs.

Molecular Toxicology

This section conducts studies on (1) chemical-induced DNA damage and cell replication as factors in neoplasia, (2) activation of cancer-causing genes (oncogenes) by carcinogens and implications for risk estimation, and (3) chemical carcinogenesis in rodent model systems as a means of low dose extrapolation to human exposure.

Recent evidence from several independent lines of investigation has merged to suggest that neoplasia results from the abnormal activation of a relatively small number of cellular genes (proto-oncogenes). Subsequent studies have established that proto-oncogenes can also be activated to cancer causing genes (oncogenes) by genetic alterations that range from point mutations to gross DNA rearrangements such as translocation and gene amplification. We have characterized activated oncogenes in spontaneous tumors of the mouse lung and liver as well as 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. Novel mutations in the ras family of oncogenes, such as mutations in the 117th codon of Ha-ras, have been identified in primary tumors, and several potentially new transforming genes have been detected. Characterization of oncogene activation in different tumor types or the same tumor type in different animal species suggest that activation of a proto-oncogene is a common pathway for tumor induction for some chemicals. These approaches may enable us to more accurately estimate risk of cancer in humans exposed to specific classes of carcinogens.

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, cell turnover and gene expression in tissues and cells that are

susceptible or resistant to carcinogen-induced neoplasia. Studies with 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 is often significantly enhanced by 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. In addition to ongoing experiments with NNK, several studies will be initiated to evaluate DNA adduct formation and loss, cytotoxicity and cell turnover in target and nontarget tissues following exposure to methylene chloride or tetranitromethane. Examination of the molecular dosimetry of DNA adduct formation, cytotoxicity and cell proliferation should allow a more accurate estimation of the carcinogenic potential of environmental chemicals during low dose exposure.

Cellular Epidemiology

This section (1) conducts cytogenetic studies on human populations exposed to cigarette smoke and halogenated aromatics focussing on the influence of metabolic activation/deactivation pathways, (2) applies ^{32}P-postlabelling methodology to detect DNA adducts in human samples and in animal model systems for the puspose of monitoring exposure to genotoxic agents and evaluating molecular dosimetry patterns, and (3) investigates the quantitative relatioinship between DNA adducts, growth factor pathways and cell transformation in models for mammary carcinogenesis.

Cytogenetic studies in the Laboratory of Biochemical Risk Analysis have developed a modified assay that greatly enhances our ability to detect genetic damage from smoking. The assay has been used to evaluate sister chromatid exchange (SCE) frequencies 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 SCEs in lymphocytes from smokers and only a small increase in samples from non-smokers. Moreover, lymphocytes from the PCB-exposed population in Taiwan were examined for cytogenetic damage using our modified SCE assay. The examination revealed that, like the data on smoking, ANF increased SCE frequency in lymphocytes from exposed but not non-exposed individuals. ANF is metabolized to a DNA reactive species in lymphocytes from smokers but not non-smokers and this adduct apparently leads to point mutations and chromosomal aberrations. The mechanism of adduct formation appears to involve a specific P-450 isozyme. This assay is a sensitive non-invasive method for detecting exposure to certain classes of polycyclic aromatic hydrocarbons.

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 marcomolecules 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/or metabolite) at the macromolecular target site which initiates 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 are applying this methodology to detect adducts in various tissues and cell types following exposure to chemicals alone or in combination.

There is considerable controversy regarding the influence of route of exposure of benzene on the toxicity of this compound, particularly its carcinogenic effects. Laboratory scientists, in collaboration with NTP are addressing this question by performing pharmacokinetic studies in animals exposed to benzene either by the oral or inhalation route. These investigations use a wide dose-response range including exposures as low as those encountered in the workplace. Key metabolites are quantified in target tissues and blood and cytogenetic effects are evaluated in relation to the pharmacokinetic and metabolism data. Follow-up studies will determine if DNA adduct concentrations are dose dependent. These studies should enhance our ability to more accurately predict risk of benzene toxicity in occupationally-exposed individuals.

Metabolism and Receptors

This section (1) evaluates metabolic activation/deactivation pathways for chemicals in human samples and animal model systems emphasizing specific cytochrome P-450 isozymes, (2) investigates signal transduction pathways in relation to the tumor promotion process in animal models emphasizing synthetic estrogens and halogenated aromatics as promoters, and (3) identifies and characterizes placental markers of human exposure to polychlorinated biphenyls and cigarette smoke.

Over the last decade research has demonstrated that many carcinogenic agents are metabolically activated by the cytochrome P-450 monooxygenase system which is comprised of numerous isozymes possessing precise substrate specificities. Our investigations are directed at evaluating selected preneoplastic cells in animal models for chemical carcinogenesis for the presence of various P-450 isozymes. Moreover, we plan on applying methodology for evaluating patterns of P-450 isozymes to detect human populations at risk for cancer following exposure to polycyclic aromatic hydrocarbons. Preliminary studies have produced a panel of antibodies to be used in these experiments and appropriate technology for cloning P-450 genes has been developed.

We are evaluating 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, DNA adducts (by ^{32}P-postlabelling), critical changes in gene

expression, including oncogene activation, and histopathological alterations including preneoplastic lesions and tumor incidence. Furthermore, the time course of these changes is being investigated. The rat two-stage model is employed as the primary model for hepatocarcinogenesis. This model uses a single dose of an initiating agent followed by chronic exposure to a promoting agent. A number of potentially important observations have been made in livers from animals at various stages of the carcinogenic process. These findings involve alterations in receptor and signal transduction pathways important to regulation of cell proliferation. Future studies are designed to localize these changes in neoplastic cells. Furthermore, attempts will be made to develop in vitro models for the purpose of obtaining data on structure activity relationships.

Scientists in the Laboratory of Biochemical Risk Analysis, the Epidemiology Branch and the Laboratory of Pharmacology have demonstrated that placentas obtained from women in Taiwan who were accidentally exposed to rice oil contaminated with 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. Autophosphorylation of the EGF-receptor was markedly diminished in placentas of exposed women and this was correlated with decreased birthweight. Concentrations of PCB and PCDF congeners were measured in placenta and blood and concentrations of two extraordinarily toxic PCDFs have been quantified. These data provide strong evidence that the PCDFs, not the PCBs, are the causative agent in this poisoning episode. 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 and dioxins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 21024-06 LBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Drug Metabolizing Enzymes in Animal Models and Human Tissue

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

PI:	Joyce Goldstein	Pharmacologist	LBRA	NIEHS
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	M. Graham	Visiting Fellow	LBRA	NIEHS
	P. McClellan-Green	Biologist	LBRA	NIEHS
	G. Lucier	Research Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

M. Negishi, Laboratory of Pharmacology, IRP; T.A. Gasciewicz, University of Rochester Medical School; K. Shiverick, University of Florida; D. Waxman, Harvard University

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors 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.)*

This project is new so the summary of work represents plans, objectives and some preliminary findings. Cytochrome P-450 is the principal monooxygenase system which catalyzes foreign chemicals to mutagens and carcinogens as well as inactivating them. This system is perturbed dramatically by exposure to hormones and foreign chemicals. The system consists of a large number of isozymes each with its own substrate specificity. Some P-450 enzymes are constitutive and some appear to be polymorphic (present or absent, active or inactive) in outbred strains of rats and in man. The polymorphisms can result in dramatic differences in the ability to metabolize certain drugs (e.g., a polymorphism for debrisoquine metabolism and sparteine metabolism in man). Antibodies and cDNA probes to the P-450 proteins are useful for determining the effects of chemicals on these enzymes and for studying polymorphisms in these enzymes. One objective of these studies is to examine phenotypic variability of P-450g, a constitutive enzyme, in male rats and humans. Our data suggest that the CD rat is a good model to study polymorphism of this cytochrome in humans. These studies revealed that rats could be divided into two distinct phenotypes containing high P-450g (10-20% of total P-450) or low P-450g (<0.5%). Surprisingly, both phenotypes of male rats appeared to contain a translatable mRNA for this enzyme athough it was absent in females suggesting that the defect might be a defective or labile enzyme in the low phenotype. P-450g was shown to metabolize aflatoxin to mutagens in a reconstituted system. A cDNA library was constructed from high phenotype P-450g in rats, and several putative cDNA clones for this protein have been selected with antibody and rescreening with a positive clone. If human tissue is available, we plan to analyze the DNA by Southern blots and restriction analysis to determine whether the phenotypic variability in one or more human P-450s is associated with liver or lung tumors.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 35005-08 LBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanisms of Carcinogen-Induced DNA Damage and Cell Transformation

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

PI:	Marshall Anderson	Res. Mathematician	LBRA	NIEHS
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	T. Devereux	Biologist	LBRA	NEIHS
	C. Hunnicutt	Biologist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Dr. Robert Maronpot, Chemical Pathology Branch
Dr. Tony Pegg - Hershey Medical Center

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	1.2	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.)*

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, cell turnover and gene expression in tissues and cells that are susceptible or resistant to carcinogen-induced neoplasia. We are concerned with the effects of dose of carcinogen on DNA adduct formation and repair, cytotoxicity and cell replication. Studies with 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 are often significantly enhanced by 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.

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 43009-04 LBRA

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	OD	NIEHS
	Suzanne Snedeker	Senior Staff Fellow	LBRA	NIEHS
Others:	Chris R. Miller	Biologist	LBRA	NEIHS
	Rong Fang-Hu	Visiting Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)*
Dr. Robert Maronpot, Chemical Pathology Branch

LAB/BRANCH
Laboratory of Biochemical Risk Analysis

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	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 spece provided.)*

Nitrilotriacetate (NTA), a metal chelator that has been used as a substitute for polyphosphates in detergents, has been shown to be nephrotoxic and promote urinary bladder carcinogenesis when administered with nitrosomines. NTA exists in water as a metal-complex, and its iron, but not its aluminum salt, has been shown to induce primary renal cell carcinomas in rats. Iron in its Fe^{++} state has been implicated in the generation of highly reactive hydroxy radicals, which may cause damage to DNA directly. Intracellular iron may promote carcinogenesis through radical formation within the cell. We are interested in using ferric-nitrilotriacetate (Fe-NTA) to study the mechanisms of metal-induced nephrotoxicity and carcinogenicity using a rat model. Specific metabolic parameters to be evaluated include: cell ultrastructure, urinary essential metal excretion, and the distribution of essential metals, activity of radical scavenging enzymes, lipid peroxidation and metallothionein (MT) levels (a metal-binding protein that may act as a hydroxl radical scavenger) in renal and hepatic tissue. We have completed a five week i.p. injection protocol to evaluate the acute, nephrotoxic affects of Fe-NTA in rats. Fe-NTA induced dysplastic/premalignant changes in renal proximal tubule cells. Iron primarily localized in these dysplastic cells. Renal iron and lipid peroxidation was increased, while the activity of catalase and tissue zinc and copper levels were depressed in the kidneys of Fe-NTA treated animals. MT levels were unchanged in renal tissue, but elevated in the livers of Fe-NTA treated animals. A long-term Fe-NTA renal tumorogenesis study is now in progress. A subset of the Fe-NTA dosed animals will be monitored in order to develop Magnetic Resonance Imaging techniques to detect and follow renal tumor growth in the intact animal. We also are investigating the excretion of low molecular weight proteins in the urine as a possible index of early renal tubular damage in rats chronically exposed to lead in the drinking water (1000 ppm) for 12 weeks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 46003-03 LBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
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	M. Anderson	Research Chemist	LBRA	NIEHS
	O. McDaniel	Bio. Lab. Tech.	LBRA	NIEHS
	J. Lambert	Bio. Lab. Tech.	LBRA	NEIHS
	D. DiAugustine	Research Chemist	LBRA	NIEHS
	Y. Liu	Visiting Fellow	LBRA	NIEHS
	T. McCoy	Bio. Lab. Tech.	LBRA	NIEHS

COOPERATING UNITS

Epidemiology Branch, DBRA
Preventive Medicine Institute - Strang Clinic
New York City, NY

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

It is the long range objective of this project to evaluate the relationships between environmental exposure to potential toxic agents and changes in biochemical, molecular or cytogenetic parameters that may be important in identifying individuals at increased risk to having an adverse health outcome. Animal models and defined human populations exposed to environmental substances are used to evaluate: (1) the utility of using lymphocytes as molecular dosimeters of environmental exposure by determining the biologically-effective dose for different classes of chemical carcinogens through measuring DNA adducts in relation to dose; (2) the relationships between pharmacokinetic parameters of activation/deactivation and the generation of biologically-effective doses, and the resulting consequences of biological endpoints such as SCEs, chromosomal aberrations and DNA damage and repair.

A cytogenetic procedure has been developed that distinguishes smokers and PCB-exposed persons from appropriate unexposed populations. Elevations in SCE frequencies in lymphocytes are seen only in exposed individuals following *in vitro* exposure of blood to the synthetic flavanoid, α-naphthoflavone (ANF), a potent P-450 inhibitor. The mechanism for this differential sensitivity is being investigated in animal and *in vitro* (CHO cell) systems. Our findings have shown that metabolic activation of ANF is required for clastogenicity. Studies are also in progress to determine the relationship between heritable differences in metabolic activation/deactivation of chemicals by human lymphocytes and DNA damage as measured by DNA-adduct levels and/or nucleoid sedimentation. DNA adducts are measured by standard techniques as well as ^{32}P-postlabelling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 46004-03 LBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	George W. Lucier	Chief	LBRA	NIEHS
Others:	D.B. Campen	Res. Biologist	LBRA	NIEHS
	G. Sunahara	Visiting Fellow	LBRA	NIEHS
	T. Goodrow	Graduate Student (GW)	LBRA	NIEHS
	Z. McCoy	Bio. Lab. Tech.	LBRA	NIEHS
	J. Goldstein	Pharmacologist	LBRA	NIEHS
	R. DiAugustine	Res. Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

University of North Carolina
Laboratory of Pharmacology, NIEHS

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	2.5

CHECK APPROPRIATE BOX(ES)

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

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

It is the long range 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, 17 α-ethinylestradiol and α-zearalanol. The objectives of these studies are to evaluate the quantitative relationships between dose of tumor promoter, receptor interactions, DNA adducts (by ^{32}P-postlabelling), critical changes in gene expression, including oncogene activation, and histopathological alterations including preneoplastic lesions and tumor incidence. Furthermore, the time course of these changes is being investigated. The rat two-stage model is employed as the primary model for hepatocarcinogenesis. This model uses a single dose of an initiating agent followed by chronic exposure to a promoting agent. A number of potentially important observations have been made in livers from animals at various stages of the carcinogenic process. These findings involve alterations in receptor and signal transduction pathways important to regulation of cell proliferation. Future studies are designed to localize these changes in neoplastic cells. Furthermore, attempts will be made to develop in vitro models for the purpose of obtaining data on structure-activity relationships.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES 46005-03 LBRA

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters 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:	Jill Stowers	Chemist	LBRA	NIEHS
	Colleen Hunnicutt	Biologist	LBRA	NIEHS
	Rachel Patterson	Microbiologist	LBRA	NIEHS
	Urs Candrian	Visiting Fellow	LBRA	NIEHS
	Bengt Widegren	Visiting Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)*
Dr. Robert Maronpot, National Toxicology Program, NIEHS
Dr. Stuart Aaronson, National Cancer Institute

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:
6.5	3.5	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.)*

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 activated oncogenes in spontaneous tumors of the mouse lung and liver as well as 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. Novel mutations in the ras family of oncogenes, such as mutations in the 117th codon of Ha-ras, have been identified in primary tumors, and several potentially new transforming genes have been detected. Relationships between biological "hot spots" for the gene activation and sequence specificity for DNA binding of several carcinogens is also being investigated. Characterization of oncogene activation in different tumor types or the same tumor type in different animal species suggest that activation of a proto-oncogene is a common pathway for tumor induction for some chemicals. These approaches may enable us to more accurately estimate risk of cancer in humans exposed to specific classes of carcinogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 46006-03 LBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	G. Sunahara	Visiting Fellow	LBRA	NIEHS
	K. Nelson	Staff Fellow	LBRA	NIEHS
	Z. McCoy	Bio. Lab. Tech.	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, DBRA; Laboratory of Pharmacology, NIEHS
Wright State University
University of Florida

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors 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.)*

Our studies have evaluated biochemical changes in placentas from humans exposed to rice oil contaminated with polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) in Taiwan. Placentas were obtained from non-smoking women four to five years after the exposure had occurred. The exposed individuals ingested approximately 1-3g PCBs and 5mg PCDFs, and many exhibited symptoms characteristic of PCB poisoning. This disease was termed Yucheng in Chinese. We examined a number of parameters including arylhydrocarbon hydroxylase (AHH) activity, cytochrome P-450 isozymes, epidermal growth factor (EGF) receptor binding properties and actions and Ah receptor. We also quantified concentrations of PCB and PCDF congeners known to be present in the contaminated rice oil. Our results revealed a dramatic elevation in placental AHH activity in samples from PCB-PCDF exposed women. This increase was associated with a parallel increase in placental microsomal protein immunochemically-related to cytochrome P-450 form 6. Placental cytosol preparations were examined for ^{3}H-TCDD binding capacity by standard receptor techniques. EGF receptor-mediated autophosphorylation capacity was significantly diminished in PCB-PCDF placentas but this effect was not associated with changes in plasma membrane EGF receptor binding properties (K_d and B_{max}). The EGF receptor autophosphorylation effect correlated well with the decrease in birthweight observed in offspring of exposed women suggesting that this biochemical event might provide a good "marker of effect" for the toxic halogenated aromatics. Two PCDF congeners (2,3,4,7,8-penta CDF and 1,2,3,4,7,8-hexa CDF) were detected in Yucheng placentas but not controls. Several PCBS were also detected (including the 2,2',4,4',5,5',-hexa CB and 2,3,3',4,4',5-hexa CB) in much higher concentrations in Yucheng placentas. Surprisingly, placental concentrations of PCBs correlated better with effects than did the PCDFs.

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 70069-05 LBRA

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

DNA Adducts in Human Lymphocytes and Hormone-Dependent Cancers

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

PI:	Richard P. DiAugustine	Research Chemist	LBRA	NIEHS
Others:	C. Thompson	Senior Staff	LIBRA	NIEHS
	B. Pentacost	Visiting Fellow	LRDT	NIEHS
	B. Brown	Biologist	LBRA	NIEHS
	I. Zajac	Chemist	LBRA	NIEHS
	G. Lucier	Research Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, DBRA
University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

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

CHECK APPROPRIATE BOX(ES)

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

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

This is a new project which means that the summary of work consists mostly of plans although some preliminary findings are included. The capability of exogenous compounds or their metabolities to form adducts with DNA is considered a crucial step in the initiation of carcinogenesis. A goal in this laboratory is to examine human tissue for DNA adducts for the purpose of assessment of exposures of humans to potential genotoxic agents. In a current study, digests of DNA prepared from human lymphocytes incubated with [^{3}H] benzo(a)pyrene revealed adduct formation by both liquid chromatography and ^{32}P-postlabeling analysis. The extent of adduct formation exhibited a wide range among individual donors, which might correlate with the individual capacity to deactivate reactive intermediates. Lymphocytes from smokers appeared to have no greater capacity to form adducts than lymphocytes from nonsmokers. We are currently analyzing lymphocyte DNA from heavy smokers and nonsmokers for the presence (in vivo) of polycyclic aromatic hydrocarbon-related adducts. The ^{32}P-postlabeling method should provide a sensitive means for detection of adducts in human tissues. This study is being extended to include the detection of DNA adducts in human breast tissue. Furthermore dose-response relationships for DNA adducts are being examined in rodent models for hormone dependent mammary and liver cancer to evaluate whether estrogen-DNA adducts are formed and/or estrogens influence DNA adduction of dietary constituents or other hormones. In the mammary studies, the relationship, if any, between DNA adducts and altered growth factor (cell proliferation) pathways is being investigated.

STATISTICS AND BIOMATHEMATICS BRANCH
Summary Statement

The Statistics and Biomathematics Branch (SBB) plays a major role in DBRA'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 Division and Toxicology Research and Testing Division as well as individuals within DBRA's Epidemiology Branch and Laboratory of Biochemical Risk Analysis.

Biostatistical Applications Section

The primary responsibility of the Biostatistical Applications Section is to carry out research dealing with the application of statistical techniques to toxicological and epidemiological problems arising within the Institute's various research programs.

At the present time much of this research effort is focused on the area of toxicology/carcinogenesis, with particular emphasis on the laboratory animal carcinogenicity studies carried out by the Division of Toxicology Research and Testing. Biostatistical research within the Section deals with various issues of experimental design (e.g., the selection of appropriate dose levels), data analysis (e.g., the development of statistical techniques that do not require cause of death information) and interpretation of experimental results (e.g., assessing inter-species correlation in neoplastic response). While much of the Biostatistical applications research effort is wholly contained within the section, some projects are sufficiently broad in their scope that they involve statisticians and mathematicians from other sections of the SBB as well (e.g., the evaluation of the carcinogenic predictability of four widely used short term tests described in the next section.). These research efforts fulfill the dual role of increasing our understanding of the various factors that influence the results of carcinogenicity testing in laboratory animals and enhancing the utility of these studies in the overall assessment of human health risk.

Biostatistical applications research in the area of epidemiological or human studies is directed toward gaining additional insight into the uses and limitations of existing study designs and methodologies, and investigating the adaptation or development of new statistical methods when such a need is indicated. Examples of research in this area include the development of methods for evaluating effects of environmental exposures on human fertility, extension of methods for detecting seasonal clustering of diseases, and the development of procedures for evaluating autonomic reflexes in newborns.

In addition to its research activities, the Biostatistical Applications Section provides computational and statistical support for various research projects within the Institute. These activities range 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 (SMS) 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. Current emphasis is on studies of genetic toxicology. The Section strives to exploit large scientific data bases, where possible, to aid and validate its research efforts.

The methodological work of SMS is illustrated by recent research on the design and analysis of epidemiological studies involving biological markers of human genotoxic exposure. The focus of this work was on statistical considerations associated with the adaptation of the Ames Salmonella assay, the sister chromatid exchange assay and other in vitro assays to studies with human subjects. As part of this effort, criteria were formulated for judging the acceptability of historical control data from observational studies of genetic toxicity.

On a more theoretical level, there has been extensive research into the asymptotic properties of a class of statistical procedures recently formulated by SMS to test nonparametrically 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.

Finally, the Section's research focus on genetic toxicology is exemplified by the completion of a three-year, 73-chemical project to assess the prediction of chemical carcinogenicity in rodents from four NTP in vitro genetic toxicity assays. Results obtained did not show significant differences in individual assay concordances with rodent carcinogenicity results, all four being approximately 60%; and did not support the short-term test battery concept.

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 in this area has been to incorporate the concepts of mutation, selection and recombination into the models. Estimates of the rate of mutation and recombination are important for both theoretical and practical reasons. One example of current research is the incorporation of selection into the coalescent process. This process, which describes the ancestral relationships of a sample of genes, is very useful in developing methods for analyzing DNA sequence data. A major result of this work quantifies the effect of selection on the mean and the variance of standard estimates of the mutation rate.

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 uses 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 sensitivity of physiological based pharmacokinetic models for estimating the effective biological dose, and the implications of a safety factor approach for estimating risk when there are individual thresholds. In the latter area, a simple method is developed to quantify the risk associated with a safety factor procedure for setting the acceptable daily intake level of a toxicant. In particular, it is shown that for some dose response relations, the standard safety factor approach results in an acceptable daily intake dose which is higher than the threshold levels of a significant proportion of the population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40004-10 SBB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 C. Gladen	Statistician	SBB	NIEHS
Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, NIEHS

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biostatistical Applications and Statistical Methodology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project encompasses the development and evaluation of statistical methods appropriate for epidemiologic research. This year, work has progressed in three areas: further development of methods aimed at evaluating effects of environmental exposures on human fertility; extension of methods for detection of seasonal or circadian clustering in the occurrence of events, e.g. diseases; and development of methods for evaluating an autonomic reflex in newborns in order to identify a possible early marker for babies at risk of sudden infant death syndrome. 1. The fertility model developed is applied to analyzing the number of non-contracepting menstrual cycles a couple requires to achieve pregnancy, allowing for the possibility that this average time to conception may be related to environmental exposures. In data gathered prospectively, allowance must be made for possible losses to follow-up, e.g. due to a change of mind about wanting the pregnancy. The model allows the investigator to estimate a ratio of fecundabilities, comparing the exposed with the unexposed. As an epidemiologic screening tool, this integrates effects an exposure might have on the gametes with effects on the hormonal function of either the male or female, and with early lethal effects on the developing pre-embryo. 2. The seasonal clustering seen in the onsets of certain diseases has offered important clues related to their etiology. In collaboration with a biostatistician at Columbia University, tables are being extended that allow one to use the "scan" statistic to test for unimodal clustering, as against a uniform distribution of occurrence times.
3. Previously developed methodology is being adapted for use in evaluating autonomic regulation of cardiac function in human newborns, based on simultaneous recordings of electrocardiograms and respiration. This will be applied to a case-control study of sudden infant death syndrome, using data provided by a collaborator at the University of London Cardiothoracic Institute.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40005-10 SBB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
Walter W. Piegorsch	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, DTRT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Statistical Methodology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is directed toward the development of new statistical methodology for use in other areas of Institute research and human health research in general. To date interest has focused primarily on the field of genetic toxicology. In the last decade there has been heightened scientific attention paid to genetic toxicology 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 studies 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. 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 and human studies in recognition of the limitations of in vitro results to anticipate or predict effects in an intact mammal.

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 41001-13 SBB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

PI:	David G. Hoel	Chief	SBB	NIEHS
Others:	Norman L. Kaplan	Mathematical Statistician	SBB	NIEHS
	Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
	Michael D. Hogan	Special Assistant to the Director	DBRA/OD	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Reproductive and Developmental Toxicology, DIR

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 of this effort 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 the 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 evaluation of alternative cancer risk models for ionizing radiation, with the implications of a safety factor approach to risk estimation when individual thresholds are present in the exposed population, with the potential relationship between toxicity, genotoxicity and carcinogenicity in laboratory rodents and the possible implications of this relationship for human carcinogenic risk assessment, with an evaluation of the nonlinearity of carcinogenesis dose-response functions, and with the sensitivity of physiologically-based, pharmacokinetic models for estimating the biologically effective dose.

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 44002-11 SBB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	LG	NIEHS
Richard R. 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

Mathematical Modeling 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.)*

Now that it is becoming common place to study genetic variation at the DNA level, it is important to develop adequate mathematical models and statistical methods for analyzing this fast growing body of data. During the past year, two important problems in this area involving mathematical modeling were studied. First, the coalescent process, which has been used extensively in analyzing neutral population genetics models, was generalized to models which allow for selection. Secondly, a population genetics model was constructed and analyzed which describes the evolution of a population of transposable elements where the mechanism for controlling copy number is based on illegitimate recombination between heterozygous copies. Two areas of statistical methodology were also investigated. A conservative statistical test of a prediction of the neutral theory of molecular evolution was developed and its properties investigated. Also, an estimator of C=4Nc was proposed where N is the population size and c is the rate of recombination per generation. Properties of the estimator C and the above test of neutral theory were investigated using Monte Carlo simulations. The remaining area of research concerns the construction of efficient computer algorithms for aligning several DNA sequences and inferring a sensible phylogeny for the sequences.

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 45001-07 SBB

PERIOD COVERED

October 1, 1987 to September 30, 1987

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
Walter W. Piegorsch	Mathematical Statistician	SBB	NIEHS
Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
Gregg E. Dinse	Senior Staff Fellow	SBB	NIEHS
A. John Bailer	Staff Fellow	SBB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biostatistical Applications

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, N.C. 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.)*

This project is concerned with the application of statistical methodology to laboratory animal experiments, with particular emphasis on long-term carcinogenicity studies. Specific areas that were the focus of research efforts during the past year included experimental design (e.g., selection of appropriate dose levels; use of dual control groups), data analysis (e.g., effect of tumor and treatment lethality on dose-related trends in tumor onset; developing new methodology that does not require cause of death information; assessing synergism in carcinogenicity studies), and interpretation of results (e.g., evaluating the effects of body weight, and time-related trends on tumor incidence; assessing inter-species correlation in neoplastic response). Other research efforts included assessing the relationship between toxicity/genotoxicity and chemically induced carcinogenesis in laboratory rodents, and investigating the possible influence of cage effects on liver tumor incidence for chemicals evaluated as hepatocarcinogens. Many of these investigations utilized the large data base of laboratory animal carcinogenicity studies carried out by the National Toxicology Program.

DIVISION OF TOXICOLOGY RESEARCH AND TESTING

DIVISION OF TOXICOLOGY RESEARCH AND TESTING
Summary Statement

The Division of Toxicology Research and Testing (DTRT), 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. DTRT 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. Three specific and continuing aims are:

- o Expanding toxicological data of chemicals nominated, selected, and studied. The number and rate of chemicals studied and evaluated depends on resources allocated.

- o Developing and validating a series of experimental designs, protocols, and biologic assays appropriate for research and regulatory needs.

- o 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; part of the Division of Biometry and Risk Assessment). 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 discipline leaders, project officers, and/or chemical managers 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, these people are responsible for the development and supervision of contracts that extend these activities or that perform in-depth toxicologic characterization of chemicals.

The NTP uses state-of-the-art testing methods and, on an ongoing basis, examines, develops, and validates new, improved technologies. 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, DTRT undertakes the appropriate methods development and validation.

Highlights of NTP activities follow:

- Toxicity and carcinogenicity testing of approximately 250 chemicals is under way at NTP contract laboratories. The tests are at various stages of completion, ranging from test design to technical report preparation.

- Thirteen draft NTP Technical Reports on toxicology and carcinogenesis studies were presented for public review by the Board of Scientific Counselors' Peer Review Panel in FY 1987. Chemicals exhibiting clear evidence of carcinogenicity in rats or mice in these studies included:

  Mirex (an insecticide)
  Malonaldehyde (a natural metabolic intermediate)
  C.I. Acid Orange 3 (a hair dye)
  Dichlorvos (an insecticide)
  Nitrofurazone (an antibacterial)
  Nitrofurantoin (an antibiotic)

- A two-year exposure study has been started to address questions regarding cellular and molecular processes responsible for the induction of lung and liver tumors in mice by a model chlorinated hydrocarbon, methylene chloride. The objective of this study is to determine whether the chlorinated hydrocarbon introduces new mutations into DNA, increases the expression of indigenous molecular lesions, or both.

- A study of the comparative toxicity and carcinogenicity of commonly occurring nickel compounds in the workplace is in progress. The studies will relate carcinogenic results to alterations in the immune system and will compare relative toxicities of water soluble and insoluble metal compounds. In addition, the studies will be used as a model for the comparison of carcinogenicity results from animal model systems with the results in humans.

- Microencapsulation, a process which permits the testing of volatile or chemically-reactive compounds in the animal diet and minimizes problems with palatability, is a new technique being studied by intramural scientists at NIEHS. Feeding studies using the microencapsulated trichloroethylene have been successfully completed. Other studies involving this process include the demonstration of bioequivalence of microencapsulated 1,1,1-trichloroethane, 2-ethylhexanol and citral.

- An in vivo transplant model of Fischer rat leukemia was developed and is being maintained by serial cell transfer, resulting in a reduction in time of tumor expression from 18 months to 2 months. The validity of this short-term model for predicting chronic potency of chemical leukogens was demonstrated with 2 chemicals and currently the model is being applied to 2 other chemicals. Additional studies are continuing on the development of stable in vitro cell lines and monoclonal antibodies from this transplantable tumor.

- The health effects study on groundwater contaminants is continuing. Developmental chemistry work to formulate a 25-chemical (those that have been most frequently found in groundwater) mixture in deionized water is near completion at a contract laboratory. Contracts for the initial toxicology studies of this mixture are being reviewed at this time with a schedule start date of early November 1987. Those studies are expected to be completed in August 1988.

- An objective evaluation of the performance of four *in vitro* short-term genetic toxicity assays in predicting rodent tumorigenicity was completed this past year. The two major deficiencies in previous efforts to evaluate these relationships (the absence of a complete matrix of results across all chemicals and test systems, and the absence of results for both carcinogens and non-carcinogens) were incorporated into this design. The results of this evaluation were published and the individual test results have been submitted for publication.

- Through a collaborative research effort, clear differences have been found in the patterns of oncogene activation between spontaneous and chemically induced liver tumors in the B6C3F1 mouse. In addition, work is proceeding which attempts to characterize the molecular biology of mononuclear cell leukemia in Fischer 344 rats. These studies should help clarify the significance of this spontaneous neoplasm which is frequently modulated during the course of NTP two-year studies.

- Through significant advances in research work with magnetic resonance imaging (MRI), it is now possible to obtain microscopic resolution (100 micrometers) of tissues in the live animal. Additional MRI studies have begun looking at the progression and repair of chemically induced toxic renal lesions.

- Progress continues in two areas of carcinogen metabolism which are particularly relevant to program goals. First, the metabolic capability of human cells in culture, used for genetic toxicity screening, has been improved by the transfection of a gene coding for a human P-450 isozyme. Also, cytofluorographic methods for detecting and isolating cells with enhanced metabolic capability have been developed. This capability will allow long-term, low-dose experiments to be conducted so that genotoxic effects can be measured with limited cytotoxicity. Secondly, human liver metabolism was compared to rodent liver metabolism of two model carcinogens. The human liver was found to be more active than rat hepatocytes in the metabolism of acetylaminofluorene and was about the same level for benzo(a)pyrene. NTP rodent liver carcinogens are now being studied with the goal that such metabolism data may aid rodent-to-human extrapolation.

- NTP Chemical disposition studies are undertaken both through in-house research and through research contracts. These studies provide information on absorption, distribution, metabolism, and excretion of chemicals or chemical classes. That information is used in the design and interpretation of toxicity and carcinogenesis studies as well as to provide data that allows for a better assessment of structure/activity relationships influencing chemical disposition or toxicity mechanisms. Through research contracts, methylene bis(thiocyanate), glyphosate, 4-vinylcyclohexene, glycol ether acetates, furfural, benzene, butadiene, and isoprene are some of the chemicals that have been investigated recently. Benzo(a)pyrene, benzidine, caffeine, and trichloroethylene are examples of those chemicals that have been investigated in human tissues (*in vitro*) to compare the metabolism to that in rat tissues. In addition, the metabolism in liver, kidney, lung, and adrenals has been evaluated.

- In-house studies have been designed to evaluate the disposition of various chemicals including hexabromonaphthalene, citral, alkyl carbamates, and furan. Teratogenicity studies conducted on mixtures of TCDD with different

dibenzofurans or PCBs found that there are common mechanisms of toxicity. Additional studies included evaluations of the senescent changes in metabolism and their relationship to the expression of toxicity, and evaluations of the relationship between the induction of cell proliferation and hyperplasia from certain chemicals.

- Immunotoxicology research has involved contracts supporting investigations into the immunotoxicity of gallium arsenide, p-nitrotoluene, m-nitrotoluene, and t-butylhydroxyquinone as well as the hypersensitivity of xylenesulfonic acid and cobaltous sulfate. In-house research has focused on model systems to evaluate the potential immunological effects of occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, or biological materials. To sequentially examine the cellular and molecular events associated with chemical-induced immunotoxicity B-cell maturation has been used. Other models assess neutrophil and alveolar macrophage function maturation potential and immunotoxicity associated with the tumorigenic process in mice that have been neonatally exposed to diethylnitrosamine.

- The primary activity in the NIEHS on-site inhalation toxicology facility during FY 1987 has involved the evaluation of the toxicity of inhaled arsine and gallium arsenide. These studies are part of a series of DTRT studies evaluating chemicals found in the semi-conductor industry. The data from these studies will be used by NIOSH to set standards for those chemicals.

- Work in metals toxicology has focused on in-house research into the similarities and differences between cadmium-binding proteins from marine molluscus and mammalian metallothionein. The role of metallothionein and lead-binding proteins in the regulation of the biological activity of lead has also been studied. In addition, this group was involved in the design and conduct of the inhalation studies of arsine and gallium arsenide.

- Developmental and reproductive toxicology contract research focused on the evaluation of several test systems to screen for teratogenesis. Studies of two of those systems (the mouse ovarian tumor cell attachment assay and the human embryonic palatal mesenchyme growth inhibition assay) were completed. A third, the use of Drosophila, is being continued. A teratology study was conducted on 1,1,1-trichloroethane to evaluate the repeatability of an observation published from another laboratroy. At higher concentrations of the test agent, the cardiovascular malformations reported in the literature were not found. Finally, through an interagency agreement with NCTR, the ovaries of female mice used in continuous breeding studies are being investigated in order to refine the methods of ovarian examinination, as well as, the interpretation of those findings. In-house research has included studies evaluating the predictiveness of changes in androgen binding protein for changes that occur in fertility, sperm count, or hormone levels. Other studies have been looking at the use of the rabbit as a model for comparison to humans, the characterization of the effects of DEHP and cimetidine on lactation and postnatal development, and the effect of neonatal exposure to DEHP on testicular function. Reproductive and developmental studies were also conducted on various metals used in the semi-conductor industry including arsine and gallium arsenide.

- The evaluation of the in vitro short-term genetic toxicity assays is continuing with two additional phases. The results of the evaluation indicate that the accuracy of the four tests is comparable in relation to

the rodent carcinogenicity results. This means that the battery of four assays is not required; a single assay (Salmonella) is adequate. In addition, these studies indicate that there may be a high probability of tumorigenicity for chemicals that are mutagenic, but that the absence of mutagenicity in vitro does not predict noncarcinogenicity.

- Efforts have been directed toward the development of assays that can reproducibly and efficiently measure chemically-induced effects of hazardous chemicals. Neoplastic transformation of mammalian cells in culture has often been proposed, and is currently being investigated, as a method for complementing the Salmonella mutagenesis assay. In addition, a new research project has been initiated looking at the modification of complex cellular interactions through the manipulation of the expression of cloned genes. This approach offers new possibilities for selecting the phenotypic substrate upon which genotoxic/carcinogenic events can be measured.

- Through the use of a system for mapping DNA damage in the centromere of yeast, it was found that the centromere is a hotspot for ultraviolet light-induced damage and that damage is repaired efficiently. A plasmid system has been developed for enhancing and genetically determining the number of functional centromere plasmids per cell in order to increase the ability to detect damage. It was found that a cell can tolerate at least a 50% increase in centromeres. The implications of this finding for the cell and for the isolation of mutagens is being investigated.

- During this fiscal year, pathology data from 59 studies submitted to the NTP by contract laboratories will be reviewed in the preparation of the NTP Technical Reports. In addition, histopathology quality assessments will be completed on three studies and Pathology Working Group reviews will be conducted on 52 studies.

- The Laboratory Animal Management Section is responsible for providing genetically defined rodents for the NTP. Both rodent production facilities and disease monitoring activities are conducted under contract. Through the use of sentinel animals to assess the health status of animals on study, it was found that more that 90% of the studies are free of viral infection.

- Fifty-seven prechronic studies were reviewed for quality of histotechnique, tissue counts, slide inventory, accuracy and completeness of diagnoses.

- Quality Assurance inspections were expanded to include support laboratories (chemistry, pathology, chemical disposition, developmental biology, and other) where work is performed under the spirit of good laboratory practice regulations. In addition to inspections at 10 Master Agreement laboratories, inspections will be conducted at about 11 support laboratories. Audits of studies in-progress will also be conducted during the site visits to Master Agreement Laboratories on 9 repeated-dose, 14 subchronic, and 10 chronic studies.

- Retrospective quality assurance audits are performed on the specimens and records from two-year studies sent to the NTP Archives. Pathology specimens (residual wet tissues, paraffin blocks, and microslides) will be audited for 42 studies, before assessment of the quality of the pathologic diagnoses begins. Records for the 2-year studies of 62 chemicals (usually 2 species per chemical), including about 27 studies conducted at Gulf South Research

Institute, and on about 42 draft NTP Technical Reports will be audited in support of the preparation of NTP Technical Reports for peer review.

CARCINOGENESIS AND TOXICOLOGIC EVALUATION BRANCH
Summary Statement

The Carcinogenesis and Toxicologic Evaluation Branch (CTEB) of the NIEHS conducts research 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 biological and biochemical research in chemical toxicity.

The major effort of the CTEB staff during FY 1986 was the design, conduct, monitoring, evaluation, and reporting of toxicity studies completed off-site at contract laboratories approved to conduct NTP studies. These studies encompassed both prechronic (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 designed to the properties of the test chemical, the needs of the requesting agency, and the usual route of exposure to the population at potential risk.

The Program Resources Group provides essential analytical chemistry and health and safety services for the toxicology and carcinogenesis studies conducted by the National Toxicology Program. Group staff procure, analyze and monitor chemicals for purity, stability, and correct dose formulation. The Chemical Health and Safety office monitors all laboratories and each study within a facility for conditions which may adversely affect the proper research and study environment. Both resources are accomplished by in-house programs and are supplemented by off-site resource contracts.

The Program Resources Group maintains a repository for over 1700 chemicals which are currently under study or which have completed studies in the various NTP programs. The Collaborative Services Group monitors Master Agreement Awards and is responsible for maintenance of the Master Agreement to enable laboratories to compete for Master Agreement Orders.

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 38 chemicals for the general in vivo chronic toxicology studies. In addition, 58 chemicals were obtained and analyzed for other programs within the National Toxicology Program such as teratology, immunotoxicology, reproductive toxicology studies, continuous breeding experiments, rat liver tumor model studies and in-house DTRT 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 five 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 74 chemicals for purity and identity. The Chemistry Group provided staff support for data auditing activities of the DTRT. This included review of completed studies to assure the chemistry performed in support of these studies was adequate and accurately reported.

- We plan to begin a two-year study to address questions regarding cellular and molecular processes responsible for the induction of lung and liver tumors in mice by a model chlorinated hydrocarbon, methylene chloride. The primary focus is to identify, characterize, and compare the oncogenes expressed in tumors isolated in control versus chemically treated mice. This research is aimed at resolving whether the chlorinated hydrocarbon introduces new mutations into DNA, increases the expression of indigenous molecular lesions, or both. (Dr. Kari)

- A project is being conducted to develop and use a system for the prolonged incubation or culture of isolated renal tubules from a wide variety of mammalian species, including humans, for comparative studies of nephrotoxicity. Culture conditions are being developed which will mimic in vivo conditions as close as possible while maintaining the ease of in vitro conditons. Once developed, this system will be used to study early events (and their progression) in chemically induced nephrotoxicity at the molecular, subcellular and cellular levels. (Dr. Rauckman)

- Processing and manufacturing of nickel have been associated with an increased risk of respiratory tract cancer in humans, but the causative agent and risk to individual nickel compounds has not been identified. The National Toxicology Program has begun a study on the comparative toxicity and carcinogenicity of commonly occurring nickel compounds in the work place including nickel oxide, nickel sulfate, and nickel subsulfide. These studies are designed to relate carcinogenic results to alterations in the immune system and tissue distribution of nickel, and to compare the relative toxicities of water soluble and insoluble metal compounds. These studies are also designed as a model for comparing carcinogenicity results from NTP animal model systems (F344/N rats and B6C3F1 mice) with results in humans. (Dr. Dunnick).

Intramural Research Highlights: Intramural research is conducted at the NIEHS by CTEB staff and collaborating scientists.

- 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 starch, gelatin or gelatin/sorbitol matrix

and determined to be stable when mixed with rodent feed. Relative bioequivalence in rats 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, have been successfully completed. Current studies include the demonstration of bioequivalence of microencapsulated 1,1,1-trichloroethane, 2-ethylhexanol and citral. (Dr. Jameson and Dr. Melnick)

- Mechanism of phthalate ester toxicity: Di(2-ethylhexyl)phthalate (DEHP) induces hepatic peroxisome proliferation in rats and mice. Increases in steady state levels of hydrogen peroxide in liver homogenates of rats treated with peroxisome proliferators correlated well with the carcinogenic potential of these chemicals. Peroxisome proliferators induced lipid peroxidation in cultured hepatocytes, indicating that oxidative stress may be associated with peroxisome proliferation. (Dr. Melnick)

- An in vivo transplant model of Fischer rat leukemia was developed and is being maintained by serial cell transfer, with a reduction in time to expression of the tumor from 18 months to 2 months. Tumor marker enzymes in enriched mononuclear cells obtained from peripheral blood samples are utilized to detect disease progression. The validity of the short-term model for predicting the chronic potency of chemical leukemogens was demonstrated with pyridine and 2-ethoxy ethanol, chemicals that were associated with positive and negative trends for leukemia in 2-year studies. Currently, the F344 rat in vivo leukemia transplant model is being used to test two additional chemicals that gave evidence of positive and negative leukemogenicity in 2-year chronic tests, 2,4,6-trichlorophenol and hexylresorcinol. Additional studies are continuing on the development of stable in vitro cell lines and monoclonal antibodies from this transplantable tumor. The proto-oncogene, c-fms, which expresses a product similar to csf-1 (granulocyte-macrophage colony stimulating factor), has been detected in this partially characterized cell line. (Dr. Dieter and Dr. French)

- Mechanism of male infertility induced by 1,2-dibromo-3-chloropropane (DBCP): The nematocide, DBCP, produces a rapid decrease in male fertility in laboratory animals after acute exposure. Based on results from recent studies, it was suggested that male infertility may be due to an inhibitory effect of DBCP at the NADH dehydrogenase step in the electron transport chain of sperm mitochondria. Further studies are planned to evaluate tissue and species specificity of this effect and chemical structure-activity relationships. (Dr. Melnick).

- Health Effects Study on Groundwater Contaminants. Developmental chemistry work on formulating a 25-chemical mixture in deionized water has been in progress since July 1986 at the Midwest Research Institute (MRI). The work is near completion and a final report is expected to be submitted to us in June 1987. The animal toxicity studies (a pilot study and a 3-month subchronic toxicity study with a 3-month recovery period in rats and mice) on the mixture is at the Best and Final stage of negotiation with a contractor. The contract is anticipated to be awarded at the end of June 1987. Following the initial chemistry work, the animal studies will start early November 1987. The animal experimentation will be completed late August 1988 and the final report will be available at the end of 1988. (Dr. Yang)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21050-04 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Evaluation of Microencapsulation As 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, Program Resources Group	DTRT/CTEB	NIEHS
Others:	T. J. Goehl	Chemist	DTRT/CTEB	NIEHS
	R. L. Melnick	Head, Experimental Toxicology Unit	DTRT/CTEB	NIEHS
	B. J. Collins	Chemist	DTRT/CTEB	NIEHS
	T. Gorski	Visiting Fellow	DTRT/CTEB	NIEHS
	A. Greenwell	Technician	DTRT/CTEB	NIEHS
	F. Harrington	Technician	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Program 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 starch, gelatin or gelatin/sorbitol matrix and determined to be stable when mixed with rodent feed. Relative bioequivalence in rats 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, have been successfully completed. Current studies include the demonstration of bioequivalence of microencapsulated 1,1,1-trichloroethane, 2-ethylhexanol, and citral.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21067-03 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT/CTEB	NIEHS
Others:	B.A. Schwetz	Chief, STB	DTRT/STB	NIEHS
	E.E. McConnell	Director, DTRT	DTRT	NIEHS
	M.D. Shelby	Head, Mammalian Mutagenesis	DTRT/CGTB	NIEHS

COOPERATING UNITS (if any)

Pulmonary Physiology Testing Laboratory, EPA

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 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.)

Following the release of methyl isocyanate (MIC) from the Union Carbide plant in Bhopal, India, and the subsequent deaths of between 2 and 5,000 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 database on this chemical. Animal studies were initiated in March of 1985 with exposure to MIC vapors accomplished in the Building 14 inhalation facility by personnel employed by Northrop, under contract N01-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, 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 21076-04 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT/CTEB	NIEHS
Others:	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
	M.I. Luster	Microbiologist	DTRT/STB	NIEHS
	G.A. Boorman	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 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.)*

The effect of inorganic or organic metals and metal complexes is of particular interest because of their prevalence in drinking water and industrial processes, use as constitutents in anticancer drugs, and their diverse target organ toxicities. Toxicological studies of various, selected metallic salts are being conducted to support and extend the results obtained at contract test facilities. Blood and target organ levels are measured to determine the disposition and steady state concentrations of the metal residues. Cellular biochemical responses provided sensitive indices of target organ toxicity that often preceeded clinical signs or microscopic evidence of pathology. Examples included mercury inhibition of glycolytic enzymes in thymic T-cells in association with immunotoxicity, and nickel inhibition of hexose monophosphate shunt enzymes in granulocyte/macrophage stem cells from bone marrow in association with myelotoxicity. Further studies are underway with titanocene dichloride, sodium chromate, zinc potassium chromate, chromium carbonyl, and ferric ammonium ferrocyanide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21078-04 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT/CTEB	NIEHS
	Thomas Goehl	Chemist	DTRT/CTEB	NIEHS
	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
Others:	Brad Collins	Chemist	DTRT/CTEB	NIEHS
	Frank Harrington	Bio. Lab. Tech	DTRT/CTEB	NIEHS
	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Tad Gorski	Visiting Fellow	DTRT/CTEB	NIEHS

COOPERATING UNITS (if any)

Midwest Research Institute, Kansas City, MO

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit; Program Resources Group

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 National Toxicology Program is exploring the feasibility of adopting microencapsulation as an alternative means of incorporating volatile, reactive, and/or unpalatable chemicals into animal feed for toxicologic studies. Oral (dosed-feed) toxicology studies are performed on those chemicals which are demonstrated to be adequately stabilized in the microcapsules, but released into the gastrointestinal tract to allow biological availability. The absorption of trichloroethylene (TCE), stabilized in gelatin-sorbitol microcapsules, was equivalent to that of neat TCE dissolved in corn oil when administered by gavage to Fischer 344 rats. In a 14-day repeated-dose study in F344 rats, similar toxic effects were produced by microencapsulated TCE given in feed as TCE dissolved in corn oil administered by gavage. These studies indicate that microencapsulation can provide an excellent alternative method for studying the oral toxicological properties of volatile chemicals in laboratory animals. 2-Ethylhexanol (2-EH) was stabilized in a starch matrix-type microcapsule. Bioequivalence studies have demonstrated that 2-EH is similarly absorbed after gavage administration of corn oil suspensions of microencapsulated 2-EH or neat 2-EH dissolved in corn oil. Future work will involve bioequivalence studies and toxicologic characterization of other microencapsulated chemicals.

GPO 914-916

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21079-04 CTEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	DTRT/CTEB	NIEHS
Others:	Konrad Tomaszewski	Visiting Fellow	DTRT/CTEB	NIEHS
	Walter Jenkins	Biologist	DTRT/CTEB	NIEHS
	Stephanie Heindel	SIS	DTRT/CTEB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogenesis and Toxicologic 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 study conducted by the National Toxicology Program, the industrial plasticizer, di(2-ethylhexyl)phthalate (DEHP), was found to be hepatocarcinogenic in $B6C3F_1$ mice and F344 rats. Since DEHP induces peroxisome proliferation but is not itself a mutagen, it has been suggested that carcinogenicity of this chemical may be due excessive peroxisomal production of H_2O_2. The effects of DEHP on hepatic peroxisomes in rats and mice have been investigated. Peroxisomal acyl CoA oxidase, the first enzyme in the β-oxidation sequence, was established as the most suitable marker for hepatic peroxisome proliferation. Maximal peroxisomal induction by DEHP occurred at a dose of 2 g/kg/day, and the no-observable effect dose was 0.6 g/kg/day. Kinetic data on the rates of formation of H_2O_2 during peroxisomal oxidation of palmitoyl CoA, and of degradation of H_2O_2 by catalase were used to estimate in vitro steady state H_2O_2 concentrations during peroxisomal β-oxidation. Increases in steady state $[H_2O_2]$ in liver homogenates of rats treated with DEHP and other peroxisome proliferators (nafenopin and di(2-ethylhexyl) phthalate) correlated well with the carcinogenic potential of these chemicals. These findings are consistent with an involvement of peroxisome proliferation in hepatocarcinogenesis. Peroxisomal enzymes activities were also found to increase in primary hepatocyte cultures incubated with mono(2-ethylhexyl) phthalate (the primary metabolite of DEHP), nafenopin, and clofibric acid. Furthermore, there was an increase in conjugated dienes, an indicator of lipid peroxidation, in treated hepatocytes. Thus, oxidative stress was associated with peroxisome proliferation in rodent hepatocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21095-01 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Development of In Vitro Propagated F344/N Mononuclear Cell Link

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

PI:	John E. French	Physiologist	DTRT/CTEB	NIEHS
Others:	M.P. Dieter	Physiologist	DTRT/CTEB	NIEHS
	S. Gangjee	Visiting Scientist	DTRT/CTEB	NIEHS
	S.A. Stefansky	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS (if any)

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The high background incidence of mononuclear cell leukemia (MNCL) in Fischer 344 rats (20 to 30%) confounds the evaluation and interpretation of possible chemical treatment related incidence of MNCL in two-year chronic toxicology and carcinogenesis studies. A F344 rat leukemia transplant model has been developed to characterize the biology of this rodent leukemia and to study the tumor biology and determine how chemical treatment effects disease expression. The development and use of in vitro propagated F344/N mononuclear leukemic cells will enhance: (1) development of monoclonal antibodies unique to MNCL for diagnostic purposes and staging of the disease, (2) the use of currently available rat cell surface antigen and receptor data to known cytochemical, morphological and cell biochemistry data and the determination of leukemic cell origin and functional lineage, and (3) the use of in vitro tests to determine the toxicity and carcinogenicity of chemicals under study in the in vivo MNCL transplant model.

In vitro propagated mononuclear leukemic cells will be developed from leukemic cells arising in the spleen from in vivo transplanted tumors derived (and periodically rederived) from F344/N rat VM-12 (isolated at necropsy at the end of a two-year study) that maintain spontaneous MNCL characteristics. Conventional tissue culture techniques based on the known growth and cell culture requirements of hematopoietic and leukemic cells are being used.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21096-01 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Identification and Isolation of c-fms Protooncogene From F344/N Rat Leukemia

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

PI:	John E. French	Physiologist	DTRT/CTEB	NIEHS
Others:	C. Walker		DIR/LPP	NIEHS
	S. Gangjee	Visiting Scientist	DTRT/CTEB	NIEHS
	S.A. Stefansky	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS (if any)

Laboratory of Pulmonary Pathobiology, DIR, NIEHS
Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

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

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

The proto-oncogene, c-fms, has been described in both mature cat macrophages and undifferentiated mouse and human tumors, and normal human bone marrow cells. The c-fms proto-oncogene is expressed during human monocyte differentiation and the c-fms product has been related to the receptor for mononuclear phagocyte growth factor (CSF-1).

The purpose of this investigation is: (1) to screen for the presence of the c-fms proto-oncogene in the in vivo serially transplanted F344/N mononuclear leukemic cell lines and, (2) to initiate studies on the isolation and expression of the gene product. This will provide insight on the origin of the tumor and aid in providing diagnostic criteria for the tumor. In addition, this tumor line may provide a valuable source of genetic material for investigations into the molecular biology of the c-fms protooncogene and its potential effect on MNCL expression. Preliminary investigation and tentative conclusions indicate the possible presence of c-fms oncogene isolated from in vivo transplanted MNCL tumor lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21097-01 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Evaluation of Chemical Myelotoxicity Using an In Vivo Leukemia Transplant Model

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

PI:	Michael P. Dieter	Physiologist	DTRT/CTEB	NIEHS
Others:	J.E. French	Physiologist	DTRT/CTEB	NIEHS
	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
	S.A. Stefanski	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 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.)

Up to 30% of the Fischer 344/N strain of rats used in the NTP 2-year toxicity and carcinogenicity studies develop spontaneous leukemia commencing at about 18 months, which reduces the sensitivity for the detection of chemical leukemogenesis. An in vivo cell transplant model was developed to transfer leukemic spleen cells from a rat with spontaneous leukemia into young, healthy recipient rats. After expression of the disease leukemic spleen cells from those rats were again used for tranplantation to maintain the leukemic cell line in vivo. The morphological and biochemical responses in organs and cells from the transplanted rats were characterized to better discriminate between age-induced and chemically-enhanced leukemia. The tumor morphology in the spontaneous and transplanted cases was identical, but the time to expression of the tumor was reduced from 18 months to 2 months. Changes in the activities of malate dehydrogenase, glucose-6-phosphate dehydrogenase, and acetylcholinesterase from the leukemic blood and spleen mononuclear cells provided consistent and unequivocal biochemical evidence of leukemia prior to other common clinical signs of the disease. These tumor marker enzymes demonstrated progressive changes in the course of leukemia that were directly associated with the severity of the disease. The validity of the model for predicting the long-term leukemogenic potency of chemicals was demonstrated by conducting short-term studies with 2-ethoxyethanol and pyridine, chemicals that respectively decreased and increased the incidence of leukemia in previous 2-year carcinogenicity tests. Similar studies are underway with additional positive and negative leukemogenic chemicals to confirm that the leukemia transplant model could detect both the presence and absence of carcinogenic activity in chemicals with structural dissimilarities, and to provide a valid data base for future investigations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21102-01 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Dermal Absorption of Diethanolamine and Triethanolamine in Rats and Mice

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

PI:	Ronald L. Melnick	Chemist	DTRT/CTEB	NIEHS
Others:	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Frank Harrington	Biologist Lab. Tech	DTRT/CTEB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.5	.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.)

Diethanolamine (DEA) and triethanolamine (TEA) are widely used in cosmetic products such as creams, skin cleaners, and shampoos. In a 14-day repeated-dose study conducted by the National Toxicology Program, TEA was found to be more toxic to the skin of rats than mice after dermal application. Dermal absorption studies of TEA in F344 rats and B6C3F$_1$ mice were initiated to help explain species differences in sensitivity to this chemical. The interscapular area of male rats and mice were clipped, and screened rings were mounted over the intended site of chemical application. ^{14}C-TEA dissolved in acetone was applied within the tissue caps to rats and mice. Blood samples were taken at eight time points over a 48 hour period after dosing, oxidized to CO_2, and assayed for ^{14}C by liquid scintillation counting. Radioactivity in urine, feces, tissue caps and skin sections from the site of application were also counted. TEA was absorbed after dermal application in rats and mice; however, the rate of absorption was greater in mice and the level of chemical retained at the site of application was greater in rats. Similar comparative dermal absorption studies of DEA in rats and mice are planned.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 30100-08 CTEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT/CTEB	NIEHS
Others:	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Frank Harrington	Bio. Lab. Tech.	DTRT/CTEB	NIEHS
	Konrad Tomaszewski	Visiting Fellow	DTRT/CTEB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Carcinogenesis and Toxicologic Evaluation Branch

SECTION

Experimental Toxicology Unit

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER.
0.75	0.25	0.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

1,2-Dibromo-3-chloropropane (DBCP) produces a rapid decrease in male fertility in laboratory animals after acute exposure. Studies were performed to determine the biochemical basis for this effect. Treatment of rats with DBCP for seven days caused a decrease in the metabolism of glucose to CO_2 in isoplated sperm, with a concomitant increase in lactate accumulation. These results indicate that DBCP may inhibit sperm carbohydrate metabolism at a site post-glycolysis. DBCP similarly inhibited the metabolism of a variety of tricarboxylic acid cycle intermediates to CO_2 in epididymal sperm isolated from Fischer 344 rats. DBCP did not inhibit the activities of NAD-dependent dehydrogenase enzymes in glycolysis or the tricarboxylic acid cycle; however, it did inhibit the rates of oxygen consumption from endogenous substrates and NAD-linked tricarboxylic intermediates. Oxidation of succinate, an FAD-linked substrate, was insensitive to DBCP. It is concluded that DBCP inhibits sperm carbohydrate metabolism at the NADH dehydrogenase step in the mitochondrial electron transport chain. Inhibition of carabohydrate metabolism by DBCP was attributed to the parent compound, since all effects were immediate and linear with time. The rapid infertility caused by exposure to DBCP may be a result of cellular ATP levels being less than that which is required for motility or penetration of the ovum by the sperm.

CELLULAR AND GENETIC TOXICOLOGY BRANCH
Summary Statement

The Cellular and Genetic Toxicology Branch conducts studies in support of the mission of the Division of Toxicology Research and Testing. These studies are generally related to the development, evaluation, and use of in vitro and in vivo short-term tests, to assess the genetic toxicity of chemicals. The Branch also conducts independent research on fundamental aspects of genetics and mutagenesis. These activities are diverse in scope but address important questions related to understanding genetic processes and consequences of genetic injury. Some significant progress has been made in the past year in both areas and is highlighted in the following summaries.

An objective evaluation of the performance of four in vitro short-term genetic toxicity assays in predicting rodent tumorigenicity was begun in 1984 and was completed in the past year. The project was originated to generate test results in the four assays for 73 chemicals that had undergone chronic toxicity/carcinogenicity assays in two rodent species. This design accommodated the two major deficiencies recognized in efforts by others to evaluate these relationships; i.e., the absence of a complete matrix of results across all chemicals and test systems; and second, results for both carcinogens and non-carcinogens. We also included other important design characteristics to insure objectivity including test protocol standardization and coded testing. In collaboration with staff of the Division of Biometry and Risk Assessment, the data were subjected to thorough statistical analysis and the results of the evaluation were published (Science 236: 933, 1987). In addition, the individual test results have either been published or submitted in peer reviewed publications.

Two additional phases of the evaluation are in progress; one is the evaluation of in vivo short-term assays (rodent bone marrow cytogenetics) with the same group of 73 chemicals, and the second is the development of another data set on a different group of chemicals in an attempt to verify the conclusions. The results of the evaluation indicate that, while the four tests showed comparable accuracy in relation to the rodent carcinogenicity results, the test results were not complementary; i.e., no single test or combination of tests improved on the sensitivity of the Salmonella assay without concomitantly decreasing the specificity of the system. The results of this study indicate that there is a high probability of tumorigenicity for chemicals that are mutagenic, but that the absence of mutagenicity in vitro is not predictive for noncarcinogens.

These results and conclusions therefore have mandated further studies to search for methods that can complement selected in vitro tests and that can be used to discriminate between non-mutagenic carcinogens and noncarcinogens. In addition to the four systems discussed above, we have also evaluated two other short-term test systems against a subset of the 73 chemicals. The sex-linked recessive lethal mutation assay in Drosophila was used to test 34 chemicals, and the in vitro unscheduled DNA synthesis assay in rat hepatocytes was used in the evaluation of 38 chemicals. The performance of both of these assays was similar in that they demonstrated a relatively high specificity but a low sensitivity for detecting carcinogens. They therefore might serve a confirmatory but not a complementary role to the Salmonella mutagenesis assay.

Neoplastic transformation of mammalian cells in culture has often been proposed as a method for complementing the Salmonella mutagenesis assay since there is substantial evidence that transformation can be induced by non-mutagens. We are continuing to evaluate the development of three cell systems (human, hamster and mouse) with particular emphasis on reproducibility and ability to discriminate between non-mutagenic carcinogens and non-carcinogens. In addition we have recently initiated a research contract to develop cellular substrates carrying selected proto-oncogenes in conjuction with retroviral regulatory sequences. Cells possessing these proviral constructs, introduced by a retroviral vector in an expressible state, will be used to attempt to identify chemicals that can specifically induce proto-oncogene expression and cause transformation.

In addition several issues relating to aneuploidy and mechanisms of chromosome segregation have been addressed using the yeast Saccharomyces cerevisiae. A highly sensitive system based on chromosome loss in mitotically growing cells has been developed and validated in two laboratories. Another system for detecting chromosome gain in mitotic and meiotic cells has also been developed. Predominant among the many chemicals that are strong inducers of aneuploidy are aprotic polar solvents such as acetone and acetonitrile. Some of the solvents are also known to be neurotoxins. Based on measured effects on tubulin aggregation in vitro, the solvents appear to act at the level of microtubules.

The Branch has also contributed substantially to characterization of the toxicity of chemicals being evaluated by the NTP on a priority basis. Most notable among these efforts were extensive in vitro and in vivo studies of methyl isocyanate and on spy dust and its metabolites. Several important studies have been conducted by laboratories under contract to the Branch including: a) identification of acrylamide as a mutagen in the mouse specific locus germ cell mutagenicity assay, and demonstration that it binds to spermatid proteins but not to DNA; b) detecting low but statistically significant mutagens in urine of "passive smokers;" and c) identification of a mouse model for human genetic disease - carbonic anhydrase deficiency.

The Branch intramural research efforts focus on specific genetic functions that are fundamental to cellular growth, differentiation or division, and that may be specific targets for chemical effects. Some highlights of recent progress are summarized. Using a system that has been developed for mapping DNA damge in the centromere of yeast, it was found that the centromere is a hotspot for ultraviolet light-induced damage and that the damage is repaired efficiently. The distribution of damage was found to be influenced by centromere structure. To increase the ability to detect damage, a plasmid system has been developed for enhancing and genetically determining the number of functional centromere plasmids per cell. As part of this project it was found that a cell can tolerate at least a 50% increase in centromeres. The consequences of this to the cell and the isolation of mutants that can effect the level of increase are being pursued. The cloned spo11 gene has been shown to be expressed only in meiotic cells and to be essential for meiosis but nonessential for somatic cells of yeast. The spo11 gene product has been expressed by recombinant DNA methods in E. coli to facilitate its biochemical characterization. New methods of cytology have demonstrated for the first time that meiotic chromosome behavior in yeast is remarkably similar to that in mammals; thus yeast is a good model system. Methods to allow protein localization to yeast chromosomes by immunocytology have been developed, allowing the connection to be made between structure and function in the meiotic nucleus.

We have made progress in two major areas in our analysis of the Fv-1 gene model for cellular regulation of retrotransposition. First, we have established and characterized several cell lines which have been transfected with an Fv-1 N tropic "packaging mutant" provirus. Retrovirus packaging cell lines with these properties are crucial to our analysis of the mechanism of Fv-1 restriction of retrovirus DNA synthesis and integration. The second area in which we have made progress involves oligonucleotide probes specific for the Fv-1 N- and B-tropic target determinant. Results indicate that endogenous MuLV related proviral elements belonging to the polytropic/MCF class contain the B-tropic determinant. Thus under the selective pressure of the Fv-1 B allele, B-tropic MuLV most likely arose by recombination between the endogenous N-tropic MuLV and sequences from this class of endogenous provirus.

In Drosophila the ends of chromosomal terminal deficiencies that had been recovered earlier have been characterized molecularly. These chromosome ends are not completely stable; they lose DNA sequences at a rate of about 75 base pairs per generation. Four stable lines have been identified. The telomeres of both the stable and unstable lines are being cloned to identify DNA sequences that are necessary for chromosome stability.

Two regions of the X chromosome, one containing the mei-41 gene and one containing mei-9 have been cloned. Work is starting on the molecular characterization of the gene. A nuclease gene on chromosome 2 genetically controlled by mei-41 has been identified and cloned based on similarities with a nuclease gene from yeast.

Progress has continued in two areas of carcinogen metabolism which are relevant to the long-term goals of CGTB. First, the metabolic capability of human cells in culture, which are used for genetic toxicity screening, has been improved by the transfection of a gene coding for a human P-450 isozyme. The human cells containing the P-450 gene are more sensitive to the mutagenic effects of certain environmental chemicals than is the parent cell system. Cytofluorographic methods of detecting and isolating cells with enhanced metabolic capability have also been developed. Acquiring replicating cells with metabolic capability will allow long-term, low-dose experiments to be conducted so that genotoxic effects can be measured with minimal cytotoxicity. The second accomplishment was that of comparing human liver metabolism to rodent liver metabolism of the model carcinogens, 2-acetylaminofluorene and benzo(a)pyrene. Although individual humans showed variation in the metabolism of these two carcinogens; they were (8 of 9 cases) more active than rat hepatocytes in metabolizing acetylamino-fluorene. For benzo(a)pyrene, rat hepatocytes showed about the same level of metabolism as the average human hepatocyte level. NTP rodent liver carcinogens are now being studied with the goal that such metabolism data may aid rodent-to-human extrapolation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21012-06 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

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.)*

The ability of human and rodent tissues to metabolize known or suspected chemical carcinogens is being investigated. The metabolic profile and genetic toxicity of the chemicals with human tissue activation are then being compared to the results from rodent tissues to aid the extrapolation of effects in rodents to potential effects in humans. Human and rodent liver and kidney cell metabolism of the model carcinogenic aromatic amine, acetylaminofluorene, has been completed. For human liver, nine individual tissue specimens have been investigated and a complex pattern of hydroxylated products observed with all cases. The same hydroxylated products were also observed with rat hepatocytes; however eight of the nine human samples were more active metabolizers than the rat hepatocytes. The interindividual variation in the overall human metabolism was about 4-fold; although variation in individual metabolites was as high as 35-fold. The ability of human hepatocytes to conjugate these hydroxylated products with sulfate or glucuronic acid was also greater than in rat hepatocytes and the human interindividual variation to conjugate was about 8-fold. Human hepatocytes were also more active than rat hepatocytes in producing metabolites which were mutagenic to Salmonella typhimurium. Studies with kidney tissues have also indicated that human cells are more active than rat kidney cells in producing hydroxylated and mutagenic acetylaminofluorene metabolites; but kidney cells from both species are less active than hepatocytes. Again, about a 4-fold interindividual variation in human kidney metabolism was observed. The results of these studies are now being extended to other NTP chemicals to determine if these data when used in combination with pharmacodynamic and genetic toxicity data can aid in rodent to human extrapolation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21013-06-CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel 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	Biologist	CGTB	NIEHS
	C. L. Innes	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Wen K. Yang, Biology Division, Oak Ridge National Laboratory

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 continued our development of a model for regulation of retrotransposition in mammalian cells based on the Fv-1 gene restriction system. Specifically, we have engineered a cell line which packages retrovirus vectors in an Fv-1 N tropic virion. The Fv-1 target molecule present in the virion results in an inhibition of certain preintegration steps during viral DNA synthesis in cultures of mouse cells with the $Fv\text{-}1^{bb}$ allele. The model system involves retrovirus vectors which are unable to replicate, thus providing a measure of a single round of successful reverse transcription and integration. This system will allow an analysis of the mechanism of the Fv-1 gene restriction previously unavailable. Biological activity of the Fv-1 packaging line has been evaluated by release of replication defective transforming virus or vectors containing a dominant selectable marker. Results indicate that the packaging construct has a propensity to regenerate infectious virus, most likely by recombination with the vector or endogenous retrovirus elements in the host mouse cell. New strategies are being developed to create packaging lines which are stable and unlikely to recover the ability to replicate autonomously. Fluorescent antibody assays and RNA blot hybridization analysis have demonstrated a low efficiency of viral gene expression in various packaging lines compared to virus infected cells. Attempts are being made to improve this by utilizing high efficiency transcription promoters. Recently we have employed oligonucleotide probes specific for the Fv-1 target determinant which allows a determination of Fv-1 tropism of endogenous retroviral elements. Preliminary results indicate that many endogenous nonecotropic proviral genomes contain the B-tropism specific sequence and are likely to be the source for generating B-tropic virus.

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 21016-06 CGTB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

PI: M. A. Resnick Supv. Research Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*
Terry Chow, National Research Council, Canada

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

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

Several DNA metabolic processes are influenced or controlled by genes whose products function in DNA repair. The RAD52 gene of Saccharomyces cerevisiae plays a central role in many of the events in recombination and repair. It controls the repair of ionizing radiation-induced DNA double-strand breaks, radiation-induced spontaneous mitotic recombination, and recombination during meiosis and is required for mutagenesis by some alkylating agents. Utilizing an antibody raised against a Neurospora crassa deoxyrobonuclease, we had shown that an antigenically-related enzyme could be identified from yeast and that this enzyme is controlled by the RAD52 gene. The protein has been purified and is a single-strand exo-endonuclease and a double-strand exonuclease with MW = 72,000. It is not the product of the RAD52 gene. Using a λgt11 vector expression ibrary 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 identifed that hybridizes with this segment. Introduction of this into a Rad+ strain leads to enhanced synthesis of cross-reacting material. The genes appear to be essential based on mutation of the cloned sequence and introduction into the genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21032-03 CGTB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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:	William 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

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. Prostagladin 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 system. Initial experiments showed hydrogen peroxide to be a good substrate for this enzyme. However, mutagenicity results are confounded by the interaction of hydrogen peroxide with sodium pyrvate, a media component in this assay. Minimal treatment conditions and optional concentrations of purified enyzme have been determined. 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 21035-03 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	C. N. Giroux	Senior Staff Fellow	CGTB	NIEHS
Others:	M. Dresser	NRC Biotechnology Associate	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, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	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 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 have been 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 cells, as visualized by both light and electron microscopy. These new methods demonstrate for the first time that meiotic chromosome behavior in yeast closely parallels that in higher eukaryotes. Chromatin condensation and decondensation proceed in step with chromosome pairing, synapsis, and desynapsis. In concert, these events produce the classical stages of leptotene, zygotene, pachytene, and diplotene; demonstrating the utility of yeast as a model system for analysis of chromosome structure and function. A combination of cytological and molecular cloning techniques has demonstrated that the SPO11 gene of yeast is required for chromosome pairing and/or synapsis during meiosis. Antibodies which recognize the synaptonemal complex in yeast and the mouse are being screened for by these new methods in order to identify protein components of the synaptonemal complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21039-03 CGTB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	R. Graetzer	IPA	CGTB	NIEHS
	A. 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, North Carolina 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.)*

The mechanism of sister chromatid recombination is poorly understood at the genetic and molecular level. Since it may be important in repair, mutagenesis and gene amplification, we have initiated studies to characterize the process and investigate the genetic control in the yeast Saccharomyces cerevisiae. Sister chromatid recombination is measured by recombination between two fragments of the HIS3 gene which share a 300 base pair region of homology. They are arranged in such a way that recombination will generate a complete gene. We have shown that several of the genes involved in DNA repair are required for the levels observed in wild type cells. Mutations in genes that enhance or depress homologous chromosome recombination have similar functions on the sister chromatid recombination process. DNA damaging agents can considerably enhance the process although the cells are resistant to x-ray induced exchange. Mutants have been isolated which can either lead to enhanced levels (esr) or depressed (dsr) levels of exchange. 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.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21045-05 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	C. N. Giroux	Senior Staff Fellow	CGTB	NIEHS
Others:	H. 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, North Carolina 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 specific genes of yeast for which mutants are available. Using this system, the SPO11 gene was physically isolated; this represents the first molecular cloning of a meiosis specific gene from any organism. The DNA sequence of the SPO11 gene has been determined and a candidate polypeptide coding sequence of 398 amino acids has been identified and confirmed by hybrid gene fusions. This sequence predicts a strongly basic amino terminal domain. An in vitro engineered gene disruption has been used to demonstrate that the SPO11 gene is essential for meiosis but is not required for vegetative growth or normal progression through the cell cycle. The cloned SPO11 gene has been shown to be expressed only in meiotic cells. Thus, mutation in a single gene is sufficient to disrupt meiotic differentiation and proper chromosome behavior, giving rise to mostly inviable or grossly aneuploid products. The SPO11 gene product is being expressed by recombinant DNA methods in E. coli to facilitate its biochemical characterization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21048-04 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI: C. N. Giroux Senior Staff Fellow CGTB NIEHS

COOPERATING UNITS *(if any)*

Bernard Kunz, Department of Microbiology, University of Manitoba, Canada

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(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. Using this system, the spontaneous mutation rate in the target SUP4-o tRNA suppressor gene has been determined to be 2.7×10^{-7} events per cell division. The distribution (or spectrum) of mutations occuring spontaneously in the target gene has been determined and demonstrates that all types of single base substitutions as well as deletions may be detected reliably in this system. 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. As a test of induced mutagenesis, we have characterized mutations induced by U.V. irradiation of yeast cells harboring the assay plasmid. U.V. induced all types of base substitutions in the SUP4-o target gene, although transitions predominated. The base pair substitutions occur at sites of adjacent pyrimidines, suggesting that they were targeted by U.V. photolesions. Hotspots for U.V. mutagenesis were detected in the target gene whereas no hotspot for spontaneous mutation has been observed.

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 21049-05 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

DNA Synthesis and Metabolism During Meiosis

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

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	A. Sugino	Visiting Scientist	LGM	NIEHS
	J. Westmoreland	Biologist	CGTB	NIEHS

COOPERATING UNITS *(If any)*

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Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 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 chromsomal DNA are detected at any time during meiosis while changes are observed in various mutants. Recombination is an important process in repair and in recombinaton. We are investigating proteins that might be involved in both processes. Previously we had shown that a RAD52 controlled nuclease increases nearly 10-fold, implicating it in meiotic recombination. We have purified a protein from cells that are undergoing meiosis that is able to carry out a strand exchange reaction. This reaction which involves the displacement of one of two strands from a duplex by another homologous single-strand DNA molecule is generally considered to be one of the basic steps in recombination that take place within cells. The protein has a MW = 38,000 and does not have ATPase activity nor is ATP required for the reaction. The appearance of the protein requires the RAD50 gene product and is meiosis specific. Strains that are homozygous for mating type (and therefore do not undergo meiosis) do not accumulate this protein during meiosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21051-04 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	James M. Mason	Geneticist	CGTB	NIEHS
Others:	Masayoshi Watada	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Duke University
University of California, Davis
National Research Council, Canada

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.3	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.)*

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. Most of our recent work has centered on the mutants for the mei-41 gene. These mutants are sensitive to a wide variety of mutagenic agents, defective in postreplication repair and meiotic recombination, female sterile, and have fragile chromosomes. The mei-41 gene is a hot spot for EMS and P-element insertion mutagenesis, and shows a high frequency of interallelic meiotic recombination, suggesting that the gene is physically large. To confirm this hypothesis and to better understand the structure and regulation of the gene, it has been cloned and is being characterized molecularly. The mei-41 gene controls the activity of a specific DNA endo-exonuclease that is antigenically related to endo-exonucleases from yeast and Neurospora. A DNA sequence that codes for the nuclease in yeast has been hybridized in situ to Drosophila salivary gland chromosomes. A single site of hybridization has been identified in region 56D on chromosome 2; mei-41, however, is located in region 14C on the X chromosome. We are currently looking for second chromosome mutants that lack the nuclease activity, and cloning the nuclease gene using the yeast sequence as a probe.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21052-05 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Metabolism of Xenobiotics to Mutagens by Non-Hepatic Enzyme Systems

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

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	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, NIEHS

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Prostaglandin H synthase (PGH) is an oxidative microsomal enzyme system separate from the cytochrome P450 system most often used for oxidative metabolism of xenobiotics. PGH derived from ram seminal vesicles has been used in the past to metabolize a number of polycyclic aromatic hydrocarbon bay-region epoxides and aromatic amines to mutagens for Salmonella typhimurium. It was recently shown that PGH can metabolize a number of aromatic amines of the carboline and aza-imidaozoarene classes that are formed during the cooking of beef to DNA-binding intermediates. Four of these chemicals, Trp-P-1, Trp-P-2, IQ, and MeIQ, which are potent mutagens in the presence of rat liver S-9, were tested with PGH. Mutagenicity was not obtained at doses comparable to those used with S-9. All four chemicals were direct-acting mutagens at doses 1000x those used with S-9. At these higher doses, PGH slightly enhanced the direct mutagenicity of Trp-P-2, had no effect on Trp-P-1, and decreased the mutagenicities of IQ and MeIQ. The direct-acting and PGH-mediated mutagenicity were highly dependent on pH and buffer composition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21053-04 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI: James M. Mason Geneticist CGTB NIEHS

COOPERATING UNITS *(if any)*

University of California, Irvine

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

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.)*

This project is designed to determine the relationship between DNA repair, chromosome structure and mutagenesis in Drosophila melanogaster. A mutation which increases the mutation frequency (a mutator) has been identified and characterized. This mutator greatly reduces the efficacy of a repair pathway for x-ray induced chromosome breaks, thereby allowing a previously undescribed repair pathway to be observed. By this newly identified repair pathway individual broken chromosome ends are "healed", allowing the recovery of terminal deletions. The distribution of chromosome breaks that can be healed has been examined at several levels. (1) When females are irradiated and crossed to males carrying a translocation between the X and Y chromosomes, long terminal deletions can be recovered, suggesting that, at a gross level, breaks can be recovered anywhere in the euchromatin, without regard to proximity to the nearest telomere. (2) The mutator increases the recovery of breaks in the heterochromatin, but the breaks are not recovered as terminal deletions. Rather, they are recovered as rearrangements similar to the rearrangements from wild-type females. Thus, while the mutator itself does not have differential effects on euchromatin and heterochromatin, the healing process may be specific to euchromatin. (3) At the DNA sequence level, the breakpoints of the terminal deletions are distributed randomly, suggesting that specific DNA sequences are not required for the healing process to occur. (4) The breakpoints associated with the terminal deletions move (i.e., DNA sequences are being lost from the deficient chromosomes), suggesting that the neotelomeres on the broken ends are not as effective as the original telomeres and that the process of replicating chromosome ends involves loss and replacement of DNA sequences.

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 21054-04 CGTB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	J. Westmoreland	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

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, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	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.)*

Chromosome segregation requires a functional spindle apparatus, microtubules, chromosomal attachment sites and a centromere specific DNA sequence. Disruptions of any of these organelles can lead to chromosomal malsegregation and aneuploidy. We are addressing two aspects of the function of centromeres within cells: the ability of cells to modify the number of centromeres, and the ability of cells to deal with damage in the centromere. We are developing a plasmid system which allows for the genetic detection of the number of centromere-containing plasmids within a cell. This is being done by including within a centromere plasmid the gene for copper resistance CUP1. Increases in plasmid number lead to increased resistance. We have observed that cells can tolerate at least 8 additional centromeres and that this does not disturb growth or the process of meiosis. This system will enable an analysis of the relationship of the spindle apparatus organization to centromere function. Because of the systems we have available for detecting aneuploidy, it will be possible to determine consequences of altered centromere number on genome stability with a high degree of detection ($<10^{-5}$). Cells containing a large number of centromere plasmids are being used to examine repair in the centromere DNA. While it was previously possible to characterize damage and repair in this chromosomal organelle, the system was not sufficiently sensitive to precisely map damage and determine repair in relation to structure. The presence of a large number of the same centromeres will allow far more quantitative approaches to these issues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21091-02 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	A. Chaudhury	Visiting Fellow	CGTB	NIEHS

COOPERATING UNITS (if any)

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Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The effects of specific DNA lesions on cellular metabolism are poorly understood. Double-strand breaks (DSBs) is one category of lesions that is of considerable concern. They can occur as primary events as a consequence of repair. In addition they may appear during normal DNA metabolic events involving recombination. DSBs are known to be inducers of recombination.

In order to understand the consequences of DSBs on cellular DNA and their role as inducers of recombination, a system has been designed in the yeast Saccharomyces cerevisiae to study the effect of defined DNA double-strand breaks in plasmids on recombination and repair at various chromosomal sites. In this system, a diploid yeast strain with a heteroallelic marker on homologous chromosomes 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, and 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, depression 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 in being investigated in terms of effects on survival, mutation, and recombination and genetic control.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21094-01 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mutagenesis by Free Radicals

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

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Avishay A. Stark, Department of Biochemistry, Tel-Aviv University, Israel
Samuel Rogers, Department of Chemistry, Montana State University, Montana

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	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 mutagenicities of sodium bisulfite and glutathione, reported earlier, appear to be mediated through the formation of oxygen-containing free radicals. The mutagenicity of bisulfite is inversely related to its rate of autooxidation in the medium, leading to the speculation that for mutagenesis to occur, unoxidized bisulfite must be present in sufficient quantities to enter the cell prior to its oxidation. Glutathione metabolism, followed by autooxidation of the resulting dipeptide, cysteinylglycine, leads to the extracellular formation of hydrogen peroxide which is a mutagen. Glutathione (and cysteinylglycine) mutagenicity is enhanced by substances or conditions that enhance the rate of oxidation and the formation of hydrogen peroxide, and is depressed by inhibitors of cysteinylglycine oxidation and hydrogen peroxide formation and accumulation. The mutagenic responses of two other sulfhydryl compounds, cysteine and penicillamine, appear to occur via a similar mechanism as cysteinylglycine, except that the oxidation reactions take place intracellularly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21101-01 CGTB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mutant Selection Due to Differential Toxicity

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

PI:	William Caspary	Biochemist	CGTB	NIEHS
Others:	D. Daston	Biologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL 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.)*

In the lymphoma forward mutation assay, there are chemicals which show a mutagenic effect only at highly toxic doses. This leads to the concern that the observed mutagenicity may be due to "selection" of the spontaneous mutants due to a greater chemical toxicity to wild-type over mutants cells.

Studies to examine this problem can involve isolating wild-type and mutant clones and determining the relative toxicity of the chemical to wild-type and mutant cells. However, this method is indirect, i.e. the clones tested may not be representative of the cellular response in the mutation assay.

We are presently developing a direct technique of measuring mutant selection due to differential toxicity, conducted in the experiment used to test the chemical for mutagenic activity. Using this technique, we have shown that certain nucleotide analogs select for as well as induce mutants. These studies are being extended to examine other compounds which only show mutagenic activity at doses which also cause high 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 21103-01 CGTB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluations of Genetic Toxicity Test Results

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

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Barry Margolin	Supervisory Mathematical Statistician	SSB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cellular and Genetic Toxicology Branch

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

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

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

A database of results from the Salmonella, chromosome aberration, and sister chromatid exchange assays has been created using chemicals tested for carcinogenicity by the NCI and the NTP. This database allows the evaluation of each short-term assay with respect to its ability to predict carcinogenesis or the other short-term assays results. It was shown that no assay can detect all carcinogens. In this database, a positive result in Salmonella is the best predictor of carcinogenicity in rodents. In fact, its predictivity is equivalent to the predictivity of rat carcinogenesis from mouse carcinogenesis, and vice versa. No battery of assays composed of the assays mentioned above was superior to Salmonella, alone, for predicting carcinogenicity. Also, none of the assays were complementary to each other. The Salmonella assay differed markedly in its responses to chemicals of different classes; it was relatively insensitive to chlorinated hydrocarbons and phthalate esters, but highly sensitive to nitro and azo compounds. These analyses will lead to an assessment of the ability of short-term genetic toxicity assays to predict rodent carcinogenicity, and to the design of strategies for the deployment of short-term tests.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60102-09 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Dr. F. Kari , CTEB, NIEHS
Dr. L.T. Burka, STB, NIEHS

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

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

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

A number of chemicals of interest have been tested for mutagenicity using Salmonella typhimurium in a number of different protocols. The carcinogen furan, a potent inducer of mutations in the ras oncogene in mice, was originally reported nonmutagenic in Salmonella. We have tested furan, and a synthesized metabolite, in Salmonella and found weak mutagenic activity for both chemicals in the absence of exogenous metabolic activation only. Modifications will be made in the test protocol in an attempt to enhance the responses, and to help determine the specific metabolite(s) responsible for furan's mutagenic activity.

HC blue 1 and 2 are both direct-acting mutagens but only HC blue 1 is a rodent carcinogen. The carcinogenic HC blue 1 is also a more potent mutagen than HC blue 2. In an attempt to determine the reason for the absence of a carcinogenic response with HC blue 2, the chemicals are being studied in bacterial strains lacking one of the nitroreductase enzymes. The mutagenicities of both chemicals require bacterial nitroreductase, and the depression of mutagenicity in nitroreductase deficient strains of Salmonella is not reversed by the addition of rat liver S-9.

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 60122-08 CGTB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	D. Trinh	Guest Researcher	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Dr. J. Nitiss, Harvard University, Cambridge, MA
Dr. J. C. Game, University of California, Berkeley, CA

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

CHECK APPROPRIATE BOX(ES)

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

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

DNA repair systems identified in mitotic cells of the yeast Saccharomyces cerevisiae are being examined for their protection of cells undergoing meiosis and the role of the corresponding genes in normal meiosis. The RAD50, RAD52 and RAD57 genes are essential in the repair of DNA double-strand breaks in mitotic cells. We have shown that they are also required for meiosis. Mutations abolish normal meiotic recombination; RAD50 acts early in meiosis. Rare single-strand interruptions (SSIs) are observed in rad52 and rad57 strains which 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, suggesting specific sites or regions involved in normal meiotic recombination. We are investigating the distribution of the breaks using complete sets of fragments of entire chromosomes. DNA is gently isolated, restricted, and displayed using alkaline agarose gel electrophoresis. The gels are probed with individual fragments to determine if the rare breaks seen with alkaline sucrose gradients can be identified within specific sequences of the chromosome. The rad52 and rad57 mutants are concluded to be defective in meiotic recombination; however 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 and rad57 are exposed to growth medium, recombinants are not recovered. This is due to the extended time necesary for recombinants to form, suggesting that rad52 and rad57 are blocked at an intermediate step. The rad57-1 temperature sensitive allele has allowed us to investigate this more extensively and determine the timing of events. 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 (CPB) 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 CPB 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 (NTP). 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.

The Tumor Pathology Section is responsible for the accuracy and validity of the pathology data derived from the two-year chronic studies. This section coordinates the histopathology quality assessment activities, organizes and conducts Pathology Working Group (PWG) reviews of the data from chronic studies, and evaluates and interprets the data for preparation of the NTP Technical Reports. During fiscal year (FY) 1987 the Tumor Pathology Section will have evaluated all aspects of the pathology data submitted to the NTP by the contract laboratories from 59 studies (Pathology Data Reviews). In addition, histopathology quality assessment will have been completed on 3 studies and PWG reviews will have been conducted on 52 studies.

Staff in the Tumor Pathology and Experimental Pathology Section (Clinical Pathology) are providing pathology support to the comprehensive toxicology studies of arsine gas. This collaborative effort involves staff from the intramural program of National Institute of Environmental Health Sciences (NIEHS) as well as the CPB. Arsine is widely used in a number of industrial processes and there is increasing use of arsine in the semiconductor industry for the production of gallium arsenide chips. A series of acute and subchronic inhalation studies in rats, mice, and hamsters that include histopathology and clinical pathology evaluations will be completed in FY 1987.

The Laboratory Animal Management Section provides genetically defined rodents of known microbial status for the NTP toxicology and carcinogenesis testing program. Two rodent production facilities are currently under contract to produce F344 rats and B6C3F1 mice. The rodent production is being modulated to provide an adequate number of animals to all new study starts and to decrease any excess production. Two diagnostic laboratories are conducting disease monitoring of NTP rodent production colonies and the animals on chemical toxicity and carcinogenicity studies. Sentinel animals in the toxicity and carcinogenicity studies are being used to assess the health status of animals on study. This program was useful in control of diseases in animals on study and currently more than 90% of the studies are free of viral infections.

Quality of the NIH-07 diet for the chemical toxicology studies is controlled by establishing limits on contaminant concentrations. These limits are being achieved by monitoring each batch of ingredient used in the

manufacture of NIH-07 diet and each lot of diet selected for the toxicology studies. Studies to optimize the diet and feeding procedures for rodents in chemical toxicology studies are in the solicitation process. Projects to improve the mouse feeder and to develop unique and permanent identification methods and devices for positive identification of rodents in toxicology studies are in progress.

The Toxicologic Pathology Section assures the quality of anatomic pathology from acute, prechronic and interim sacrifice studies in mice and rats, establishes diagnostic nomenclature for toxicologic pathology, defines organ specific toxic lesions and correlates treatment related toxic lesions in target organs with neoplastia in the same organs in chronic toxicologic studies. Cross-study comparison allows correlation of target organs with the chemical structure of the test compound. Attempts are made to identify preneoplastic lesions and study the biologic behavior of toxic induced proliferative lesions. During FY 1987, 57 prechronic studies were reviewed by the Toxicologic Pathology Section. Studies are evaluated for quality of histotechnique, tissue counts, slide inventory, accuracy and completeness of diagnoses. Review of the study includes six randomly selected animals sacrificed at the termination of the study from the highest dose groups with at least six survivors and six randomly selected controls from each sex. All slides are reviewed from all animals that are moribund sacrificed or die prior to termination of the study. All identified target organs are reviewed in all subsequent lower doses until a no-effect level is established. All tumors are reviewed. These findings are reviewed by the Section Head, and a PWG chairperson is designated. The chairperson reviews pertinent slides and identifies discrepancies in diagnosis between the study pathologist and the quality assurance (QA) pathologist. The PWG, consisting of four to six pathologists, reviews all differences of opinion in diagnoses, evaluates target organ effects, correlates clinical signs, significant abnormalities in clinical pathologic parameters, and differences in body and/or organ weights with morphologic changes, and makes dosage recommendations to the chemical manager based on pathologic data. The PWG also evaluates the quality of work performed by the study pathologist, QA pathologist and the histotechnique at the contracting laboratory. A summary report of these findings is prepared by the PWG chairperson and action items are returned to the study pathologist for reconsideration.

The collaborative research effort with Dr. Marshall Anderson has continued. In addition to obtaining tumor tissue from ongoing NTP two-year carcinogenicity studies, additional tumor tissues are being generated under contract using animal model systems. Clear differences in the patterns of oncogene activation have been documented between spontaneous and chemically induced liver tumors in the B6C3F1 mouse. One of the CPB staff members, Dr. Steven Stefanski, is currently working in Dr. Anderson's lab attempting to characterize the molecular biology of mononuclear cell leukemia in Fischer 344 rats. This work should help clarify the significance of this spontaneous neoplasm that is frequently modulated by treatment during the course of NTP two-year studies.

Research work with magnetic resonance imaging (MRI) has made significant technologic advances. It is now possible to obtain microscopic resolution of tissues in the live animal using the improved magnets in the Radiology

Department at Duke Medical Center. New MRI studies have commenced to examine the progression and repair of chemically induced toxic renal lesions.

A new collaborative research with the Division of Biometry and Risk Assessment, Carcinogenesis and Toxicology Evaluation Branch and Systemic Toxicology Branch has been undertaken to study the early cytologic events associated with exposure of female B6C3F1 mice to methylene chloride vapors. As a result, the CPB has developed expertise in autoradiography in order that cell turnover studies can be done in the mice exposed to methylene chloride. These results will be correlated with the known tumor response in the lung and liver of mice chronically exposed to methylene chloride.

Another new area of active research interest is in the field of image analysis. The CPB has just begun to explore the use of image analysis techniques to quantitate pathology, perform microdensitometry on autoradiographic and immunocytochemical slides, and to analyze small animal magnetic resonance images.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21074-03 CPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	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:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Ethylene glycol (EG) or ethylene glycol monomethyl ether (EGMME) was administered by gavage to both sexes of B6C3F1 mice for 4 consecutive days at total doses of 200, 400, and 1000 mg/kg body weight. Exposure to EG produced hypocellularity and suppression of granulocyte-macrophage progenitor colony formation in both sexes on days 1 and 5 postexposure. Values returned to normal by day 14 in the female mice but not in the males. Erythropoiesis, as measured by ^{59}Fe incorporation and quantitation of erythroid precursors in culture, revealed no effect in female mice and affected male mice at the high dose only. In contrast, EGMME exposure in female mice resulted in inhibition of erythropoiesis. There was also a pronounced effect on white blood cells with decreased peripheral counts, and decreases in the number of CFU-C's cultured from marrow cells. The effect of EGMME was seen at the lower dose levels and was sustained through the 14-day evaluation period. In addition, EGMME caused a 20% decrease in testicular weight, which was shown microscopically to be a segmented degeneration of seminiferous tubules, an effect not found with EG. This study demonstrates that EGMME is more myelotoxic in mice than EG and that pancytopenia is more pronounced in males, while erythropoiesis is more affected in females. These results were submitted in 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21080-03 CPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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:	M. B. Thompson	Veterinary Pathologist	CPB	NIEHS
Others:	R. R. Maronpot	Veterinary Pathologist	CPB	NIEHS
	G. A. Johnson	Radiologist	Dept. of Radiologist	Duke Univ. Med.Center

COOPERATING UNITS *(if any)*

Duke University Medical Center
Durham, N.C.

LAB/BRANCH

Chemical Pathology Branch

SECTION

Experimental Pathology Section

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.)*

Magnetic resonance imaging (MRI) experiments are being conducted at Duke University Medical Center to explore the application of the technique during toxicologic studies. Animals (primarily rats) are anesthetized with a gaseous anesthetic (halothane), given complete and ventilation support, extensively monitored via electronic sensors to determine physiologic status and imaged for variable lengths of time (<1 hour to >4 hours) using a 30 cm bore, 2 Tesla MRI devise. Animals are imaged repeatedly (1 to 4 times a month) during a study. Investigations currently underway include imaging animals that are being treated to develop hepatic neoplasms to determine at what stage the neoplastic process can be detected and if the progression or regression of the tumors can be monitored. Additionally, studies are being conducted to explore the ability of MRI to detect acute renal damage in the rat. During 1987, a 7 Tesla magnetic imaging system will become operational and will provide resolution approximately 10 times greater than that currently available. This will permit the use of MRI in studies using mice in addition to rats. Three scientific articles have been published thus far in FY 1987. These relate to technical developments in small animal imaging, multiple imaging of the rat brain and imaging of the live, developing, chick embryo.

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 21082-02-CPB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	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:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

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

Ethylene glycol monomethyl ether (EGMME) has been reported to cause hematopoietic abnormalities in man. We evaluated the effect of EGMME exposure in B6C3F1 mice during the perinatal period. Offspring from mice that had been exposed during pregnancy and lactation showed marked depression of hematopoiesis. This indicates that in mice EGMME can cross the placenta and/or be in the milk in sufficient amounts to cause myelotoxicity. It has also been shown that mice exposed to EGMME postnatally have suppressed bone marrow cellularity and progenitor cells 8 weeks postexposure which returned to normal values by 16 weeks. Studies were designed to determine whether EGMME exposed mice that recovered had evidence of residual marrow stem cell injury. B6C3F1 mice were subcutaneously injected with EGMME on days 1-5 after birth at doses of 0, 100, 200 and 400 mg/kg/day, allowed to recover, and stressed with 200 rads whole body irradiation at 15 and 21 weeks postexposure. Mice that had been exposed to EGMME were more sensitive to irradiation and recovery of marrow cellularity and progenitor cell numbers occurred more slowly than in unexposed controls. Mice exposed to EGMME and subsequently stressed by irradiation had significant residual marrow damage at a time when marrow cellularity and peripheral blood counts had apparently recovered. This indicates that EGMME can cause persistent residual damage of bone marrow progenitor cells in mice, an effect that would not be apparent with routine hematological techniques. These results were submitted in 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21083-02 CPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	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:
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.)*

The effects of a 4-day inhalation exposure (6 hr/day) to 0, 1 and 3 ppm methyl isocyanate (MIC) on bone marrow parameters in female mice were examined at 5, 8, 21 days and 1 year following exposure. The MIC exposure was associated with bone marrow suppression 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. Hematopoietic parameters returned to normal by 21 days in the 1 ppm dose group, but not in the 3 ppm dose group. MIC is a highly reactive chemical that appears to exert its effect directly on the lining epithelium of the nasal cavity and major airways, and there was no histological evidence of a systemic effect. There was no significant effect on bone marrow cellularity and CFU-C in mice 1 year following acute exposure at the doses of 3 and 10 ppm for 2 hours. In conclusion, MIC exposure appears to cause acute cell death of lining epithelium of the nasal passages and major airways with transient alterations of bone marrow parameters that are likely related to the pulmonary injury either directly or secondary through the thymus. These results were accepted for publication in Environ. Health Persp. 72, 1987 (In press).

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 21098-01 CPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Adverse Effects of Lindane in B6C3F1 Mice

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

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	C. W. Jameson	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:
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.)*

Lindane (r-Hexachlorocyclohexane; r-Benzene hexachloride) is a popular insecticide. It is of interest to investigate the possible damaging action of this insecticide which is found in significant concentrations in everyday food (WHO, 1973). Male and female B6C3F1 mice are given Lindane at doses of 0, 10, 20 or 40 mg/kg daily for 3 consecutive days by gavage. Animals are killed on days 1, 2, 5 and 28 after the final treatment to study the histopathology, hematology and myelotoxicity of Lindane. We also examine the recovery of mice and the residual marrow effects following stress of radiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21099-01 CPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Hematopoietic Effects in Female B6C3F1 Mice Exposed to Arsine Gas

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

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	B. A. Fowler	Ph.D.	STB	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:
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.)*

Arsine gas is a potent hemolytic agent. Concern about semiconductor workers prompted an in-depth study of arsine at NIEHS. The purpose of this study is to determine the hematopoietic effects of prolonged exposure to 0, 0.5, 2.5, and 5 ppm arsine 6 hours/day via inhalation for 14 days. Body weights of exposed mice were comparable to controls, but a marked, dose-related splenomegaly was observed. Arsine exposure produced significant dose-related decreases in red blood cells, hematocrit and hemoglobin, with increases in white blood cells and the mean corpuscular volume of red blood cells. Furthermore, erythropoiesis as measured by quantitation of erythroid precursors in culture revealed significant reduction of CFU-E/2 X 10^5 cells for all treated groups and of CFU-E/femur at 5 ppm dose group. There was no alteration in bone marrow cellularity and less significant effect on granulocyte-macrophage progenitors. In conclusion, arsine exposure at well-tolerated concentrations produces a dose-related stress on the hematopoietic system characterized by a hemolytic anemia. A further 90-day study of asine at 0, 0.025, 0.5 and 2.5 ppm (6 hr/day) via inhalation is currently underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21100-01 CPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Residual Hematopoietic Effect of Ochratoxin A(OCT A) in Mice Exposed to Irradiation

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

PI:	H. L. Hong	Biologist	CPB	NIEHS
Others:	G. A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	C. W. Jameson	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:
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.)*

The purpose of this study was to determine whether residual damage could be demonstrated by subsequent stress at a time when hematopoietic stem cells sustained injury or nearly recovered. Six-week-old female B6C3F1 mice were injected intraperitoneally with total doses of 0, 20, or 40 mg/kg of OCT A on alternate days over a week, and then stressed with 200 rads whole body irradiation at 10 and 52 days postexposure. There was a significant suppression of marrow granulocyte-macrophage progenitors (CFU-C) in both OCT A-treated groups which returned to normal levels after 1 week for 20 mg/kg group and 4 weeks for 40 mg/kg group. Peripheral leukocytes were significantly reduced in both irradiation control and OCT A-treated groups throughout 4 weeks following the first radiation with a return to normal levels on week 5. However, recovery of a more profound reduction (60%) on leukocytes in OCT A-treated mice was slower following the second radiation. Furthermore, the suppression of CFU-C revealed a significant OCT A dose-related effect on recovery following first and second radiation. Hematopoietic stem cells in mice exposed to OCT A 10 days prior to 200 rads WBI were more sensitive to irradiation and hematopoietic recovery was delayed further with each newly repeated stress. This indicates that mice exposed to OCT A have residual marrow damage that can be demonstrated by sublethal radiation. These results are in preparation (1987).

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. Groups within the Systemic Toxicology Branch (STB), in combination with those of other branches in the Division of Toxicology Research and Testing (DTRT), 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: Chemical Disposition, Developmental and Reproductive Toxicology, Immunotoxicology, Inhalation Toxicology, and Metals Toxicology. Each group is summarized below; for more details and specific accomplishments, consult the individual presentations on the following pages.

Chemical Disposition: Studies of chemical disposition within the NTP are conducted through outside contracts as well as an in-house program. 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 NTP studies. 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 relationships which influence chemical disposition or mechanisms of toxicity and provide basic information which will facilitate the extrapolation of laboratory data to humans. Through contracts or interagency agreements, the metabolism and disposition of a variety of chemicals has been investigated, including methylene bis(thiocyanate), glyphosate, 4-vinylcyclohexene, glycol ether acetates, furfural, benzene, butadiene, isoprene, and others. Methods have also been evaluated, through contracts, by which human tissues can be used to study the metabolism of chemicals. Chemicals such as benzo(a)pyrene, benzidine, caffeine, and trichloroethylene have been investigated in human tissues to compare the metabolism to that in rat tissues. Metabolism in liver, kidney, lung, and adrenals has been evaluated. While the metabolic profile is generally similar, there were definite differences of quantitative and qualitative natures between the species.

Studies in-house have been designed to evaluate the disposition of such chemicals as hexabromonaphthalene, citral, alkyl carbamates, 2-butoxyethanol, butyl acrylate, furan, tetrahydrofuran and selected halogenated dibenzofurans and dibenzodioxins, including octachlorodibenzo-p-dioxin. To evaluate the mode of action of TCDD, teratogenicity studies were conducted on mixtures of TCDD with different dibenzofurans or PCB's; effects were found to be additive, suggesting common mechanisms of toxicity. Studies to evaluate the senescent changes in metabolism and their relationship to the expression of toxicity have continued. Also, studies have been conducted to evaluate the relationship between the induction of cell proliferation and hyperplasia from chemicals like ethyl acrylate, and the induction of carcinogenic effects in the forestomach of rats.

Developmental and Reproductive Toxicology: Studies of the potential of chemicals to adversely affect development and reproduction have been conducted through contracts as well as an in-house program of research. Studies were completed which evaluated the performance of two test systems regarding their potential as a screen for teratogenesis; this included the mouse ovarian tumor cell attachment assay and the human embryonic palatal mesenchyme growth inhibition assay. These two test systems were evaluated in two different laboratories using a fixed list of known animal teratogens and non-teratogens to assess the sensitivity and specificity of these systems. We also continue to evaluate Drosophila as a potential test system for predicting teratogenesis, using the same series of chemicals as were used for the other two in vitro test systems. A number of chemicals were evaluated in our contract to conduct the continuous breeding reproduction study or teratology studies. Among the latter was a teratology study conducted on 1,1,1-trichloroethane where the test agent was given to rats in the drinking water in an effort to evaluate the repeatability of an observation published from another laboratory. Our study at higher concentrations of test agent failed to reproduce the finding of cardiovascular malformation reported in the literature. As a complement to our continuous breeding reproduction studies and as an effort to be involved in the evaluation of ovarian function as a target for toxic effects, we are investigating the ovaries of female mice used in our continuous breeding studies through an interagency agreement with NCTR. This information should be useful guidance regarding methods of examining the ovaries and the ability of this examination to predict and correlate with functional changes observed in continuous breeding studies.

Intramural projects continue in the areas of male fertility, postnatal toxicity, and teratology. Studies were conducted to evaluate the predictiveness of changes in androgen binding protein for changes that occur in fertility, sperm count, or hormone levels. Di-pentyl-phthalate has been used as a model chemical to pursue this evaluation. In an effort to integrate animal model data with results in human semen evaluations as conducted through NIOSH, the rabbit has been investigated as a model for comparison to the human. Studies have been conducted on DEHP and cimetidine to better characterize the effects of these chemicals on lactation and postnatal development. Also, an evaluation was conducted to determine the effect of exposure of neonates to DEHP on testicular function at sexual maturity. The role of Sertoli cells in the expression of testicular toxicity continues to be investigated using cultured cells and test chemicals which are either known to affect testicular function or known not to affect testicular function. In conjunction with other work groups within the STB, reproductive and developmental studies were also conducted on some of the metals used in the semi-conductor industry, including arsine and gallium arsenide, in animals exposed by the inhalation route in the NIEHS inhalation facility.

Immunotoxicology: Effects of chemicals on the function of the immune system have been evaluated through contracts and through in-house research. Through contracts, the immunotoxicity of gallium arsenide, p-nitrotoluene, m-nitrotoluene, and t-butylhydroxyquinone was investigated. Additional chemicals, xylenesulfonic acid and cobaltous sulfate, were tested for hypersensitivity. In-house studies were also designed to evaluate the potential effects on the immune system which could result from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, or biological materials. Studies were done to develop and utilize B-cell maturation as an in

vitro model to sequentially examine the cellular and molecular events associated with chemical-induced immunotoxicity. Other model systems were evaluated which would allow assessment of neutrophil and alveolar macrophage function maturation potential, and ability to respond to physiological activation. Immunotoxicity associated with the tumorigenic process in mice neonatally exposed to diethylnitrosamine was also investigated.

Inhalation Toxicology: This group conducts studies to evaluate the toxicity of chemicals where human exposure is primarily expected to be 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 within the in-house inhalation facility. Major studies have been conducted this year to evaluate the toxicity and the mechanism of toxicity of inhaled arsine and gallium arsenide. These studies have been conducted in collaboration with other groups within the branch as well as elsewhere in DTRT. These studies are part of a series of studies to evaluate the toxicity of chemicals that are found in the semi-conductor industry. These studies focus on the adverse effects on hematopoiesis, the immune system, porphyrin metabolism, and reproduction and development. The data which are generated in these studies are extremely critical to NIOSH to facilitate their evaluation of the setting of standards for these chemicals.

Metals Toxicology: Studies are conducted in-house through this group to characterize the toxicity of metals and the mechanisms of defense of the body against toxicity from metals. The similarities and differences between cadmium-binding proteins from marine molluscs and mammalian metallothionein have been evaluated. Studies were also conducted to evaluate the role of metallothionein and lead-binding proteins from rat, kidney and brain in regulation of the biological activity of lead. This group led the design and conduct of the inhalation toxicity studies to evaluate the toxicity of arsine and gallium arsenide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21003-07 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	David W. Brewster	Guest Researcher	NRSA Postdoctoral Trainee	
	Laurie Couture	Bio. Lab. Tech.	Graduate Student, UNC	
	Yolanda Banks	Guest Worker	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	0.7	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.)*

Chlorinated dibenzodioxins (CDDs) and dibenzofurans (CDFs) are found world-wide as environmental pollutants. Previous studies from our laboratory have indicated that metabolism is a prerequisite for elimination and is a detoxification process. Persistence is related to lack of metabolism, but can also be modulated by body composition. These compounds are well absorbed after oral exposure, although 2,3,4,7,8-pentaCDF (4-PeCDF) is not absorbed as well as 2,3,7,8-tetraCDF (TCDF) in either the rat or the monkey. These chemicals, as well as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 1,2,3,7,8-pentaCDF (1-PeCDF) are only poorly absorbed from the skin (~20% of an applied dose). In the rat, metabolism follows the following order: TCDF>1-PeCDF>TCDD>4-PeCDF. This is inversely related to the persistence of these compounds with 4-PeCDF having the longest whole-body half-life. 4-PeCDF, which has been shown to be present in the human population, is nearly as toxic as TCDD in the monkey.

We have also examined the disposition and toxicity of octachlorodibenzodioxin (OCDD). It is poorly absorbed after oral exposure (<10% absorption). However, what is absorbed concentrates and persists in the liver. Repeated exposure results in a linear accumulation of OCDD in the liver and adipose tissue. The whole body half-life is between 3-5 months in the rat, suggesting that steady-state conditions would never be achieved upon continuous, low-level exposure. We exposed rats for as long as 13 weeks, 5 days per week, to OCDD to determine if subchronic exposure would result in any toxic effects. In fact, OCDD caused the same toxic syndrome as that seen upon acute exposure to TCDD: induction of specific hepatic monoxygenases, fatty changes and vacuolization of the liver, a mild, non-regenerative anemia, and increases in bile acids. Thus, OCDD, while only .01-.001X as potent, is TCDD-like in its actions upon repeated exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21004-07 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	Susan Borghoff	Chemist	Graduate Student, UNC	
	Laurie Couture	Bio-Lab. Technician	Graduate Student, UNC	
	Yolanda Banks	Guest Worker	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:
1.2	0.2	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The aging process can result in altered sensitivity of an organism to the effects of environmental toxins. We are interested in examining the alterations that occur with advancing age in the processes of absorption, distribution, metabolism, and excretion of toxicants and how such changes may affect the toxicity of such chemicals. Our model system is the aging male Fischer rat, a colony of which is being maintained with ages from 1-36 months. We have studied gastrointestinal absorption and observed that while active transport declines with age, passive diffusion does not appear to change. Body composition, which can play a major role in the distribution of compounds changes with age, with a decrease in the lean body mass being accompanied by an increase in adipose tissue. For lipophilic compounds, this results in an increased depot volume and thus enhanced retention. A decrease in renal elimination has been reported for many drugs. However, we have also observed a decline in the rate of bile flow, resulting in a decreased rate of excretion in feces as animals age.

Previous work has shown that the phase I reactions, especially oxidations, may increase, decrease, or remain unchanged with age. We are conducting a systematic examination of age-related changes in the phase II (conjugation) reactions. We have observed that glutathione conjugation does not change with age. However, glucuronidation by the enzyme that metabolizes chloramphenicol and bilirubin undergoes an age-related decline. This results in higher levels of the unconjugated chemical in the body. However, we did not observe any enhanced toxicity in the aging rat. Examination of other conjugation pathways as a function of age are currently in progress, as are correlations of altered metabolism with toxic effects. We are also planning to conduct studies on the effects of age on dermal absorption and metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21009-06 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Pharmacologist	STB	NIEHS
Others:	J. J. Heindel	Expert	STB	NIEHS
	Pia Lindstrom	Biologist	STB	NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch
Program Resources Branch

LAB/BRANCH
Systemic Toxicology Branch, DTRT

SECTION
Developmental & Reproductive Toxicology Group

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

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

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK *(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.

The data indicate that serum ABP is more sensitive than other measures in the first 2-3 weeks after a single exposure, but other endpoints are more sensitive at longer times post-dosing. Increased serum ABP does appear to predict future testicular damage.

We initiated studies to evaluate the usefulness of the rabbit as a model for human semen characteristics, using control and treated rabbits. Endpoints so far have been fertility and number; these will expand as the studies progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21026-06 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Other:	James D. McKinney	Research Chemist	LEC	NIEHS
	Christopher Miller	Guest Worker	DTRT	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:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

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

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

This project is completed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21031-03 STB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

P.I.: Michael P. Moorman Engineering Officer DTRT NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH: Systemic Toxicology Branch

SECTION: Inhalation Toxicology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has progressed very little this year. A simple package of software developed to simulate physiologically based models is being tested on elementary models. An apparatus for measuring partition coefficients has been built and is being tested. Partition coefficients for arsine will be measured so that a simulation can be used in conjunction with the arsine studies which are in progress. A collaboration with the proposed skill center on physiologically based modeling is planned in conjunction with a two-year methylene chloride study. A closed exposure system will be used to measure global pharmacokinetic parameters at various time points during the study. The effects of changes in these parameters will then be evaluated using existing models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21033-03 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	Usha Gundimeda	Visiting Fellow	DTRT	NIEHS
	Janet Diliberto	Biologist	DTRT	NIEHS
	Yolanda Banks	Guest Worker	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:
2.5	1.2	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.)*

An understanding of pharmacokinetic factors can assist greatly in both dose-setting for toxicity studies and in the interpretation of the results. Selected chemicals on-test by the NTP are nominated for disposition studies. The absorption, both oral and dermal, distribution, metabolism, and excretion of these chemicals are studied in rats and other species as needed. The effect of dose on disposition is determined, as is the effect of the route of exposure. These studies help to predict the results obtained upon chronic exposure. Xenobiotics to be studied are radiolabeled with ^{14}C or ^{3}H by custom syntheses. Distribution and excretion are compared after iv, oral, and/or dermal exposures at several doses, the highest being 1/10th of the LD_{50}. Disposition after an iv dose is examined at multiple time points after treatment. The excreta, expired air, and volatiles are analyzed for radioactivity which is resolved into parent compound and metabolites by organic solvent extraction and chromatography. Metabolites are then characterized by chemical and/or enzymatic means. Current work has focused on the disposition of citral ("oil of lemon"), a common flavoring and fragrance. It is completely absorbed after oral exposure, and well absorbed dermally, although volatilization makes dermal absorption incomplete. Urine is the major route of excretion although biliary elimination results in some enterohepatic circulation as well as some fecal excretion. Oxidative metabolism results in the production of substantial amounts of $^{14}CO_2$. Citral is rapidly metabolized with little parent compound being detected in the blood within 10 minutes of administration. There are at least eight metabolites, some of which are common to urine and feces. Both glucuronides and sulphate conjugates appear to be produced. Studies are ongoing to further characterize the metabolites of citral.

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 21034-03 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

C.F. Chignell and R. Hall, Laboratory of Molecular Biophysics; D. R. Winge, University of Utah; J.S. Garvey, Syracuse University; E. Gould, NMFS/NOAA

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Metals Toxicology 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.)*

Previous studies from a number of laboratories have demonstrated the presence of cadmium-binding proteins (CdBP) from marine molluscs which exhibit both similarities and differences with mammalian metallothionein (see Environmental Health Perspectives 65:3-224, 1986 for review). Comparative biochemical studies on these proteins are of particular interest both with respect to understanding the regulatory roles of these molecules in toxic-essential metal homeostasis and elucidating possible pathways for metallothionein evolution. The present studies have examined *in vivo* competition for binding sites on CdBP of the scallop *Placopecten magellanicus* following exposure to cadmium concentrations of 0, 17.7, 35.4 ppb or 17.7 ppb Cd plus 5.0 ppb Cu (2:1 Cd/Cu molar ratio) in seawater for 8 weeks. Results of these studies indicate that Cd showed dose-related binding to CdBP fractions and that animals receiving the Cd plus Cu dose regimen had approximately two times less Cu than Cd bound to these protein fractions on a molar basis. Competitive radioimmunoassays using polyclonal antibodies to mammalian metallothionein (MT) were used to evaluate possible immunological relationships between MT, the scallop CdBP, and the low molecular weight CdBP from the oyster *Crassostrea virginica*. Data from these studies indicated that the scallop CdBP was competitive in the RIA relative to mammalian MT suggesting shared antigenic determinants with mammalian metallothionein, while the oyster CdBP which is closer in size to metallothionein was only minimally reactive. Results of these studies suggest that despite an apparent size difference, the 45K CdBP from scallops possess similarities to mammalian MT in regard to both immunological determinants and *in vivo* regulation of Cd and Cu.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21038-05 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	H. B. Matthews	Research Chemist	DTRT	NIEHS
OTHERS:	J. M. Sanders	Bio. Lab. Technician	DTRT	NIEHS
	S. V. Vo	Bio. Lab. Technician	DTRT	NIEHS
	S. C. Tsao	Visiting Fellow	DTRT	NIEHS
	L. T. Burka	Research Chemist	DTRT	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:
1.7	0.7	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.)*

Studies of chemical metabolism and disposition are designed to provide both applied knowledge in support of chronic toxicity tests conducted by the National Toxicology Program and basic knowledge of those chemical structure and property relationships which determine toxicity. Studies are designed to determine the absorption, tissue distribution, metabolism and clearance of a variety of chemicals. Recent studies of alkyl carbamates in rats and mice have revealed a marked species dependent variation in the rate of metabolism and clearance of these compounds with the mouse having a much greater capacity for clearance than the rat. However, the capacity of both species for clearance is easily saturated and dose dependent kinetics may account for toxicity associated with exposure to these and similar compounds. Studies of a glycol ether, 2-butoxyethanol, have determined that this compound has a unique toxicity to red blood cells causing rapid and massive hemolysis. This toxicity has been determined to be age dependent, increasing dramatically as the age of the exposed animal increases. The causative factor which accounts for the toxicity of 2-butoxyethanol has been determined to be a metabolite, 2-butoxyacetic acid, and toxicity has been modulated by inhibitors of metabolism and clearance. A study of the major acrylate monomer used in the plastics industry, butyl acrylate, has shown that this compound is rapidly and near completely absorbed from the gastrointestinal tract. Most of the dose is metabolized by rapid hydrolysis of the ester linkage and clearance is also rapid with minimal residues remaining in tissues. As part of the work with butyl acrylate a new metabolite not previously identified for this class of chemicals has been isolated and identified.

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 21046-04 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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, DTRT

SECTION
Developmental & Reproductive Toxicology 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.)*

Studies were conducted to characterize the toxicity of drugs and chemicals to neonates relative to adults, and to evaluate the role of lactation in the induction of neonatal toxicity. The effects of chemicals on the quality and quantity of milk produced were determined, and the chemical species excreted into the milk were identified and quantitated. A study was completed in which the concentrations of di-(2-ethylhexyl) phthalate (DEHP) and its monoester metabolite, mono-2-ethylhexyl phthalate (MEHP), were determined in the milk and plasma of lactating rats after oral doses of DEHP to the dams. High levels of DEHP and lower, but significant, levels of MEHP were present in the milk of DEHP-treated dams. Increases in peroxisomal enzyme activities were observed in the pups suckling the DEHP-treated dams. Milk composition and mammary gland nucleic acid content (DNA and RNA) were also affected by DEHP treatment. In another study, neonatal male rats were treated for one week with DEHP and the fertility and testicular parameters in the rats was assessed in mating trials after different periods of recovery. The concentrations of the H2-receptor antagonist, cimetidine, in the milk and plasma of lactating rats was determined and the effect of maternal dosing with cimetidine during lactation on the activity of a microsomal monooxygenase in the pups was evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21057-03 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 DTRT, 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:
0.00	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.)*

This project is completed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21059-03 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT, NIEHS
Others:	H.B. Matthews	Research Chemist	DTRT, NIEHS
	Robert Maronpot	Pathologist	DTRT, NIEHS
	Gregory Eatmon	Biological Aid	DTRT, NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is completed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21060-03 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT, NIEHS
Others:	L.T. Burka	Research Chemist	DTRT, NIEHS
	H.B. Matthews	Research Chemist	DTRT, 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:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is completed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21070-04 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	Richard Morrissey	Biologist	DTRT	NIEHS
	Martha Harris	Biologist	DTRT	NIEHS
	Eric Haskins	Bio. Lab. Technician	DTRT	NIEHS
	Janet Allen	Bio. Lab. Technician	DTRT	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:
1.2	0.3	0.9

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.)*

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is the most toxic man-made chemical known. TCDD has been shown to be highly teratogenic in multiple species. In the mouse, TCDD causes cleft palate and hydronephrosis at doses where no overt toxicity is evident in the mother. We have recently discovered that hydronephrosis is caused by an effect on the epithelial cells of the developing kidney, a response which is more sensitive than cleft palate. The pattern or teratogenic effects in the mouse is so characteristic of TCDD that it can be used as a method of determining whether or not compounds are TCDD-like or not. We have been able to demonstrate that several polychlorinated dibenzofurans, 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzofuran (1-PeCDF), 2,3,4,7,8-PeCDF (4-PeCDF), and 1,2,3,4,7,8-hexachlorodibenzofuran (HCDF) all cause cleft palate and hydronephrosis in the developing mouse fetus. A persistent and common environmental PCB, 2,3,4,5,3',4'-hexachlorobiphenyl (HCB) causes the same response. Thus, all of these chemical can be assigned a "toxic equivalency factor" relative to TCDD. Thus, if TCDD is assigned a potency factor of 1, the relative potencies of TCDF, 1-PeCDF, 4-PeCDF, HCDF, and HCB are .05,.03, .1,.01,and .00003. Combination of these compounds results in additivity of effects, supporting the conclusion that these chemicals act by a common mechanism. Other compounds which have been suggested to be TCDD-like are also under investigation. Perfluorodecanoic acid, a long chain-totally fluorinated fatty acid developed for potential use as a flame retardant, causes thymic atrophy, wasting, and delayed lethality. However, it does NOT cause cleft palate or hydronephrosis. Current studies also indicate that its toxicity does not segregate with the Ah locus. In addition, treatment of mice with PFDA does not cause a depression in thyroid hormone levels, another effect seen after TCDD exposure. Thus, we conclude that PFDA is not a dioxin analog.

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 21075-04 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	H. B. Matthews	Research Chemist	DTRT	NIEHS
	P. Srinivas	Visiting Fellow	DTRT	NIEHS
	S. Vo	Laboratory Technician	DTRT	NIEHS
	C. P. Kool	Chemist	DTRT	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:
2.5	1.3	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.)*

Knowledge of the metabolism and disposition of a xenobiotic is often critical in understanding the toxic effect of the compound. The fidelity of extrapolation of results from animal testing to possible human health effects is also greatly enhanced when metabolic pathways are known. Investigation of the mechanistic aspects of metabolic pathways allows greater understanding of how metabolism of a xenobiotic might lead either to detoxification or to a reactive species with greater toxicity. As more is learned about mechanisms of metabolism more accurate prediction of the possible metabolic pathways for new compounds should be possible. The disposition and metabolism of furan, a hepatotoxic heterocyclic compound, is being studied in male F344 rats. Following an oral dose of 14C-furan, tissue concentration of radioactivity was highest in liver at 24 hr; the next highest tissue level was found in kidney. The structure of the metabolites of TCDF, a highly toxic contaminant often found in PCB's, is under investigation. One metabolite, a hydroxylated tetrachloro compound, has been identified using GC-MS and chemical synthesis. An investigation of the metabolism of dimethylvinyl chloride indicated that the apparent specificity for metabolic oxidation of the methyl group trans to the chlorine is not the result of a regiospecific oxidation. The all trans configuration of the isolated urinary metabolites seems to arise from a subsequent reaction which gives trans product regardless of the stereochemistry of the reactant.

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 21081-02 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Pharmacologist	STB	NIEHS
Others:	P. Lindstrom	Biologist	STB	NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch
Data Management and Analysis

LAB/BRANCH
Systemic Toxicology Branch, DTRT

SECTION
Developmental and Reproductive Toxicology 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
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project has been terminated, and work related to this project has been incorporated into project no. Z01 ES 21009-06 STB.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21084-02 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	Sr. Staff Fellow	DTRT	NIEHS
Others:	H.B. Matthews	Research Chemist	DTRT	NIEHS
	Robert Maronpot	Pathologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Chemically induced cancer is a major concern of the National Toxicology Program (NTP). The mechanisms by which cancer is induced following exposure to chemicals are diverse and not well understood. Further, there are very few good model or test systems for studies of chronic insult by chemicals. Present work by this group has focused on the induction of cancer in the forestomachs of experimental animals receiving oral doses of test chemicals. This work was designed to follow the development of the early stages of these lesions in an effort to characterize their development from early lesions to fullscale cancers. The purpose of this work has been to determine the relevance of these lesions to human exposure to chemicals and evaluate the suitability of this lesion as a short term test for chemical toxicity and carcinogenicity. Results of this work have shown that a series of chemicals which have been characterized as stomach carcinogens induce hyperplasia and hyperkeratosis on acute exposure at doses which induced cancer in chronic studies. These lesions were dose dependent and significantly increased over those observed in controls in every case in which a known forestomach carcinogen was studied. Further, structurally related chemicals which did not induce cancer in chronic studies did not induce similar lesions when administered at comparable doses. This work is being extended to further characterize the development of these lesions following exposure to additional chemicals and to more closely examine development of these lesions in the course of repeated dosing over a period of several months and to determine the effect of coadministration of other drugs and chemicals.

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 21085-02 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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 DTRT, NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is completed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21086-02 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	Sr. Staff Fellow	DTRT	NIEHS
OTHERS:	L. T. Burka	Research Chemist	DTRT	NIEHS
	H. B. Matthews	Research Chemist	DTRT	NIEHS
	R. R. Maronpot	Pathologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	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.)*

Glycol ethers have solvent properties of both alcohols and ethers and are thus very valuable and widely used as industrial solvents. However, the smaller members of this group have recently been incriminated as having selected toxicity to the reproductive system. This fact has increased the use of the already widely used ethylene glycol monobutyl ether (2-butoxyethanol; BE) so that current use of this compound approaches one quarter billion pounds per year. Despite its wide use this chemical has been the subject of relatively little study to characterize its acute or chronic toxicity. The present investigations of the fate of BE in higher animals have determined that it is readily absorbed from the gastrointestinal tract and rapidly metabolized to butoxyacetic acid (BAA), BE-glucuronide and BE-sulfate as well as CO2. Additional work done on this project has demonstrated that, in the rat, BE administration causes severe acute hemolytic anemia as evidenced by a drastic decrease in red blood cells, hemoglobin and hematocrit. Further, this work has demonstrated that sensitivity to the toxicity of BE is age dependent becoming increasingly severe with increasing age. Work has also demonstrated that BE induced anemia can be prevented by prior administration of inhibitors of alcohol dehydrogenase such as pyrazole. This and additional findings indicate that BE metabolism via alcohol and aldehyde dehydrogenase is a prerequisite for hematotoxicity. The ultimate metabolite BAA is believed to account for BE toxicity and studies of BAA in vitro have demonstrated that incubation of BAA with whole blood results in a dramatic increase in hematocrit due to red blood cell swelling followed by hemolysis. Work continues on the characterization of BE toxicity and its relevance to human health.

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 21087-02 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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, DTRT, 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:
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is completed.

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 21088-02 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	DTRT, NIEHS
Others:	H. B. Matthews	Research Chemist	DTRT, NIEHS
	Steven Vo	Biological Lab Technician	DTRT, 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:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is completed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21089-01 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanism of Action of Testicular Toxicants

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

Co-PI:	Jerrold J. Heindel	Expert	STB	NIEHS
Co-PI:	Robert E. Chapin	Pharmacologist	STB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, DTRT

SECTION

Developmental & Reproductive Toxicology 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 perturb male reproductive function. The objectives of these studies are to define subcellular target sites in testicular somatic cells in primary culture. For FY87, efforts have focused on effects of mono-2-ethylhexyl-phthalate, 2,5-hexanedione, and the active metabolite of tri-o-cresyl phosphate (TOCP), saligenin cyclic o-tolyl phosphate, on Sertoli cells in primary culture. Since TOCP needs to be metabolized to an active intermediate in vivo, and because the testis has more of this active metabolite than most other tissues in the body, studies have been initiated to evaluate the capability of Leydig cells to activate TOCP in vitro, and to investigate the relationship of this activation to the Sertoli cell response to the saligenin in vitro. Endpoints for these studies have included overall energy balance, intermediary metabolism control, and "throughput," enzyme activity, cytoskeletal distribution by immunostaining. The emphasis continues to be on the dose- and time-relationships between these endpoints.

Second messengers (cyclic AMP, calcium, and inositol trisphosphate) are important regulators of cellular function. We have recently initiated a series of studies to determine whether testicular toxicants exert some of their effects by altering these second messenger systems. Compounds for these studies are the same as above: MEHP and 2,5-HD.

The significance of these studies is that they have identified structures and processes within these somatic testicular cells which are vulnerable to toxicants. A greater knowledge of where and how compounds work will further our understanding of how the cells work, and could help avoid toxicity for novel compounds in the future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21090-02 STB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	P.L. Goering	NRSA Fellow	DTRT	NIEHS
	G. Rosenthal	NRSA Fellow	DTRT	NIEHS
	G. Boorman	Chief, CPB	DTRT	NIEHS
	B. Schwetz	Chief, STB	DTRT	NIEHS
	R. Morrissey	Research Pharmacologist	DTRT	NIEHS
	M. Moorman	Engineering Officer	DTRT	NIEHS

COOPERATING UNITS *(if any)*
Northrop Services (Dr. Bernard A. Adkins, Jr.)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Metals Toxicology Group

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.)*

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 have been exposed to arsine gas at concentrations of between 10-5000 ppb for 14, 28, or 90 days. All groups exposed to 10 ppm or higher experienced 100% mortality within 4 days while those exposed to 5 pm showed no overt signs of toxicity. A dose-dependent increase in spleen weights and a slight increase in liver weights was observed at necropsy. Microscopic examination of spleens from exposed rats showed sequestration of erythrocytes within the red pulp, hemosiderin accumulation within macrophages, and increased erythropoiesis. Blood samples showed small decreases in packed cell volume (PCV) and a marked increase in ALAD activity. Urine samples showed elevated levels of coproporphyrin and 7 and 8 carboxyl uroporphyrin isomers. Female rats appeared less sensitive than male rats in the 14-day studies. The data suggest that alterations in the heme biosynthetic pathway may be used as early biological indicators of ongoing arsine toxicity to the hematopoietic system. These data were also correlated with complete blood cell analyses which demonstrated decreased RBC count, hemoglobin concentration and PCV. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count were also increased. Marked polychromasia, mild to moderate anisocytosis and poikilocytosis, an increased number of Howell-Jolly bodies and nucleated red blood cells, and reticulocytosis were observed. The above observations were consistent with an arsine-induced regenerative anemia. Although the data from these studies disclosed common hematologic responses for the two species, the changes were more severe in mice than rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21093-01 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanism of Dioxin Toxicity

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

PI:	Linda S. Birnbaum	Research Microbiologist	DTRT	NIEHS
Others:	David W. Brewster	Guest Worker	DTRT	NIEHS
	Charles D. Hebert	Biologist	DTRT	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:
1.7	0.7	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.)*

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin; dioxin) is the most toxic man-made chemical. It has a broad range of toxic effects which are both species and tissue specific. Wasting, thymic atrophy, and delayed lethality are among its more common effects. It has also been shown to be a potent tumor promoter, both in the skin and liver. Polychlorinated dibenzofurans (PCDFs) act like TCDD and may be considered as "dioxin equivalents." In order to determine whether this class of compounds are also tumor promoters, and if the toxic equivalency factors derived from acute and short term studies apply to chronic effects, we are investigating the ability of 2,3,4,7,8-PentaCDF and 1,2,3,4,7,8-hexaCDF to promote MNNG-induced skin tumors in HRS hairless mice, in comparison to the results observed with TCDD.

Recent studies on the mechanism of TCDD toxicity have indicated effects on certain growth modulating receptors, i.e., epidermal growth factors, glucocorticoid. We are investigating the effects of TCDD exposure on Transforming Growth Factor β (TGF β) and its receptor in hairless mouse skin, which has been shown to be a good model for chloracne, a sensitive indicator of TCDD toxicity in humans. We are also studying TGF and its receptors in a human squamous carcinoma line which is responsive to TCDD toxicity. Since effects on the growth factors (or receptors) may involve second messengers, we are initiating studies on the effects of TCDD on various second messenger systems.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30044-11 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
OTHERS:	Michael P. Moorman	Engineering Officer	DTRT	NIEHS
	Richard A. Sloane	Biologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Northrop Services, Incorporated

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.95	.75	.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.)*

Fischer 344 rats and B6C3F1 mice have been exposed to arsine gas in a series of short-term inhalation studies. The collaboration of many different laboratories within the NIEHS has made it possible to investigate a variety of toxicological endpoints including histological, reproductive, hematological, immunological, and biosynthetic effects. This work will continue with an interaction study involving gallium arsenide. Subchronic exposures to methylene chloride have been conducted in preparation for a two-year chronic study. A closed exposure apparatus is being developed to measure changes in global pharmacokinetic parameters during the course of the two-year study. The ability to define the exposure environment continues to be enhanced by refinements to the facility data system. Measurements of both environmental and physiological variables are being organized in a central data base where they will be accessed by standard software packages for analysis. Work is continuing on the design of a new inhalation facility. The layout of the facility has been completed. The design of the air system is in progress.

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 30106-13 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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
	T. Eling	Research Scientist	LMB	NIEHS
	M. Thompson	Research Scientist	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Systemic Toxicology Branch, DTRT

SECTION

Immunotoxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.8	1.0	5.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Immunotoxicology Group studies the adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and biological materials. The ongoing objectives include efforts: (1) to evaluate and examine the influence of selected drugs or environmental chemicals on the immune response; (2) to relate alterations in immunological functions with general and specific organ toxicity; (3) to relate changes in immunological functions with altered host resistance following challenge with tumor cells or infectious agents; 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) Development and utilization of B cell maturation as an in vitro model to sequentially examine the cellular and molecular events associated with chemical-induced immunotoxicity. General methodology includes the use of flow cytometry as well as methods for examining second messenger, cellular proliferation, and cellular differentiation. Chemicals and drugs that have been examined include benzidene (and other compounds that modulate arachidonic acid products), tetrachlorodibenzo-p-dioxin, polycyclics, pertussis toxin, and methotrexate. (b) Development of model systems which allow assessment of neutrophil and alveolar macrophage function, maturation potential, and ability to respond to physiological activation. Endpoints for these assays include production of soluble mediators, surface markers, and effector cell function. (c) Evaluation and examination of immunotoxicity associated with the tumorigenic process in mice neonatally administered a liver promotor (diethylnitrosamine), in mice exposed to a contaminated drinking water sample, and in mice exposed to antiviral drugs used in the treatment of AIDS (i.e., AZT, DDC, and DDA).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70200-13 STB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	DTRT	NIEHS
Others:	D. Gilg	Visiting Fellow	DTRT	NIEHS
	P. Goering	NRSA Postdoctoral Fellow	DTRT	NIEHS
	G. DuVal	NRSA Postdoctoral Fellow	DTRT	NIEHS

COOPERATING UNITS *(if any)*

C.F. Chignell, Laboratory of Molecular Biophysics

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Metals Toxicology Group

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.)*

The roles played by metallothionein (MT) and more recently discovered lead-binding proteins (PbBP) from rat kidney and brain in regulating biological activity of Pb have been studied. MT mediates Pb inhibition of δ-aminolevulinic acid dehydratase (ALAD). Pretreatment of rats with Zn activated liver ALAD and attenuated the inhibition of ALAD by Pb *in vitro* and *in vivo*. MT isolated from Zn-pretreated rats injected with ^{203}Pb disclosed that both Zn and ^{203}Pb co-eluted in the MT fractions. Addition of purified liver ZnMT to purified bovine liver ALAD reaction mixtures increased enzyme activity by 2-fold and prevented inhibition of ALAD by Pb. Apothionein alone decreased the activity of Zn-activated ALAD and attenuated Pb inhibition of the enzyme. Studies of incubates containing ^{65}ZnMT demonstrated that Zn was transferred from MT to ALAD. Fractionation of incubates containing ^{203}Pb demonstrated that ZnMT sequestered Pb away from ALAD. These data suggest that MT may regulate the activity of some Zn-metalloenzymes by controlling Zn availability and that it may also alter the interation of Pb with ALAD by decreasing the cytosolic pool of free Pb. Purification studies were conducted on the 10K PbBP from rat kidney. Amino acid analyses demonstrated a high glutamic and aspartic content with significant levels of phenylalanine and leucine but little cysteine. These data indicate that the 10K PbBP is not an MT but rather a novel carboxyl-rich, metal-binding protein with a unique amino acid composition. Western blot analyses using rabbit polyclonal antibodies to PbBP were conducted. The antibodies were specific for the PbBP and did not cross-react with MT or calmodulin and the protein was present in only kidney and urine. The data provide further evidence that PbBP is a novel renal protein which plays a major role in metal metabolism.

GPO 914-918

INTRAMURAL PROJECT NUMBER LISTING

Z01 ES 10004-08 LMB
Z01 ES 21003-07 STB
Z01 ES 21004-07 STB
Z01 ES 21009-06 STB
Z01 ES 21012-06 CGTB
Z01 ES 21013-06 CGTB
Z01 ES 21016-06 CGTB
Z01 ES 21024-06 LBRA
Z01 ES 21026-06 STB
Z01 ES 21031-03 STB
Z01 ES 21032-03 CGTB
Z01 ES 21033-03 STB
Z01 ES 21034-03 STB
Z01 ES 21035-03 CGTB
Z01 ES 21038-05 STB
Z01 ES 21039-03 CGTB
Z01 ES 21045-05 CGTB
Z01 ES 21046-04 STB
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