NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

FY 1989 ANNUAL REPORT

October 1, 1988 through September 30, 1989

DIVISION OF INTRAMURAL RESEARCH

LABORATORY OF MOLECULAR AND INTERGRATIVE NEUROSCIENCE

INTRAMURAL RESEARCH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22103-06 CMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	D. E. Blackmore	Head, Veterinary Medicine	CMB, NIEHS
	E. H. Lebetkin	Bio Lab Tech, QAL	CMB, NIEHS
	S. D. Matheson	Bio Lab Tech, QAL	CMB, NIEHS
	G. F. Caviness	Bio Lab Tech, QAL	CMB, NIEHS
	W. M. Yearby	Bio Lab Tech, VM	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.)*

Sentinel animals are an essential component of animal health surveillance programs, providing the primary means of detecting adventitious agents in laboratory animal colonies. An optimal program attempts to maximize exposure of the sentinel animals to infectious agents and to minimize the time required for detection. Our study compared the classical aerosol exposure method with a technique utilizing sensitive strains of mice exposed to both aerosols and soiled bedding from the research colony.

Eight cages of mice containing 12 mice (3 each of 4 different strains) per cage were housed without filter bonnets on the bottom shelf of 4 out of 12 racks in an animal room which had a history of sporadic mouse hepatitis virus (MHV) infections. Half of the cages received a composite sample of bedding used previously by experimental mice in the room and the other half received fresh unused bedding. The sentinel mice were bled at monthly intervals for MHV serology and observed twice weekly for clinical changes. After 5 months, all of the mice in cages receiving used bedding had seroconverted to MHV and three of the groups were positive for Myobia musculi mites. In contrast, only 2 of the 4 groups of mice which received fresh bedding were positive for MHV and all were negative for mites. In addition, 2 of the groups of mice receiving used bedding seroconverted 3 weeks before any of the groups receiving fresh bedding.

These findings indicate the importance of exposing sentinel mice to used bedding to enhance transmission of MHV and mites. This study has been accepted for publication in the July 1989 issue of Laboratory Animal Science.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 22109-01 CMB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Comparison of Tissue Response to Complete Freund's and RIBI Adjuvant

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

PI:	D. E. Blackmore	Head, Veterinary Medicine	CMB, NIEHS
Others:	H. L. Amyx	Chief	CMB, NIEHS
	P. C. Gardinier	Biol Lab Tech, VM	CMB, NIEHS
	J. A. Clark	Biol Lab Tech, VM	CMB, NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch, DTRT, NIEHS

LAB/BRANCH
Comparative Medicine Branch

SECTION
Veterinary Medicine

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

Complete Freund's adjuvant, a water in oil emulsion, is the most common adjuvant used to stimulate antibody response in rabbits. It's use is often associated with undesirable side effects at the inoculation site, such as inflammatory lesions, tissue necrosis, and even local sloughing. The RIBI Adjuvant System, an oil in water emulsion, is the most frequently used alternative to complete Freund's adjuvant. RIBI utilizes bacterial cell walls and byproducts which have been purified to eliminate the toxicity and allergenicity associated with the intact tubercle bacillus contained in complete Freund's adjuvant.

This study will examine intradermal, subcutaneous, intramuscular and intraperitoneal routes of inoculation in the rabbit, comparing the two adjuvants at varying dosage levels. Rabbits will be clinically evaluated for pain and distress, and gross and histopathologic collections will be made and examined at 1, 2, 3, or 4 weeks postinoculation. Rabbits scheduled for sacrifice on fertility studies will be used. Collaborations with investigators in other laboratories will be initiated to evaluate and compare antibody response to antigens under the varying experimental conditions. We hope to obtain a profile of the method(s) which result in maximum antibody response with minimum undesirable tissue reactions, benefitting the experimental animal and improving the scientific result.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES-22110-01 CMB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alopecia and Dermatitis in C57BL/6N Mice

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:	D. E. Blackmore	Head, Veterinary Medicine	CMB, NIEHS
	E. H. Lebetkin	Bio Lab Tech, QAL	CMB, NIEHS
	S. D. Matheson	Bio Lab Tech, QAL	CMB, NIEHS
	G. F. Caviness	Bio Lab Tech, QAL	CMB, NIEHS
	W. M. Yearby	Bio Lab Tech, VM	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.)*

C57BL/6N mice, an important strain in several NIEHS studies, develop an alopecia which usually arises at 4 to 6 months of age. This condition often progresses into a protracted dermatitis and may become severe enough that the animals develop ulcerative skin lesions and suffer premature moribidity and mortality. We have initiated a project directed at discovering a nutritional basis for the progressive skin disorders.

Mice were divided into groups of 15 and fed either the standard NIH-31 diet (control diet) or a test diet made by fortifying the NIH-31 diet with vegetable oils, animal fat, thiamine, riboflavin, pyridoxine, cyanocobalamin, biotin, zinc oxide, or other vitamin and mineral mixtures. The mice have been on the study for 11 months to date. Preliminary indications are that obvious clinical differences are observed between groups and that the alopecia and degenerative skin conditions can be controlled by combinations of the vitamin and mineral mixtures. These findings suggest that C57BL/6N mice may require dietary changes as they age to compensate for natural degenerative skin changes that occur, probably due to strain dependent genetic predisposition. It further suggests that modifications to laboratory animal diets to compensate for aging might need to be considered for other laboratory species as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80001-17 LCMP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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

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

PI:	R. Philpot	Research Chemist	LCMP	NIEHS
Others:	R. Gasser	Visiting Associate	LCMP	NIEHS
	J. Schulze	Visiting Fellow	LCMP	NIEHS
	D. Duignan	IRTA Fellow	LCMP	NIEHS

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

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Molecular Pharmacology Section

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.)*

Three of the most highly expressed drug-metabolizing enzymes in rabbit lung are cytochrome P-450 isozymes 2 (IIB) and 5 (IVB) and the flavin-containing monooxygenase. These three enzymes together can metabolize a wide variety of exogenous chemicals, including a number that contain sulfur, nitrogen, or phosphorous as well as carbon. These enzymes may be involved in the activation of certain chemicals that result in pulmonary-specific toxic effects. Because similar enzymes are also present in liver, the observation of tissue-specific effects suggests that different forms of the enzymes may actually be expressed in liver and lung. With respect to cytochrome P-450, we have shown that identical forms of isozyme 5 are expressed in the two tissues and no evidence for multiple forms has been found. On the other hand, at least one form of isozyme 2 is expressed in liver, but not in lung. The other two forms of the enzyme are found in both tissues. Only with the flavin-containing monooxygenase do we find marked differences between the pulmonary and hepatic enzymes. The pulmonary enzyme is only 56% identical to the hepatic enzyme with respect to primary sequences. A similar relationship is found between the enzyme from rabbit lung and the one from pig liver. These findings demonstrate that the evolutionary branching point between the lung and liver enzymes occurred prior to speciation. Both of the enzymes are products of single genes, although inthe case of the pulmonary enzyme multiple mRNA species are detected. The relationships among these mRNAs is under investigation. A single gene is also found in the case of the enzyme from pig liver. All three flavin-containing monooxygenases show a high degree of structural conservation for a number of hydrophobic areas and for the pyrophosphate binding sites. These sites, which are centered around glycine-x-glycine-x-x-glycine peptides, are characteristic of a number of flavin-containing enzymes from lower organisms as well as from mammals. The differences between the catalytic activities of the lung and liver enzymes will now be investigated through the construction of hybrid proteins and the use of expression systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80031-13 LCMP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	LCMP	NIEHS
Others:	D.S. Miller	Expert	LCMP	NIEHS
	P.M. Smith	Visiting Fellow	LCMP	NIEHS

COOPERATING UNITS *(if any)*

University of Florida; Duke University

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Comparative Membrane Pharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	2.25	4.75

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.)*

Transport of solutes across epithelial membranes is a vital function of many organs, e.g., kidney, choroid plexus, liver and gut. Epithelial transport depends upon individual transport systems located in apical (BBM) and basolateral (BLM) membranes. Their complex organization, functional importance and exposed location make epithelial membranes particularly susceptible to toxic effects of foreign chemicals. Recent research has focussed on the renal organic anion (OA) transport system, which determines the extent of elimination of many toxic xenobiotics. This work has documented the intimate interdependence of metabolism and subsequent excretory transport in determining both fate and toxicity of specific pollutants, e.g., benzo(a)pyene. Mechanistic studies using both isolated membrane vesicles and intact renal tubules have shown that OA entry across the BLM is mediated by highly specific anion exchange for either glutarate or α-ketoglutarate. The outwardly directed dicarboxylate (DC) gradient may be maintained by metabolic production within the tubular cells or by Na-dependent DC uptake which indirectly couples OA transport to the energy stored in the out>in sodium gradient across the BLM. In choroid plexus intracellular production of DC, rather than Na driven recycling is apparently used to maintain the in>out DC gradient needed to drive OA transport. Because of the complexity of this mechanism, it is sensitive to foreign agents at a number of specific sites. For example, lithium reduces OA transport by inhibiting Na-dependent uptake of DCs; whereas, mercury or foreign monovalent anions may inhibit by direct actions on the OA/DC exchanger.

Membranes also play important roles in information transfer between the cell and its environment. Amphibian oocytes were used to examine the nature of polypeptide hormone (e.g., insulin, growth factors) action. These studies have shown that insulin acts directly on both membrane and intracellular sites to alter protein, RNA, and glycogen synthesis. Effects of intracellular and extracellular insulin were additive, suggesting separate modes of action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80042-03 LCMP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	J.W. Putney, Jr.	Chief	LCMP	NIEHS
Others:	A. Hughes, F. Menniti, K. Oliver	Staff Fellows	LCMP	NIEHS
	H. Takemura, G. Bird	Visiting Fellows	LCMP	NIEHS
	D. Kwan	Visiting Scientist	LCMP	NIEHS
	K. Nogimori	Guest Researcher	LCMP	NIEHS
	M. Rossier	Special Volunteer	LCMP	NIEHS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Calcium Regulation Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	6	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 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 Ca^{2+}. An early event in the action of receptors of this class is the hydrolysis of a membrane lipid, phosphatidylinositol 4,5-*bis*phosphate to yield two putative second messengers, diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3). DG activates a specific kinase in cells, called protein kinase C, and IP_3 releases Ca^{2+} from an intracellular organelle. The general approach in this project is to combine HPLC measurements of the formation and metabolism of inositol phosphates with real time measurements of cytosolic Ca^{2+} using intracellular fluorescent Ca^{2+} indicators. Recently, the actions of a non-phorbol ester tumor promoter, thapsigargin, have been investigated in some detail. This agent acts to inhibit the active transport of Ca^{2+} by the intracellular organelle on which IP_3 acts. These studies have revealed new information on the mechanism of regulation of intracellular Ca^{2+}, and also have provided information on the mechanisms by which Ca^{2+} transport at the surface membrane of the cell is regulated. The intracellular organelle on which both IP_3 and thapsigargin act is known to be distinct from the endoplasmic reticulum in cells, and has been termed a "calciosome". Efforts are currently being directed toward its purification and characterization. Since Ca^{2+} is believed to play a central role in mechanisms of chemically-induced cell injury, these studies should provide insights into the mechanisms underlying the pathophysiological consequences of exposure to toxins and other environmental agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80043-02 LCMP

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ion Channel Modulation by Signal Transduction Systems

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

PI:	D.L. Armstrong	Senior Staff Fellow	LCMP NIEHS
Others:	M. Austin	Biologist	LCMP NIEHS
	R. White	Staff Fellow	LCMP NIEHS

COOPERATING UNITS *(if any)*
Drs. Angus Nairn and Paul Greengard, Laboratory of Molecular and Cellular Neuroscience, Rockfeller University, New York, New York

LAB/BRANCH
Laboratory of Cellular and Molecular Pharmacology

SECTION
Calcium Regulation Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.15	1.15	1.00

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.)*

Voltage-activated calcium channels in the plasma membrane of excitable cells play a major role in regulating the intracellular concentration of calcium. In this way they transduce the electrical effects of modulating other ion channels into a chemical signal that can alter cell function. Recently, the predominant calcium channel in a wide variety of vertebrate cell types has been characterized with patch-clamp techniques. Like many other ion channels, these dihydropyridine-sensitive calcium channels are modulated by cAMP-dependent protein phosphorylation. We have investigated the phosphorylation dependence of individual dihydropyridine-sensitive channels under voltage-clamp in cell-free patches of native membrane from a rat pituitary tumor cell line (GH_3). Our data suggest that the enzymatic addition or removal of phosphate esters on the channel protein by endogenous kinases and phosphatases profoundly alters the response of these channels to depolarization of the membrane. Experiments with exogenous protein kinases and their inhibitors, purified to homogeneity by affinity chromatography, support that conclusion. Phosphorylation by the cAMP-dependent kinase leads to short bursts of openings with an average duration of ~1 ms. Subsequent phosphorylation by the calcium-/calmodulin-dependent protein kinase type II produces much longer openings of ~10 ms duration, like those produced by BAY K 8644. In the absence of ATP-Mg, the kinases have no effect and the channel opens very rarely, if at all, in patches of native membrane. Additional data suggest that the phosphorylation dependence of calcium channel activity underlies both the modulation by dihydropyridines and the rapid inactivation produced by intracellular accumulation of calcium, two additional properties that distinguish these channels from other voltage-activated calcium channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80044-01 LCMP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Mechanisms of Embryonic Neural Induction

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

PI:	D. Armstrong	Senior Staff Fellow	LCMP	NIEHS
	D. Miller	Expert	LCMP	NIEHS

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Calcium Regulation Section and Comparative Pharmacology 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.)

In some of the most famous experiments in embryology, Spemann and Mangold demonstrated that the potential for brain formation is not restricted to specific cells in the ectoderm of early amphibian embryos, but is induced by cell contact with the underlying chordamesoderm. The central concept which emerged from their work of cell interactions determining cell fate remains a cornerstone of modern embryology. Nevertheless, over 50 years later, both the molecular signal that induces neural differentiation and the method of its communication remain to be discovered. We have begun to reinvestigate the problem of neural induction with modern microinjection techniques in embryonic ascidians, primitive marine chordates with a simple, archetypal development. Microelectrodes will be used to inject specific pharmacological probes into single, identified blastomeres. The effect of these compounds on neuronal development will be determined by simultaneously filling the blastomeres in the presumptive neuroectoderm with fluorescent tracers of cell lineage (conjugated dextrans) and of cell coupling across gap junctions (Lucifer yellow). Lithium's ability to block neural induction will be analyzed by experimentally separating its effects on cell coupling, cyclic nucleotide metabolism and sodium pumping. These experiments are designed to illuminate one of the most fundamental unsolved problems in biology: how does one cell alter the fate of its neighbor during vertebrate embryogenesis?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80045-01 LCMP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

GTP-Binding Proteins and Signal Transduction: Structure and Function

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

PI:	M. Rodbell	Senior Research Scientist	LCMP	NIEHS
Others:	F. Ribeiro-Neto	Visiting Associate	LCMP	NIEHS
	K. Haraguchi	Visiting Associate	LCMP	NIEHS

COOPERATING UNITS *(if any)*

Rocky Mt. Laboratory, MT, National Institute of Allergy and Infectious Diseases

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Signal Transduction Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A family of GTP-binding proteins (α-proteins) are linked to membrane receptors and serve as transducers of hormone/neurotransmitter action in most eukaryotic cells. Receptor-occupation leads to enhanced binding of GTP. It is thought that α-proteins are linked to membranes and/or receptors through a complex of two proteins, designated β/γ, that form heterotrimeric complexes called G-proteins. Extraction of rat brain, liver, and adipocyte membranes with octyl-ß-glucoside (OG) results in solubilization of the β/γ complex and insoluble forms of the α-proteins. The α-proteins sediment with cytoskeletal proteins and display characteristics of polymeric structures: they readily cross-link with various cross-linking agents and their solubility in OG depends on temperature and divalent cations in a manner similar to actin, tubulin, and other cytoskeletal proteins. When activated by non-hydrolyzable analogs of GTP ($GTP\gamma S$), the polymeric structures are soluble in OG and α-proteins are no longer cross-linked. In contrast, the β/γ complexes do not cross-link with α-proteins and remain soluble in OG under all conditions. These findings suggest that α-proteins associate with membrane receptors as oligomeric or polymeric structures; β/γ complexes may serve to link the oligomers to the membrane in a manner similar to functions of ankyrin in annealing spectrin and actin-like proteins to cell membranes. Pertussis toxin blocks hormone/neurotransmitter action on several types of α-proteins (α_o, α_i). The toxin, using NAD as co-substrate, catalyzes ADP-ribosylation of α-proteins. However, recent studies show that the toxin induces shifts in the electrophoretic mobility and enhances the immunogenicity of α-proteins in the absence of NAD.. Studies with site-directed mutants of the toxin's catalytic subunit revealed mutants lacking or with weak ADP-ribosylating activity but retains the shift- and immunogenic-enhancing activities. The latter activities may be responsible for the blocking effects of hormones on α-proteins; they involve SH-residues in both the toxin's catalytic unit and the α-protein substrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80046-01 LCMP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Regulation of Inositol Lipid Signalling Mechanisms

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

PI:	S.B. Shears	Visiting Scientist	LCMP	NIEHS
Others:	P.J. Hughes	Visiting Fellow	LCMP	NIEHS
	C. Rubiera	Guest Worker	LCMP	NIEHS

COOPERATING UNITS *(if any)*

University of Oviedo, Spain; University of Michigan Medical Center

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Inositol Lipid Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Activation of cell-surface receptors releases inside cells inositol (1,4,5) trisphosphate ($I(1,4,5)P_3$), a ubiquitous signal playing a pivotal role in cell regulation through initiation of calcium fluxes. Enzymes that metabolize and thereby deactivate $I(1,4,5)P_3$ are crucial to the regulation of cell signalling. Moreover, increasing evidence points to the ensuing metabolites themselves having important roles in signal transduction. This project aims to understand how metabolism of inositol phosphates is regulated by extracellular agents such as hormones, toxins (including carcinogens) and clinically important drugs; inositol phosphate fluxes in isolated cells and cell lines, and the influence of extracellular agents, will be monitored to seek possible control points. Techniques are being developed for the rapid purification of enzymes in their modified and regulated state from stimulated, freeze-clamped cells. As evidence emerges that cAMP kinase, C-kinase and tyrosine kinases interact with this pathway, it is important to develop methods that, during protein purification, inhibit reversal of these effects without non-specifically perturbing inositol phosphate phosphatases and kinases, so as to understand control at a molecular level. Evidence from this laboratory indicates an endogenous non-protein factor may also regulate inositol phosphate metabolism; its identity and significance will be pursued. Growing evidence (largely from this laboratory) also points to feedforward and feedback regulation by inositol phosphates themselves. As the complexities of this system are unravelled, we will better understand and treat the effects of extracellular toxins on cell signalling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60099-10 LG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	B. Yukihiro Hiraoka	Visiting Fellow	LG, NIEHS
	Tetsuo Takano	Visiting Fellow	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Genetics

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The genomic structure of human LDH-A, LDH-B and LDH-C genes as well as mouse LDH-A gene have been characterized. The protein-coding sequences of mammalian LDH-A, LDH-B and LDH-C genes are interrupted by six introns, and their relationships between protein structure and exon organization are illustrated. The developmental and tissue-specific expression of mouse LDH-A, LDH-B and LDH-C genes have been studied during spermatogenesis and oogenesis. This information will allow more accurate evaluation of genetic mutation events caused by mutagens 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 61019-09 LG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The complete sequence of 354 amino acids of mature human prostatic acid phosphatase was determined by structural analyses of both protein and cDNA. Human prostatic and lysosomal acid phosphatases exhibited 50% sequence homology, including five Cys and two putative N-linked glycosylation sites. The collaborative protein chemistry laboratory with UNC has already provided lots of research services on protein microsequencing and peptide synthesis to other scientists at the NIEHS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61032-06 LG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	Jun M. Versola	Biological Aid (SIS)	LG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Genetics

SECTION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The complete primary structure of 333 amino acids from mouse LDH-B_4 (heart) isozyme has been determined by sequencing both protein and cDNA. A comparison with human LDH-B sequence revealed eight (2.4%) amino acid differences: four differences are clustered within the random-coil region of amino-terminal 20 residues, two substitutions at residue numbers 52 and 132 are located in the β-sheet, and two changes at residue numbers 236 and 317 are positioned in α-helix.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65021-17 LG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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

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

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

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project are (1) to detect natural and induced mutations in mice, (2) to achieve understanding of the molecular events occurring in the process of mutation induction, and (3) to relate these events to the life, form and function of the mammalian organism. Our project is relevant to the problem of human exposures to environmental chemicals; particularly, the increased risk of genetic disease in the offspring of exposed individuals. We have detected a variety of mutations at specific biochemical loci with electrophoretic methods, characterized several normal and mutant genes (and gene products), and examined the offspring of mutagen-trated and control parents for the physical manifestation of polygenic mutations affecting the skeleton. Last year we developed an expanded set of skeletal characters and an independent method for evaluating metrical variation using X, Y coordinate data obtained with a microscope, computer and digitizing tablet. We applied these methods to the left mandibles of 1030 progeny from ethylnitrosourea-treated and control mice. One treated group showed a very highly significant increase in variability. This year we applied our method to an additional three series of bones, including the right mandible and the left and right humerus. Analysis of these data are presently in progress. So far, results suggest the morphometrical analysis provides a superior method for investigating the problem of genetic risk in a model system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65033-06 LG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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.
E. J. Eisen, NCSU, Raleigh, N. C.
Clement Markert, NCSU, Raleigh, N. C.

LAB/BRANCH
Laboratory of Genetics

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 research is to study mutagenesis at the DNA level in mammals and to evaluate genetic and biochemical events in certain mutants as models of human genetic diseases. A major problem in mutagenesis is that the level and specificity of response is very different between indicator organisms; predictions about induced mutagenesis may not be relevant. Significant variation is due to the diversity of the marker genes; a single sequence needs to be used as a target in the various organisms and tissues. Our analysis is based on variance among single copies of the ΦX174 virus containing am3 and cs70 mutations as a shuttle vector in different species. The accomplishments are as follows. 1) Pilot experiments with transgenic cell cultures suggest that the approach is sensitive enough to study mutations at the single copy level in vector DNA recovered from the host. 2) Transgenic mice containing the ΦX vector have been produced in the inbred C57BL/6 strain and the transgene has been transmitted to offspring. Each hemizygous founder was found to contain more than 50 copies per genome. Mating schemes are in progress to expand the number of copies per genome and to produce mice homozygous for the vector at each allelic insertion site. 3) Methods have been developed to efficiently recover the vector from the transgenic mice and measurements of background mutation rates are in progress. 4) Experiments to produce a second type of transgenic mouse containing a different ΦX vector with an expanded indicator region are about to begin. The use of integrated viral vector in transgenic mice can combine a theoretical study of mechanisms of mutation in several model organisms with an assessment of mutagenic hazard. A single DNA sequence can be exposed and analyzed as naked DNA, as a single stranded virus, double stranded in bacteria, and as vector DNA in the nuclear genome of mammalian cells or transgenic mice. In addition, such an approach may allow us to examine in vivo mutagenesis and repair in many somatic tissues and gametogenic tissue during development or as a function of aging and various conditions of environmental exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 10004-10 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Elizabeth Murphy	Senior Staff Fellow	LMB	NIEHS
	Louis Levy	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Charles Steenbergen, Department of Pathology, Duke University, Durham, NC; Professor Leonard S. Gettes, Dept. of Medicine, UNC Medical School, Chapel Hill, 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:
1.7	1.2	.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.)*

In vivo and *in situ* NMR studies are carried out on systems ranging from cell suspensions to perfused organs, to intact, anesthetized experimental animals in order to determine the mechanisms by which environmental chemicals and other types of stress irreversibly injure cells. Physiological, biochemical, and magnetic resonance measurements are carried out in parallel when possible, both to validate the techniques used, and more importantly, to correlate various metabolic changes in order to determine which factors may play a causative role. Our recent studies have focused on an examination of the role of ionic and volume changes occurring during cell injury produced by a variety of factors. Increases in cytosolic calcium, sodium, magnesium, H_i, and a decrease in ATP are observed during injury induced by ischemia and some toxins. The decrease in pH appears to stimulate sodium uptake via sodium-proton exchange. The increase in cytosolic sodium can be blocked by inhibitors of Na-H exchange such as amiloride. The increase in Na_i appears to stimulate an increase in Ca_i via Na-Ca exchange; if the increase in Na_i is blocked with amiloride the rise in Ca_i is largely attenuated. Cell swelling is an important parameter in the development of cell injury. Changes in Mg_i have been postulated to alter volume regulatory pathways. We have obtained preliminary data suggesting that in red blood cells, swelling and shrinkage are accompanied by changes in cytosolic magnesium ion concentration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 30003-18 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Ronald P. Mason	Research Chemist	LMB	NIEHS
	C. Tyler Burt	Expert	LMB	NIEHS
	Robert Chapin	Research Chemist	STB	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:
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.)*

Previously developed methods were applied to the identification of the metabolite of di-(2-ethylhexyl) phthalate apparently responsible for the initiation of acute testicular atrophy in rats (collaborative study with Dr. Chapin). Factors influencing the phosphorus NMR spectra of natural phospholipids produced by a variety of bacterial species were studied in collaboration with Dr. Burt. Opportunities for misassignment of NMR peaks were related to temperature- and concentration-dependence of chemical shifts. Obtaining useful mass spectra of spin-trapped adducts of lipoxidase-dependent fatty acid free radicals required the development of a quenching technique free of side reactions. In contrast, it was possible to obtain interpretable infrared spectra without quenching (collaborative study with Dr. Mason). Newly developed techniques of radio-HPLC made it possible to determine that the spin trapping agent POBN reacts with linoleic acid in the presence of lipoxygenase to produce a much more complex mixture of products than is suggested by ESR spectra alone. ESR-inactive products include both di-adducts and polymeric material.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50046-11 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	Piotr Bilski	Visiting Fellow	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:
2.8	2.3	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 project 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. UV-irradiation of the anti-psoriatic drug anthralin (AN) resulted in the generation of the superoxide anion radical ($O_2^{\cdot -}$), which was identified by spin trapping with 5,5-dimethylpyrroline-1-oxide (DMPO). In the absence of oxygen, the drug abstracted hydrogen atoms from the solvent ethanol. However, 1,8-dihydroxyanthraquinone (1,8-DHAQ), the major AN photoproduct, was much more active than AN itself in generating superoxide and ethanol radicals. This suggests that AN photosensitization may be due to 1,8-DHAQ and not AN. Other photosensitizers which also generate free radicals upon UV-irradiation include anthracyclines, the anthraquinone-based dye disperse blue 35, tetracyclines, the salicylanilides and porphyrins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50080-07 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
OTHER:	Carol E. Parker	Chemist	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	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:
.90	.45	.45

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 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; 7) continuous flow FAB/MS and FAB/MS/MS under both positive and negative ion conditions; and 8) tandem MS in combination with positive and negative ion FAB, EI and CI MS.

During the past year approximately 725 MS analyses have been performed on a service basis (not including collaborative work).

In addition to mass spectrometric services provided to other NIEHS scientists, we have been called upon to provide high performance liquid chromatographic (HPLC) support. With the addition of capillary zone electrophoresis (CZE) capabilities in this lab, these service aspects are expected to increase in importance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50082-06 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Studies on Tumor Promoters and Antipromoters

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

PI: Phillip W. Albro Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	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.)*

Current objectives include assessment of the effects of hepatic tumor promoters and antipromoters on components of the plasma membrane. Current emphasis is on effects of the compounds on protein kinase C and inositide-specific phospholipase C. Phorbol diesters are traditionally used as biochemical tools in the study of activation, translocation and down-regulation of protein kinase C, but give misleading results when liver tissue or hepatocytes are studied. We have examined the rapid metabolism of the phorbol diesters in hepatocyte cultures. While some of the metabolites are effectively inert (as has been assumed for all the metabolites), other hepatic metabolites bind to the plasma membrane and to protein kinase C even more tightly than do the parent phorbol diesters. We are presently concerned with identification of the active metabolites, which appear to be more highly oxidized than the simple hydrolysis products formed in most other tissues. These metabolites are readily formed either hepatocyte suspensions or liver slices, and are not rapidly eliminated from the cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50087-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Reza Dabestani	IRTA	LMB	NIEHS
	Colin F. Chignell	Chief, LMB	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Ann G. Motten, Duke University, Durham, NC.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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

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. We are also developing a nano-second laser flash photolysis unit to carry out time-resolved transient absorption and emission spectroscopy on excited state intermediates (precursors to $^1O_2$) of potential photosensitizers. This instrumentation has permitted us to delineate the photophysics of $^1O_2$ production from a number of photosensitizers including phenothiazines, tetracyclines, benzoxazoles and anthrapyrazoles. Anthralin (AN), a potent anti-psoriatic drug and tumor promoter, was found to be a poor generator of $^1O_2$. However, 1,8-dihydroxyanthraquinone (1,8-DHAQ), the major AN photoproduct, was about one-fifth as active as rose bengal in generating $^1O_2$ upon UV-irradiation. This suggests that AN photosensitization may be due to 1,8-DHAQ and not AN. Factory workers exposed to the anthraquinone-based dye Disperse Blue 35 often develop photocontact dermatitis. Only one component of the dye (which contains more than 10 different compounds), 4,5-diamino-1,8-dihydroxyanthraquinone, generated a significant amount of $^1O_2$.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50088-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Kathy T. Knecht	Biologist	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

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:
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. The $\cdot CO_2^-$ radical adduct also was observed in urine following the intragastric administration of CCl_4 or $CBrCl_3$ and spin trap. 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. Metabolism of halocarbons to the $\cdot CO_2^-$ 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. A good 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 the free radical intermediates that are causally related to halocarbon-induced hepatotoxicity. Recently, the $\cdot CCl_3$ and $\cdot CO_2^-$ radical adducts also have been detected in the bile from anesthetized rats. Hypoxia or pretreatment with phenobarbital has been reported to enhance the hepatoxicity of CCl_4 *in vivo*; these treatments also produced an increase in the biliary concentration of the PBN/$\cdot CCl_3$ radical adduct and in the $\cdot CCl_3$-derived PBN/$\cdot CO_2^-$ radical adduct as well. ESR analysis of bile from animals treated with free radical traps and other xenobiotics, such as ethanol, may prove useful in monitoring hepatic free radical-adduct formation *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50089-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
Other:	Mark Burkitt	Visiting Fellow	LMB	NIEHS
	William D. Flitter	Visiting Fellow	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	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:
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.)*

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. The reaction of the hydroxyl radical, generated by a Fenton system, with pyrimidine deoxyribonucleotides was investigated using the ESR technique of spin trapping. The spin trap t-nitrosobutane was employed to trap secondary radicals formed by the reaction of the hydroxyl radical with these nucleotides. The results presented here show the hydroxyl radical attack on thymidine, 2-deoxycytidine 5-monophosphate and 2-deoxyuridine 5-monophosphate produced nucleotide-derived free radicals. The results indicate that ·OH radical attack occurs predominantly at the carbon-carbon double bond of the pyrimidine base. The ESR studies showed a good correlation with previous work produced by authors who used x- or γ-ray irradiation to generate the hydroxyl radical. A thiobarbituric acid assay was also used to monitor the damage produced to the nucleotides by the Fenton system. These results showed qualitative agreement with the spin trapping studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50090-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Herbert Sipes	Research Chemist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	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:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50091-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	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:
1.2	0.7	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 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 a 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). The results of a series of experiments with sulfhydryl reagents and C-13-labelled phenylhydrazine led us to assign this DMPO radical adduct to the trapping of a hemoglobin-derived thiyl free radical. In addition to phenylhydrazine the hydrazine-based drugs isoniazid, iproniazid, phenelazine, and hydralazine were examined. Of the four drugs, only phenelzine and iproniazid were able to induce the formation of the DMPO/hemoglobin thiyl free radical adduct *in vivo*, whereas only phenelzine and hydralazine were able to form this adduct *in vitro*. We were able to decrease the *in vivo* iproniazid-induced adduct formation by pretreating the rats with bis-*para*-nitrophenylphosphate, an arylamidase inhibitor. Our results support the idea that iproniazid is hydrolyzed in the liver to a more reactive metabolite, most likely isopropylhydrazine, which is subsequently released into the blood stream. DMPO/hemoglobin thiyl free radical formation is not limited to hydrazines, but forms when either hydroperoxides or aromatic hydroxylamines react with oxyhemoglobin. This species may be an *in vivo* indicator of free radical damage to red blood cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50092-02 LMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Programer	CSL	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biophysics

SECTION
Molecular Biophysics

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

The project has been combined with Z01 ES 50113-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50093-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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, Division of Phyisology, Department of Cell Biology, Duke University Medical Center, Durham, NC. 27710

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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Z01 ES 50110-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50094-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	Michael S. Perlman	Senior 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.

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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Z01 ES 50111-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50095-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	Scott A. Gabel	Biologist	LMB	NIEHS
	Donald G. Davis	Expert	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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Z01 ES 50112-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50096-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

COOPERATING UNITS *(if any)*

Dr. Charles Hill, Dept. Poultry Sci., N.C.S.U., Raleigh, NC; Drs. N. Sharp & J. Kornegay, Dept. of Companion Animals & Special Species, Dr. S. VanKamp, Dept. of Food Animals, N.C. State School of Veterinary Medicine, Raleigh, NC

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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:
1.0	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.)*

Non-cyclic phosphodiesters, which are generally found to be present at the millimolar level in tissues from a wide variety of organisms, constitute a relatively abundant class of phosphorus-containing metabolites. Glycerophosphoryl choline (GPC) and glycerophosphoryl ethanolamine (GPE) are most frequently observed in mammalian tissues, however serine ethanolamine phosphodiester (SEP) and threonine ethanolamine phosphodiester (TEP) analogs are found in other species such as chickens and fish, respectively, as a consequence of the relatively high abundance of these compounds and the presence of an NMR detectable phosphorus nucleus in a unique spectral range, *in vivo* ^{31}P NMR provides a unique opportunity for studying the role of these compounds. an extensive series of NMR measurements has suggested that a principal function of these compounds may be to act as inhibitors of lysolecithianse, and in turn as regulators of membrane composition and structure. During the past year, enzymatic studies were carried out demonstrating that 50% inhibition of rat liver lysolecithianse is observed at 1 mM and 5 mM levels of GPC and SEP, respectively. Further studies of the effects of food and water deprivation on phosphodiester content and lipid composition of chicken kidney were also carried out. The SEP content of the kidney was found to more than double after 24 hours, and preliminary lipid analyses suggest a loss of phospholipid. We have recently noted that GPC levels are extremely high in semen. Studies of samples from a dog model of muscular dystrophy indicate significant changes in GPC levels, as well as marked phosphodiesterase activity. Further studies on the relationship of these changes are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50097-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
OTHER:	Jos de Wit	Chemist	LMB	NIEHS
	Arthur Mosley	Chemist	LMB	NIEHS
	Carol E. Parker	Chemist	LMB	NIEHS
	Bernard Escoffier	Visiting Fellow	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Z01 ES 50107-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50098-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
OTHER:	Sunita Verma	Visiting Fellow	LMB	NIEHS
	Leesa Deterding	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Carl 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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The project has been combined with Z01 ES 50108-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50099-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	Bernard Escoffier	Visiting Fellow	LMB	NIEHS
	Sunita Verma	Visiting Fellow	LMB	NIEHS
OTHER:	Jos de Wit	Chemist	LMB	NIEHS
	L.T. Burka	Research Chemist	DTRT/STB	NIEHS
	F. Kari	Research Chemist	DTRT/CTEB	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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Z01 ES 50106-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50100-03 LMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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
OTHER:	Leesa Deterding	Chemist	LMB	NIEHS
	John Dino, Jr.	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*
Andrea Dietrich, Guest Worker, Dr. L.M. Ball, A. Bartczak, Dr. A. Gold, Dept. Env. Sci., Professor D.G. Kaufman, Dept. of Pathology, UNC, Chapel Hill, NC; Drs. S. 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:

CHECK APPROPRIATE BOX(ES)

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

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

This project has been combined with Z01 ES 50106-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50101-03 LMB

PERIOD COVERED: October 1, 1988 to September 30, 1989

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

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Steven R. McGown	Chemist	LMB	NIEHS
	L.T. Burka	Chemist	DTRT/STB	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:
0.15	0.05	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this project is to identify the glucuronidase/sulfatase-treated, methylated biliary metabolites in rats orally administered 2,3,7,8-tetrachlorodibenzofuran (TCDF) which is a highly toxic contaminant sometimes found in commercial chlorinated phenols and polycyclic biphenyls. The first step in this project was the mass spectral characterization of synthetic potential metabolites along with determination of their GC retention windows.

The GC/MS data for 2-methoxy-3,7,8-trichlorodibenzofuran, 3-methoxy-2,7,8-tetrachlorodibenzofuran 1-methoxy-2,3,7,8-tetrachlorodibenzofuran, 3-methoxy-2,4,7,8-tetrachlorodibenzofuran and 4-methoxy-2,3,7,8-tetrachlorodibenzofuran were obtained. These data were used as standards to compare with the isolated biliary metabolites. Two components of methylated rat bile extract were determined to be tetrachlorodibenzofuran metabolites. On the basis of their mass spectra and retention times, we have been able to establish the identities of the major metabolites to be 4-methoxy-2,3,7,8-tetrachlorodibenzofuran and 3-methoxy-2,7,8-trichlorodibenzofuran. In addition a small amount of unmetabolized TCDF was identified in bile extract. These results were confirmed by analysis of the biliary metabolites of a second rat. Based on these identifications, it appears that the preferred site of metabolism in TCDF is near the furan oxygen with oxidation of the C-H bond taking precedence over oxidation of the C-Cl bond.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50103-03 LMB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	DBRA/EB	NIEHS
OTHER:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Steven McGown	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:
0.25	0.05	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.

We have examined blood and cerumen samples for 2,3,7,8-tetrachlorodibenzofuran and hexachlorodibenzofuran using selected ion monitoring at a mass resolution of 5,000. Instrument sensitivity during these analyses was such that 50 femtograms of analyte could be detected on an absolute level with a signal to noise of *ca.* 20:1. Taking into account sample volume and recovery yields, the practical limits of detection in the actual samples was *ca.* 2.4 picogram per sample (2.4 parts per trillion). No TCDFs or HCDFs were observed in these samples. To verify these results, selected samples will be analyzed using the selected decomposition monitoring capabilities of the concept I-SQ hybrid mass spectrometer. This technique is less sensitive than the high resolution selected ion monitoring technique but is more selective. These results are also consistent with the PCB results obtained by RTI in which no PCBs were observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50104-03 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Elizabeth Murphy	Senior Staff Fellow	LMB	NIEHS
	Louis Levy	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Melvyn Lieberman, Division of Physiology, Department of Cell Biology, Duke University Medical Center, 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:
1.2	0.8	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.)*

There has been considerable recent interest concerning the role of ionized cell magnesium (Mg_i) in the regulation of cell function. Grubbs and coworkers have suggested that hormones may modulate ionized magnesium levels in the cell, which in turn may regulate the chronic sensitivity of adenylate cyclase. Furthermore, Mg_i has been shown to modulate many ion channels and may therefore play an important role in cell physiology and pathophysiology. We have loaded isolated rat liver cells and embryonic chick heart cells with our recently synthesized fluorescent magnesium indicator, FURAPTRA. Basal Mg_i levels were 0.59 ± 0.04 mM (n=5) and 0.48 ± 0.03 mM in liver and heart cells, respectively. We have examined possible mechanisms responsible for regulating Mg_i. In chick heart cells we observed that an increase in cytosolic calcium resulted in a significant increase in cytosolic magnesium, most likely due to competition for intracellular binding sites. This raises the possibility that hormones or toxins that elevate Ca_i may also elevate Mg_i. Toxic agents frequently decrease cellular ATP levels, a major chelator of cytosolic magnesium. We therefore investigated the effect of ATP depletion on Mg_i. These studies were performed in a perfused rat heart loaded with our recently developed fluorinated, NMR sensitive magnesium indicator (5F APTRA). We observed a three fold increase in Mg_i during a time in which ATP fell from ~ 10 mM to 4 mM. This increase in Mg_i is in a range which will alter calcium uptake by the sarcoplasmic reticulum, as well as plasmallemal Na-Ca exchange and K and Ca channel activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50105-02 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Analysis of Airway Epithelium Prostaglandins

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
OTHER:	Steven McGown	Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. David Henke, Dept. of Pulmonary Medicine, University of North Carolina Medical School, 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:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Z01 ES 50106-01 LMB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50106-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Collaborative Projects in 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
Other:	Carol E. Parker	Chemist	LMB	NIEHS
	Sunita Verma	Visiting Fellow	LMB	NIEHS
	Leesa Deterding/Steven McGown	Chemist	LMB	NIEHS
	William Wilson	Research Chemist	LMIN	NIEHS
	Leo T. Burka	Research Chemist	STB	NIEHS
	Frank Kari	Research Chemist	SBB	NIEHS
	Charles Jameson	Research Chemist	CTEB	NIEHS

COOPERATING UNITS *(if any)*

Drs. L.M. Ball, A. Bartczak, A. Gold, Dept. Env. Sci., Dr. D. Henke, Dept. Pulmonary Medicine, UNC Medical School, Chapel Hill, NC; Prof. Buhler, Oregon State University, Oregon

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Mass Spectrometry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.55	0.35

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Collaborative projects in the environmental health sciences includes those projects in which the mass spectrometry work-group collaborates with other groups, both within and without the institute to solve problems of mutual interest. These projects typically involve on-line separation and identification of complex mixtures and often involve use of all instrumental techniques available in the MS lab including thermospray LC/MS (TSP/LC/MS), FAB/MS and FAB/MS/MS (including the use of continuous flow techniques) and GC/MS.

A typical project is the identification of the metabolites of H.C. Blue No. 1 and H.C. Blue No.2. H.C. Blue No. 1 is a known carcinogen which differs only slightly from the non-carcinogenic H.C. Blue No. 2. The metabolic profiles in mice of these two compounds differ significantly. We are currently employing TSP/LC/MS in the analysis of the metabolic products and have identified glucuronide conjugates, dealkylated, nitro reduced and acetylated metabolites. When the metabolic profiles of these compounds have been elucidated, the differences noted will provide significant information relating to the mechanism of carcinogenicity of H.C. Blue No. 1.

Other projects, which are included in this heading, include the identification of the metabolites of 12-HETE in murine lymphocytes (dihydroxyeicosanoids), the determination of toxic senecionine alkaloids and their microsomal metabolites by TSP/LC/MS, analysis of airway epithelium prostaglandins and the determination of bradykinin in bovine milk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50107-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Development of Nanoliter Capillary LC/MS Techniques

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

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
Other:	Leesa Deterding	Chemist	LMB	NIEHS
	Arthur Moseley	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	Sunita Verma	Visiting Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor J. Jorgenson, Department of Chemistry, UNC, Chapel Hill, NC; Dr. Dr. P. Thibauis, Atlantic Research Laboratory, National Research Center, Canada; Dr. P. Kassel, MIT, Mass. General Hospital

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:
1.6	0.4	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.)*

A perennial problem in the mass spectrometric analysis of both biological and environmental samples is that the absolute level of analyte is extremely low. One approach to this problem is to develop low volume-high flux delivery systems for the mass spectrometer. We have undertaken the development of interfaces for nanoliter capillary systems and MS. These capillary systems offer the same advantages over wider-bore LC systems that capillary GC offers over packed-column GC, a high flux of analyte into the MS but with a significantly lower total analyte level necessary. Current developments are in three major areas, EI/CI instrumentation, continuous flow FAB (CF-FAB), and capillary zone electrophoresis (CZE). In the area of EI/CI instrumentation we have developed an interface for use with magnetic sector high voltage instruments. This interface permits the analysis of significantly more polar analytes, such as dipeptides and nucleosides, than can be achieved with low voltage instrumentation. We have also successfully interfaced LC with an ion trap detector which is notorious for being extremely sensitive to high source pressures as are normally encountered in LC/MS. We have developed a coaxial interface for CF-FAB for both open tubular (10μ id) and packed (50μ id) nanoliter columns. Detection limits for typical compounds using this approach are ca. 100 times lower than for conventional CF-FAB. For example we have achieved attomole detection limits for peptides and femtomole to low picomole detection limits for carbohydrates and nucleosides. These detection limits are now approaching biologically relevant levels for many compounds. We are currently exploring the coupling of this approach with micro-dialysis techniques for real-time monitoring of biochemicals in body fluids. We have developed the first successful on-line CZE/FAB/MS interface using the basic coaxial CF-FAB interface design. Separation efficiencies of 500,000 theoretical plates and detection capabilities at the low femtomole level have been successfully achieved. This is an extremely exciting area which offers great promise for the determination of low levels of polar compounds and the determination of proteins with M.W. of over 100,000 by MS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50108-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Development of Tandem Mass Spectrometry for Structure Elucidation

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

PI:	Kenneth Tomer	Research Chemist	LMB	NIEHS
Other:	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	Arthur Moseley	Chemist	LMB	NIEHS
	Sunita Verma	Visiting Fellow	LMB	NIEHS
	Leo Burka	Research Chemist	STB	NIEHS
	Thomas Eling	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

Prof. A. Spatola, University Louisville, KY; Drs. M.L. Gross and R.L. Ceray, University Nebraska; Dr. P. Thiabult, Atlantic Res. Lab., National Research Council, Canada

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	0.5	.65

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 major program in the mass spectrometry laboratory at NIEHS is the application of tandem mass spectrometric techniques (MS/MS) to the structure elucidation of compounds of interest in the environmental health sciences. The structure determination of these compounds is basic to understanding the interactions of compounds within the body, especially those due to altered metabolism and those arising through the interactions of xenobiotics and biomolecules. These techniques are important because samples of interest are often complex mixtures and because the ionization techniques applicable to these samples often provide little or no structural information.

Our approach to the development of MS/MS techniques is twofold; structure elucidation and increasing the sensitivity of the technique. Current projects in the area of structure determination include: 1) glutathione, cysteine and N-acetylcysteine conjugates of xenobiotics, including identification of conjugates excreted from challenged animals; 2) determination of the structures of backbone-modified peptides in which the amide-linkage has been replaced by another functionality such as CH_2S; 3) carcinogen-modified nucleic acid constituents such as an adduct between benzo[a]pyrene and guanosine; and 4) compounds within the arachidonic acid cascade including HETEs and leukotrienes. The major effort in increasing MS/MS sensitivity has been in the combination of high flux/low level introductory systems such as OTLC and CZE. We have successfully lowered the MS/MS acquisition levels several orders of magnitude for a number of analyte types including peptides, nucleotides, carcinogen-modified nucleosides, phospholipids and carbohydrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50109-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Peroxyl Free Radical Formation by Chloroperoxidase and Lipoxygenase

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:	Walee Chamulitrat	Visiting Fellow	LMB	NIEHS
	Thomas Eling	Research Chemist	LMB	NIEHS
	Michael Hughes	Research Chemist	LMB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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

The decomposition of organic hydroperoxides as catalyzed by chloroperoxidase was investigated with electron spin resonance (ESR). Tertiary peroxyl radicals were directly detected from incubations of *tert*-butyl hydroperoxide or cumene hydroperoxide with chloroperoxidase at pH 6.4. Peroxyl, alkoxyl, and carbon-centered free radicals from tertiary hydroperoxide/chloroperoxidase systems were successfully trapped by the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide, whereas alkoxyl radicals were not detected in the ethyl hydroperoxide/chloroperoxidase system. The classical peroxidase mechanism is proposed to described the formation of peroxyl radicals. In the case of tertiary peroxyl radicals, their subsequent self-reactions result in the formation of alkoxyl free radicals and molecular oxygen. In case of the primary ethyl peroxyl radicals, decay through the Russell pathway forms molecular oxygen. Evidence for the production of singlet molecular oxygen was found.

The lipid peroxyl radicals from the peroxidation of polyunsaturated fatty acids by soybean lipoxygenase were directly detected by the method of rapid-mixing, continuous flow ESR. When air-saturated, pH 9.0 borate buffer containing linoleic acid or arachidonic acid was mixed with lipoxygenase, fatty acid-derived peroxyl free radicals were readily detected with a characteristic *g*-value of 2.014. Fatty acids without at least two double bonds, *e.g.*, steric acid and oleic acid, did not give the corresponding peroxyl free radicals, suggesting that the formation of a bisallylic carbon-centered radical preceded that of peroxyl radical. The doublet feature of the arachidonate peroxyl spectrum was proven (by selective deuteration) to be a hyperfine coupling due to a γ-hydrogen, which orginated as a vinylic hydrogen of arachidonate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50110-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

NMR Studies of Cellular Metabolism

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:	Michael Perlman	Senior Staff Fellow	LMB	NIEHS
	Louis A. Levy	Research Chemist	LMB	NIEHS
	Donald G. Davis	Expert	LMB	NIEHS

COOPERATING UNITS *(if any)*

Professor Joseph J. Blum, Chairman, Division of Physiology, Dept. of Cell Biology, Duke University Medical Center, Durham, NC 27710

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:
1.4	0.9	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 program is aimed at the development and application of *in vivo* NMR spectroscopic methods for studying metabolism and its perturbation by chemical toxins. A principal focus of these studies has been the development of NMR active, intracellular indicator molecules to allow determination of metabolic parameters of interest in intact cells. Research has stressed the use of fluorinated indicators as a consequence of the inherent sensitivity of fluorine for NMR detection and the essential absence of background fluorine resonances from untreated cells. During the past year, it was found that useful information could be obtained by studying the transmembrane distribution of simple fluorinated compounds such as trifluoroacetate and trifluoroacetamide. The distribution can be determined in red blood cells without the need to physically separate the cells from the suspension medium as a consequence of the chemical shift difference between intra and extracellular resonances. The trifluoroacetamide is distributed according to cell volume, while the trifluoroacetate distribution reflects membrane potential. Additional development work on fluorinated calcium indicators has resulted in significant improvements in sensitivity, and further synthetic effort in this area is being carried out. In addition to the work on metabolic indicators, direct observation of cell metabolism is carried out. Recent studies have focused on the hepatic metabolism of amino sugars which have been proposed to be useful anti-tumor agents. Two dimensional proton-phosphorus correlated spectroscopic studies have allowed unambiguous analysis of the UDP sugar composition of complex mixtures obtained from the liver of treated rats. NMR studies of the metabolism of *Leishmania braziliensis*, responsible for causing the disease *Leishmania*, have also have carried out.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50111-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

NMR Studies of Biomolecular Structure, Function, and Dynamics

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

PI:	Robert E. London	Research Chemist	LMB	NIEHS
Other:	Donald G. Davis	Expert	LMB	NIEHS
	Michael E. Perlman	Senior Staff Fellow	LMB	NIEHS

COOPERATING UNITS *(if any)*

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

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:
2.5	1.8	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.)*

One pathway to understanding the biological functions of proteins, nucleic acids, polysaccharides, and other macromolecules lies in determining their structure and dynamics at the molecular level. Recent advances in NMR technology together with computer based methods and graphics provide a means to obtain quantitative, three-dimensional structure information about proteins and nucleic acids in the 10-20 kD MW range. This strategy represents the only approach available for obtaining detailed structural data for molecules in solution. During the past year we have developed new spectral assignment methods using the so called reverse detection approach, in which the spectrum of the sensitive reporting nucleus (the proton) is presented in one dimension and the spectral components of other NMR active isotopes (N-15, C-13, P-31) are arrayed in the second dimension. As a consequence of the increased dispersion in the two dimensions, spectra of moderately large enzymes such as lysozyme (MW=14,000) can be unraveled and interpreted and, due to the enhanced sensitivity, spectral information about rare and insensitive isotopes such as N-15 (nat. abundance 0.4%) obtained without isotopic enrichment. One recent application development utilizes the spin coupling interactions between the carbonyl carbons and amide protons along the backbone of a polypeptide in order to make sequence specific assignments for the peptide bradykinin. A specific structural study which has been in progress for more than a decade involves dihydrofolate reductase, a target enzyme of anti-folate drug therapy. Despite extensive research on this enzyme, its catalytic mechanism remains largely undetermined. Studies utilizing (5-N-15) and (6-C-13) labeled dihydrofolate, folate and dihydrobiopterin have been carried out in order to more fully characterize the interaction between the enzyme and its substrates. Among other results, the data provide no support for models in which there is an initial protonation at N-5.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50112-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Magnetic Resonance Imaging Studies of Heavy Metal Distribution

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

PI:	Robert E. London	Research Chemist	LMB	NIEHS
Other:	C. Tyler Burt	Expert	LMB	NIEHS
	Xiaoming Wan	Visiting 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:
2.0	1.3	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.)*

It has recently become possible to obtain spatially resolved "images" of the nuclear spins of biological and chemical materials. Magnetic resonance imaging or "MRI" is rapidly evolving into an important diagnostic tool for a wide range of human pathologies. Such imaging studies have been almost exclusively limited to the detection of protons, which in turn provide images of the abundant protonated molecules in biological tissues: fat and water. Since image intensity is dependent on the density of protons in a given sample volume, as well as on the nuclear relaxation properties of these protons, it becomes possible to study the distribution of species which can alter these nuclear relaxation parameters. We have utilized this aspect of MRI to study the distribution of manganese (II) ions in rat brain. Interest in evaluating this distribution is based on the neurotoxicity of Mn, which at excess levels can produce Parkinsonian type symptoms in humans. Magnetic resonance images of the brain of rats given various i.p. doses of manganese chloride showed localized, time dependent changes due to the accumulation of manganese ions. The major increases in intensity of T1 weighted images were observed in the ventricles, and in the pituitary and pineal glands. Since manganese frequently acts as a calcium antagonist, such accumulations could lead to toxicological effects by antagonizing the action of calcium ions. The rapid appearance of manganese in the ventricular cerebrospinal fluid indicates that manganese readily crosses the filtration barrier of the choroid plexus. Although the large majority of MRI studies involve observation of protons, some studies have been carried out on other nuclei as well. During the past year we have carried out both spectroscopic and imaging studies on the distribution of cesium ions. The unique sensitivity of the cesium resonance shift to the local chemical environment allows intra and extracellular resonances to be distinguished, as well as opening up the possibility for observing separate resonances from intracellular organelles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50113-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Studies on the Free Radical Metabolite of Acetaminophen

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	Programmer/Analyst	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:
1.2	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.)*

Acetaminophen, a mild analgesic and a antipyretic drug is both hepatotoxic and nephrotoxic at high doses. Under therapeutic dose conditions tissue glutathione is known to protect the liver by reacting with quinoneimine form of acetaminophen. It is also suggested in the literature that glutathione can detoxify the acetaminophen phenoxyl radical. In this work we have investigated the mechanism of detoxification of the acetaminophen phenoxyl radical. Acetaminophen was oxidized by horseradish peroxidase system to generate the acetaminophen phenoxyl radical. This radical reacts with both glutathione and ascorbate.

$$RO^{\bullet} + GSH \rightarrow ROH + GS^{\bullet} \quad (1)$$

$$GS^{\bullet} + GS^{-} \rightarrow [GSSG]^{-} \quad (2)$$

$$[GSSG]^{-} + O_2 \rightarrow GSSG + O_2^{-} \quad (3)$$

$$RO^{\bullet} + AH^{-} \rightarrow ROH + A^{-} \quad (4)$$

In the presence of glutathione both the phenoxyl radical and the disulfide radical anion were detected (eq. 1 & 2). In the presence of ascorbate, only the ascorbyl radical was detected (eq. 4). These observations suggests that ascorbate rather than glutathione is more likely to be involved in the detoxification mechanism *in vivo*. The rate of formation of the disulfide radial anion was also measured by the oxygen-consumption measurements (eq. 3), and the GSSG formed was determined by optical measurements. The carcinogenic action of 4-nitroquinoline N-oxide has been attributed to hydroxyaminoquinoline N-oxide and its oxidation products. Therefore we studied the reaction of hydronitroxide quinoline N-oxide radical generated by horseradish peroxidase, with glutathione and ascorbate. We found that ascorbate reduces the hydronitroxide free radical completely, but glutathione reacts very slowly. The disulfide radical anion was not detected in this case, thus showing that ascorbate is a better free radical reducing agent than glutathione, and may be more important in deactivation *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50114-01 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Xenobiotic Metabolism in Lower Species

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

PI: Phillip W. Albro Research Chemist LMB NIEHS

COOPERATING UNITS *(if any)*

Comparative Medicine Branch

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Metabolism

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.)*

This project has two objectives: (1) To explore the metabolic capabilities of invertebrate species, with emphasis on the ability to metabolize common environmental pollutants. Initially we are studying compounds whose metabolism is well understood in mammals, in order to make comparisons. (2) To investigate the possibility that some types of metabolism studies, especially those which must be performed *in vivo*, can be effectively accomplished in species having less developed nervous systems (and are thus presumably less subject to pain and distress) than the more commonly used rodent species. We are presently studying *Lumbricus terrestris*, the common earthworm ("night crawler") because it has been relatively neglected in studies of metabolic capabilities, and because it is typically exposed to pollutants in landfills and therefore may have experienced local selection for resistant and non-resistant varieties. This species performs essentially all of its digestion and metabolism in the gut, which contains a wide range of hydrolytic and oxidative enzymes including what appears to be a P-450 cytochrome. Our initial studies involving plasticizers and halogenated pesticides suggest that some of these compounds are metabolized in a manner similar to what occurs in mammals, while others are metabolized quite differently.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 50115-01 LMB

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: A Computerized Spin Trapping Data Base

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

PI:	Anson A.S.W. Li	Staff Specialist	CSC	NIEHS
	Colin F. Chignell	Chief	LMB	NIEHS

COOPERATING UNITS *(if any)*

Dr. Garry R. Buettner, University of Iowa

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:
0.7	0.2	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Spin trapping is a powerful and convenient technique for the study of free radical reactions. The breadth of applications ranges from clinical studies to high-energy physics. Over 1500 references to the technique have accumulated in Chemical Abstracts. STDBII, a spin trapping database, has been implemented on an IBM PC/AT. The package operates with no 'add-ons'. The program is powerful yet user-friendly; the command structure is similar to the familiar 1-2-3 light-bar menu; search strategy employs the method of Query-by-Example (QBE); logical combination of any fields is accomplished by using AND, OR, NOR, and EXCEPT. Presently, STDBII (4.0) contains files for 5,5-dimethylpyrrolidine-N-oxide (DMPO), alpha-phenyl-N-tert-butyl nitrone (PBN), 2-methyl-2-nitrosopropane (MNP), alpha-(4-pyridl-1-oxide)-N-tert-butyl nitrone (POBN), nitrosodurene (ND) and 3,5-dibromo-nitrosobenzene sulfonate (DBNBS). Data for other less popular traps are included in a catch-all file. Our goal is to incorporate all published work that relates to spin trapping. Presently, the database files have more than 1100 references with over 2500 parameter entries. The STDBII files contain information on: 1) spin trap used; 2) radical trapped; 3) hyperfine splittings reported; 4) solvent; 5) g-value, if reported; 6) a terse summary on how the radical was produced and observed; 7) full bibliographic data; and 8) retraction on anything by the author. STDBII helps researchers: 1) in identification of spin adducts from the sometimes unique hyperfine splitting parameters; 2) as a key to the spin trapping literature 3) as a vehicle to correct published errors. STDBII is now available to researchers both inside and outside NIEHS. The package includes a user manual that lists all of the compiled information on spin trapping. Scientists who do not presently have access to an IBM/PC can still benefit from STDBII because all of the database entries are printed in the STDBII User Manual.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80008-15 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Wayne Glasgow	IRTA	LMB	NIEHS
	Mike Luster	Research Microbiologist	TRTP	NIEHS
	Julie Angerman-Stewart	Biologist	LMB	NIEHS
	Jan Capps	Bio. Lab. Technician	LMB	NIEHS
	Carl Barrett	Research Chemist	LMC	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Prostaglandin Biochemistry

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.6	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.)

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 investigated the mechanism responsible for the inhibition of PHS by phenylbutazone (Pb). Pb must be oxidized by PHS peroxidase for inhibition to occur. The metabolite Pb-peroxide was not a substrate for PHS peroxidase nor was it an inhibitor of the enzyme. Instead, the data indicate instead that intermediate Pb-alkoxyl or peroxyl radicals are responsible for the inhibition and that the hydroperoxide undergoes a Russell reaction to yield the corresponding alcohol. We have also investigated the mechanism for the inhibition of PHS by eugenol. Although engenol is a substrate for the peroxidase, inhibition is not via a reduction in peroxide tone but rather by competition with the substrate arachidonic acid. We have also studied the role of arachidonic acid metabolism in the response of cells to growth factors. For BALBc cells, PGs are required for EGF but not PDGF stimulated DNA synthesis. In contrast PGs are potent inhibitors of EGF-stimulated DNA synthesis in Syrian hamster embryo (SHE) cells. Also supp+ cells make more PGs than supp-, suggesting a possible relationship to the suppressor genes in these cells. In response to EGF both the BALBc cells and SHE cells metabolize linoleic acid to 9/13-hydroxyoctadecadienoic acid (9/13-HODD), which when added to these cells enhances DNA synthesis. Inhibition of the 15-lipoxygenase, that catalyzes this oxidation, inhibits DNA synthesis. The data indicate that EGF-stimulated DNA synthesis requires the linoleic acid metabolites and that growth factors either activate or induce the synthesis of the 15-lipoxygenase. Studies are currently underway to further investigate these problems. These findings suggest a possibly important role for arachidonic and linoleic acid metabolism in regulating cell growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80035-13 LMB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Cooxidation of Xenobiotics by the Prostaglandin Synthetase

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

PI:	Thomas Eling	Research Chemist	LMB	NIEHS
Other:	Ronald Mason	Research Chemist	LMB	NIEHS
	David Thompson	Staff Fellow	LMB	NIEHS
	John Curtis	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:
4.1	3.1	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The 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, are metabolized to mutagens by PHS. PHS dependent oxidation occurred by a free radical mechanism and resulted in the formation of DNA adducts which can be used as *in vivo* markers for PHS-dependent oxidation. We have further studied the formation of amine mutagens by PHS using bacterial tester systems having different levels of acetylase activity. Our data indicate that acetylase plays an important role in the formation of free radical mutagens from aromatic amines, including bladder carcinogen such as benzidine derivatives. We have also examined the interaction between bisulfite oxidation and the carcinogen benzo[a]pyrene (BP) -7,8-diol. Enhanced epoxidation is observed but the formation of sulfonates of BP-7,8-diol are seen in the reaction of sulfur trioxide anion radical with BP-7,8-diol. We further studied peroxidase catalyzed GSH conjugate formation and showed that this reaction occurs with a number of chemicals that contain a conjugated double bond adjacent to a aromatic ring. The reaction appears to be a general mechanism for conjugate formation. We have also shown that P-450 metabolites of BP will enhance this reaction which serves as a mechanism for detoxication of carcinogens. We have also started a new study on the dealkylation of aromatic amines by peroxidases using as model compounds the calcium ion indicator Quin-2 and its analogues. Our investigation of the activation of the heterocyclic aromatic compounds by PHS has continued. Our data suggest that PHS is a versatile enzyme system that can catalyze a variety of reactions which are important in the conversion of chemicals to carcinogenic metabolites in extra hepatic tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 23000-02 LMC

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Events in Hepatocarcinogenesis of B6C3F1 Mice

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

PI:	R.W. Wiseman	NRC Fellow/Sr. Staff Fellow	LMC	NIEHS
Others:	E. Hou	Biologist	LMC	NIEHS
	C.J. Cochran	Biological Lab. Tech.	LMC	NIEHS

COOPERATING UNITS *(if any)*
DIR/LMC, NIEHS (J.C. Barrett)
University of Wisconsin (A. Messing)
DIR/LRDT, NIEHS (E.M. Eddy & E.F. Goulding)
DTRT/CGTB, NIEHS (W.D. Caspary)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Chemical Carcinogenesis

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.50	0.25

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

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

This project's goal is to define alterations in proto-oncogenes and tumor suppressor genes that play a role in B6C3F1 mouse hepatocarcinogenesis. PCR amplification and DNA sequencing have been employed to characterize the activating mutations in nine unusual H- and K-ras proto-oncogenes of chemically induced B6C3F1 mouse hepatomas that lacked H-ras 61st codon alterations. In each tumor a CG->GC transversion was observed at the 1st position of codon 13; this result is quite surprising based on predicted mutagenic specificity and the absence of any 12th codon ras mutations in over 200 hepatomas examined to date. The presence of a single oncogene in transgenic mice is generally insufficient for malignant transformation. Since B6C3F1 hepatomas frequently contain mutated H-ras genes, we asked whether ras activation is a secondary genetic event during hepatocarcinogenesis in transgenic mice carrying a SV40 large-T antigen/metallothionein enhancer construct (provided by A. Messing, U.W.). H-ras genes of these hepatomas were amplified by PCR and sequenced, but no mutations were detected; this study will be extended with additional transgenic constructs. Inactivation of tumor suppressor genes is another common genetic alteration in human cancers. This has been detected by loss of heterozygosity in specific chromosomal regions using restriction fragment length polymorphism (RFLP) analysis. We have extended these studies to DNA from chemically induced B6C3F1 hepatomas with several RFLP probes, including the retinoblastoma gene, but no losses of heterozygosity have been detected to date. In order to generate tumors in additional tissues for RFLP analyses a panel of transgenic mouse lines containing oncogenes under the control of various tissue specific transcriptional regulatory elements is being constructed in collaboration with M. Eddy and G. Goulding (LRDT). A collaboration has also been initiated with W. Caspary (CGTB) using a contract mechanism to generate and map a large number of new RFLP probes for B6C3F1 mice. NTP bioassay tumors from a variety of tissues will be screened with these probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25001-12 LMC

PERIOD COVERED
October 1, 1988 - September 30, 1989

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

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

PI:	J.C. Barrett	Research Chemist	LMC	NIEHS
Others:	P. Lamb	Biologist	LMC	NIEHS
	R. Wiseman	NRC Fellow	LMC	NIEHS

COOPERATING UNITS *(if any)*
Laboratory of Reproductive and Developmental Toxicology, Dir (Dr. J. McLachlan)
Mt. Sinai Hospital (Dr. N. Suzuki)
Nippon Dental University, Tokyo (Dr. T. Tsutsui)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Cellular Carcinogenesis

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 (DES), asbestos, arsenicals and benzene) usually do not induce gene mutations. We have examined the ability of these chemicals to induce morphological transformation, gene mutations and chromosome mutations in Syrian hamster embryo (SHE) cells in culture. We have previously proposed that the mechanism of action of DES is related to its ability to induce numerical chromosome changes, i.e., aneuploidy. Currently, DES-induced aneuploidy is being examined in the newborn mouse genital tract to test whether these changes occur in vivo in the target tissue. The mechanism of another important human carcinogen, asbestos, was also examined. We have proposed that asbestos induces cell transformation due to its ability to induce chromosomal changes. We have identified a possibly novel transforming oncogene in human mesotheliomas, and currently we are cloning this gene. Sodium arsenite and sodium arsenate are inactive as gene mutagens but are potent inducers of cell transformation, chromosome aberrations and gene amplification. Benzene induces cell transformation but is a weak gene mutagen. This chemical is a very effective inducer of aneuploidy in this system. These results further support our hypothesis that cell transformation involves a chromosomal mutation and suggest an important role for carcinogen-induced aneuploidy in carcinogenesis. Di(2-ethylhexyl)phthalate (DEHP), a commonly used plasticizer, induces peroxisome proliferation in liver cells and hepatocellular carcinomas in rodents. We have shown that DEHP induces morphological transformation, chromosome aberrations, and peroxisome proliferations of cultured Syrian hamster embryo (SHE) cells. The transformation frequency and chromosomal aberrations by DEHP was enhanced in the presence of rat liver post-mitochondrial supernatant. The results suggest a possible involvement of genetic damage by DEHP metabolites in the induction of transformation of SHE cells. No clear relationship between induction of peroxisome proliferation and cell transformation was observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25029-05 LMC

PERIOD COVERED
October 1, 1988 TERMINATED February 28, 1989

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	Guest Worker	LMC	NIEHS
Others:	Bartel Turk	Guest Worker	LMC	NIEHS

COOPERATING UNITS (if any)
University of Virginia (T. Parsons)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Cellular Carcinogenesis

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

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

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

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

Tumor-derived Syrian hamster embryo (SHE) cell lines, induced in vitro by treatment with chemical carcinogens, contained increased levels of pp60$^{c\text{-}src}$ kinase activity compared to preneoplastic parental cell lines and normal SHE cells. The increased kinase activity did not result from an increase in the pp60$^{c\text{-}src}$ content of the SHE cell lines, but represented a 4-11 fold increase in pp60$^{c\text{-}src}$ kinase specific activity. Both the extent of phosphorylation and the velocity of pp60$^{c\text{-}src}$ phosphotransferase activity were increased in the tumor-derived cell lines. SHE cell lines producing chicken pp60$^{c\text{-}src}$ were isolated following co-transfection with plasmids bearing the chicken c-src and neo^{r} genes. Chicken pp60$^{c\text{-}src}$ expressed in an asbestos-transformed tumor-derived cell line showed an approximate 3-fold activation of tyrosine kinase activity compared to chicken pp60$^{c\text{-}src}$ expressed in the preneoplastic cell line. We suggest that these results indicate that activation of pp60$^{c\text{-}src}$ is mediated by trans-acting cellular factors present in the tumor-derived cells. Analysis of pp60$^{c\text{-}src}$ in normal SHE cells, preneoplastic cell lines and tumor-derived cell lines showed no alteration in the phosphorylation of tyr-527 or tyr-416, two tyrosine residues whose phosphorylation states have been associated with modulation of kinase activity. In addition, a strong correlation was observed between the activation of endogenous pp60$^{c\text{-}src}$ tyrosine kinase specific activity and the presence of additional phosphotyrosine-containing proteins. These studies indicate that the neoplastic progression of cells may be accompanied by the activation of proto-oncogene products, such as the pp60$^{c\text{-}src}$ tyrosine kinase, by mechanisms that may not directly involve genetic alteration of the proto-oncogene DNA sequence and that novel tyrosine phosphorylations may result from this activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 25031-03 LMC

PERIOD COVERED
October 1, 1988 - September 30, 1989

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	LMC	NIEHS
Others:	J. Hosoi	Visiting Fellow	LMC	NIEHS
	J. Montgomery	IRTA Fellow	LMC	NIEHS
	J. Stowers	IRTA Fellow	LMC	NIEHS
	H. Satoh	Visiting Fellow	LMC	NIEHS
	J. Boyd	Staff Fellow	LMC	NIEHS
	C. Jones & R. Whitehead	Q Appointments	LMC	NIEHS
	L. Annab	Biologist	LMC	NIEHS

COOPERATING UNITS *(if any)*
Chemical Carcinogenesis Group, LMC
Kanagawa Cancer Center (Dr. M. Oshimura)

LAB/BRANCH
Laboratory of Molecular Carcinogenesis

SECTION
Cellular Carcinogenesis

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.)*

Cancer development in humans and animals is a multistep process involving at least two classes of genes, proto-oncogenes and tumor suppressor genes. We have shown that 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. The loss or inactivation of tumor suppressor genes is an essential step in the multistep neoplastic transformation of SHE cells. Non-tumorigenic variants have been isolated that have lost (sup^-) or retained (sup^+) the ability to suppress tumorigenicity of tumor cells in cell hybrids. Fusions of sup^+ or sup^- variants with different tumor cells show different patterns of suppression indicating that a family of tumor suppressor genes exists in these fibroblast cells. Currently, several strategies to clone tumor suppressor genes are in progress. cDNA libraries of sup^+ hamster cells have been screened with RNA from sup^+ or sup^- cells and differentially expressed cDNAs have been cloned. Two-dimensional gel analyses of proteins showed that a reduction in the expression of tropomyosin I correlates with the loss of the tumor suppressor function. A cellular phenotype associated with the loss of tumor suppressor gene function has also been found. Sup^- cells suspended in agar respond reversibly to transforming and normal growth factors by forming colonies in agar whereas sup^+ cells fail to grow. Tumor suppressor genes can be mapped to specific chromosomes by introduction of normal chromosomes into tumor cells by microcell fusion. We have shown that normal human chromosome 11 suppresses cervical carcinoma cells, lung adenocarcinoma cells, rhabdomyosarcoma cells, and Wilms' tumor cells, whereas chromosome 3 suppresses renal carcinoma and lung adenocarcinoma cells. An uterine endometrial cancer cell was suppressed by three different chromosomes (Nos. 1, 6, and 9). In addition to the tumor suppressor genes described above that are expressed in some immortal cell lines, tumorigenicity also may be limited by cellular senescence. Our results indicate that a gene(s), possibly involved in the senescence phenotype, can be mapped to human chromosome 1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 60147-06 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

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 Scientist	LMG NIEHS
Others:	R. L. Dunn	Biologist	LMG NIEHS
	R. Cornacchio	Stay-In-School Employee	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Molecular 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 determined from the frequency of mutant phage before and after replication. The specificity of the DNA replication errors can be determined by DNA sequence analysis of the revertants. Since insights into SOS-modified DNA replication requires knowledge of the factors involved in maintaining normal accuracy, the latter is investigated simultaneously. E. coli mutator and antimutator strains with known (or presumed) DNA replication defects are important tools for this purpose. We have found DNA replication in crude extracts to be extremely accurate, with error rates approaching (or identical to) estimated in vivo rates. The validity of this system to study in vivo fidelity is further evidenced by the observation of increased error rates in extracts of at least two mutator strains (mutD, mutT).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61022-08 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	LMG, NIEHS
Others:	E. A. Goode-Montgomery	Geneticist	LMG, NIEHS
	G. M. Simmons	Staff Fellow	LMG, NIEHS
	W. H. Stephan	Visiting Associate	LMG, NIEHS
	B. H. Judd	Research Geneticist	LMG, NIEHS
	S. M. Huang	Geneticist	LMG, 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

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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

CHECK APPROPRIATE BOX(ES)

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

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

The main goal of this project is to study the population biology of transposable genetic elements (parasites of the genome) using Drosophila as a model system in conjunction with quantitative theoretical analysis. During this period, the research has focused on two topics: 1) What is the primary mechanism containing the numbers of transposable elements? and 2) Is the evolutionary diversity observed between copies of elements at the DNA sequence level consistent with quantitative models of the dynamics of the elements in natural populations? The cloning and DNA sequencing of copies of the transposable element hobo from sampled individuals, populations and species is ongoing. The genetic and molecular characteristics of spontaneous deletions arising from unequal crossing over is ongoing. The role of heterozygosity on the rate of unequal crossing over is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61024-07 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMG, NIEHS
Others:	J. F. Sterling	Biologist	LMG, NIEHS
	J. P. Graves	Biologist	LMG, NIEHS
	W. Gibson	Research Chemist	LMG, NIEHS
	S. S. Carpenter	Biological Aid (SIS)	LMG, NIEHS
	T. J. Maness	Biological Aid (SIS)	LMG, NIEHS
	S. Lingle	Biological Aid (SIS)	LMG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	1.0	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.)*

We are investigating the molecular mechanism of action of the suppressor-of-sable [su(s)] system of Drosophila melanogaster: recessive su(s) mutations suppress recessive mutations at the vermilion (v) locus that are caused by insertions of the mobile element 412 in 5´transcribed but untranslated sequences. Current evidence suggests that this suppression is effected by the removal of the 412 sequences from the primary transcript utilizing cryptic donor and acceptor splice sites located within the LTRs closely adjacent to LTR-genomic DNA junction.

Genomic DNA and cDNA sequences of su(s) have been cloned and sequenced. The cDNA contains an open reading frame that could translate a putative protein of 1322aa. A portion of the protein consists of highly charged amino acids and has similarity to the human, Xenopus and Drosophila 70K U1 binding proteins and to the Drosophila suppressor of white-apricot and transformer proteins, all of which are known to be RNA binding proteins. Portions of the cloned 25 kb of DNA have been reintroduced by P element mediated transformation and allow an identification of genetic function with messages produced by the region. A segment of DNA which is homologous with only the su(s) message rescues both the primary phenotype of suppression and a secondary phenotype of cold-sensitive male sterility. Antibodies raised against fusion proteins produced by portions of the above ORFs specifically recognize the s(s) portion of the fusion protein. When these antibodies are used to probe total protein preparations from adults, they recognize a protein that is much more abundant in testis than in other male tissues or in any female tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61037-05 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMG NIEHS
Others:	R. K. Hamatake	Senior Staff Fellow	LMG NIEHS
	H. Araki	Visiting Associate	LMG NIEHS
	K. Kitada	Visiting Associate	LMG NIEHS
	H. Hasegawa	Visiting Fellow	LMG NIEHS
	J. Nakao	Visiting Fellow	LMG NIEHS
	A. B. Clark	Biologist	LMG NIEHS
	T. Sugino	Guest Worker	LMG NIEHS

COOPERATING UNITS *(if any)* Lucy M. S. Chang, Prof. & Chairperson, Dept. of Biochem., The Uniformed Ser. Univ. of Health Sci., Bethesda, MD; Dr. L. H. Johnston, Group Leader, Lab. of Cell Propagation, Nat. Inst. for Med. Res., London, England; Dr. P. Burgers, Assoc. Prof., Dept. of Biochem., Washington Univ., St. L., MO

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	3.1	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.)*

An in vitro DNA replication system using yeast 2-μm and ARS (autonomously replicating sequences) plasmid DNAs, developed in this laboratory, has been used to investigate the mechanism of DNA replication in yeast. To identify and purify enzymes and components required for yeast chromosomal DNA replication, the crude extract system has been fractionated and reconstituted with the help of several temperature-sensitive chromosomal DNA replication mutants. To aid in overproducing and purifying such DNA replication proteins, the DBF1 and 2 genes (which are required for the elongation step of DNA replication) and the TS26 gene (required for the initiation of DNA replication) have been cloned, their nucleotide sequences determined, antibodies to them raised and their regulation studied. Using complementation assay and antibodies, the purification protocol for each protein has been established. During the course of this study, it has been established that the DBF2 protein is a serine/threonin-specific protein kinase controlled by cell-division-cycle, suggesting that protein phosphorylation regulates not only the initiation of DNA replication but also elongation of DNA synthesis in yeast.

A new yeast DNA polymerase (DNA polymerase IV) has been purified to homogeneity for the first time and studied extensively, besides the previous purified DNA polymerases I, II, and III. Using inhibitors and antibodies against the purified DNA polymerase IV, it has been established that DNA polymerase IV is unique and has a different function in yeast cells. In order to study its function(s), the molecular cloning of DNA polymerase IV has been carried out using both antibodies and the amino acid sequences of oligopeptides generated from the purified DNA polymerase IV. In the meantime, mammalian PCNA/cyclin, which is under cell-cycle control and is a subunit of mammalian DNA polymerase δ, has been shown to stimulate the yeast DNA polymerase IV reaction like yeast DNA polymerase III.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 61039-05 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

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

Mechanisms of DNA Recombination and Repair in the Yeast Saccharomyces cerevisiae

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

PI:	A. Sugino	Visiting Scientist	LMG NIEHS
Others:	C. C. Dykstra	Guest Worker (NRC Fellow)	LMG NIEHS
	A. B. Clark	Biologist	LMG NIEHS
	T. Sugino	Guest Worker	LMG NIEHS

COOPERATING UNITS (if any)

Dr. F. E. Coleman-Wilson, Ass. Prof., Dept. of Microbio., Univ. of NC at Asheville, NC; Dr. K.-I. Arai, Dir., Dept. of Mol. Biol., DNAX Res. Inst., Palo Alto, CA

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Mutagenesis Section

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

An ATP-independent activity which catalyzes the transfer of one strand from a linear duplex DNA molecule to a complementary circular single strand has been detected in crude extracts from both mitotic and meiotic yeast cells. The assay requires the addition of yeast single-stranded DNA binding protein (ySSB). The polypeptide (yeast Strand Transfer Protein α, ySTPα) responsible for this activity has been purified to homogeneity from meiotic cells, characterized, and antibodies raised from a rabbit. Using the antibodies, the gene for ySTPα has been isolated, its nucleotide sequence determined, and its regulation studied. Although the gene is not essential for mitotic cell growth, it is required for meiotic homologous recombination, proving that ySTPα is one of the meiotic recombination components in yeast and that the ATP-independent reactions catalyzed by ySTPα are biologically important. The ySTPα mRNA and polypeptide are constitutively expressed in both mitotic and meiotic yeast cells. However, the polypeptide is uniquely activated during meiosis by a mechanism that has not yet been identified.

An activity (ySTPβ) similar to that of ySTPα has been purified to homogeneity from yeast mitotic cells crude extracts. From immunological and biochemical studies, it has been concluded that ySTPβ is encoded by a gene different from that of ySTPα. By using antibodies and partial amino acid sequences of oligopeptides generated from the purified ySTPβ, the molecular cloning of the gene and its nucleotide sequencing studies have been achieved.

In addition, one of the yeast DNA repair genes, RAD18, has been cloned, its nucleotide sequence has been determined, its polypeptide overproduced and purified, and its regulation studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61041-03 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	LMG, NIEHS
Others:	Naohiko Miyashita	Visiting Fellow	LMG, NIEHS
	Gail M. Simmons	Staff Fellow	LMG, NIEHS
	Wolfgang Stephan	Visiting Associate	LMG, NIEHS
	William Quattlebaum	Biologist	LMG, NIEHS
	Barbara Lange	Biologist	LMG, NIEHS

COOPERATING UNITS *(if any)*

Dr. Norman Kaplan, DBRA/SBB; Dr. Richard Hudson, Dept. of Evolution and Ecology, University of California, Irvine, CA; Dr. Martin Kreitman, Department of Biology, Princeton University

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	3.0	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.)*

The primary focus of this project is the investigation of the relative roles of mutation, recombination, genetic drift and natural selection in shaping the levels of genetic variation observed at the DNA level. Several experiments address the fundamental question: what is the quantity and quality of molecular population genetic variation? To obtain a general answer many loci (white, yellow to achete, g-6-phd, forked, vermilion, suppressor of forked and zeste) in natural populations of Drosophila have been surveyed. A specific question in these and comparative studies with other species is the consequence of large differences in the amounts of crossing over per kilobase on the molecular genetic variation. The experimental results in conjunction with theoretical studies suggest that reduced levels of DNA sequence polymorphism in chromosome regions where crossing over is reduced are caused by the "hitch-hiking" effect of rare selectively favored and linked mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61042-03 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Gene Expression During Drosophila Development

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

PI:	Michael Abbott	Staff Fellow	LMG, NIEHS
Others:	Willie Gibson	Research Chemist	LMG, NIEHS
	Krista Cartledge	Biological Aid (SIS)	LMG, NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Eukaryotic Gene Structure and Function Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.17	1.0	1.17

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The long-term goal of this project is to study the genetic control of morphogenesis. Our approach is to identify and characterize genes whose products have roles in morphogenetic processes occurring during the embryonic and post-embryonic development of Drosophila melanogaster. The specific processes under investigation are: (1) the transformation of the head of the embryo into the anterior end of the larva, (2) the rotation of the male genital disc during the pupal stage, and (3) the development of the sex-combs on the first pair of legs of the adult male fly.

One of the genes currently being investigated is head involution defective (hid). Genetic studies involving recessive mutations of hid have revealed that its expression is initially required sometime during the first half of embryogenesis for the proper development of the anterior end of the larva. Post-embryonic hid expression is required for the rotation of the male genital disc and wing morphogenesis. Further investigation into the role of this gene will involve the use of cloned hid DNA. We have cloned 70kb of DNA in the chromosomal region in which hid is located and are now searching within this DNA for the gene.

In addition to the aforementioned work, we have recently recovered 11 mutations in X-chromosome genes which affect either the rotation of the male genital disc or disrupt the formation of the male sex-combs. We are now characterizing these mutations genetically to determine how many different genes have been mutated and the precise location of each of these genes on the X-chromosome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65034-05 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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 Scientist	LMG NIEHS
Others:	R. L. Dunn	Biologist	LMG NIEHS
	R. Cornacchio	Stay-In-School Employee	LMG NIEHS

COOPERATING UNITS (if any)
R. P. Fuchs, Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France
M. Radman, Institut Jacques Monod, Paris, France

LAB/BRANCH
Laboratory of Molecular 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. DNA sequence information is gathered on all the classes of mutations that occur: 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 thus provides a way to analyze and separate the various components of mutation. We use the lacI gene of the bacterium E. coli as a mutational target. The gene codes for the repressor of the lac operon and forward mutations to lacI⁻ are scored based on their 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, producing the mutational spectrum of interest. Comparing spectra in strains affected in various DNA repair/replication pathways is a next important step. In case of defined enzymatic pathways, the spectra provide direct direct correlations between mutational classes and their responsible pathways. In case of unknown pathways, the mutational specificity may provide new insights into the affected pathway. So far, we have determined the specificity of mutation in mutH, mutL, mutS, mutT and mutD and wild-type strains of E. coli and have gained insights into the specific contributions of DNA damage, DNA mismatch repair and exonucleolytic proofreading to mutation. In case of induced mutagenesis, the specificity of mutation is a tool to identify both the nature of the premutagenic lesions and the mechanisms by which these are converted into mutations. Recent examples of this approach are the determination of the specificity of mutagenesis by ultraviolet light and the chemical carcinogen N-acetoxyacetylaminofluorene (NAAAF).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65036-05 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

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	Head, EGSFS	LMG, NIEHS
Others:	Patricia S. Davis	Chemist	LMG, NIEHS
	Shu-Mei Huang	Geneticist	LMG, NIEHS
	Katherine M. Peterson	Biologist	LMG, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Eukaryotic Gene Structure and Function Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.50	0.25	2.25

CHECK APPROPRIATE BOX(ES)

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

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

The goal of this project is to understand more fully the mechanisms of gene regulation during eukaryotic development. The approach is to study selected loci having mutations that perturb regulatory functions. The major effort is focused on the white locus of Drosophila melanogaster. The gene encodes a protein that shares sequence similarity to ATP binding proteins, the majority of which are components of membrane transport systems. We are studying the molecular characteristic of alleles that perturb the tissue specificity, pattern regulation and allelic interaction known as transvection. The objectives are to understand how the gene responds to developmental signals and what the gene product does. Three approaches to these goals are being pursued. First a family of transposon-induced mutations that upset pigment pattern and interaction with the zeste locus have been cloned and their various molecular structures determined and compared. Second, we have placed a partial cDNA sequence from the 3´end of the gene into an expression vector and have obtained protein that was used to raise antibodies. We will examine the patterns of expression relative to developmental stages and tissues among the various regulatory mutant strains. Third, we have identified a suppressor of one of the leaky mutants. That gene has now been mapped, new alleles induced and the molecular cloning begun. The objective is to determine how the suppressor locus interacts with the white locus in normal development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65037-05 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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

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

PI:	B. H. Judd	Head, EGSFS	LMG, NIEHS
Others:	Shu-Mei Huang	Geneticist	LMG, NIEHS
	C. H. Langley	Research Geneticist	LMG, NIEHS
	E. A. Goode-Montgomery	Geneticist	LMG, NIEHS

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

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Eukaryotic Gene Structure and Function Section

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

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

CHECK APPROPRIATE BOX(ES)

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

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

This project is focused on the role of transposons in the process of spontaneous mutation and chromosome rearrangement in Drosophila. With Prof. Lim we are studying a strain that showed a burst of activity by the retrotransposon gypsy, exhibiting amplified copy number and high mobility. This caused a high rate of spontaneous X chromosome mutation due to insertion/excision events. The mobilization is shown to occur very early after fertilization, causing somatic and germline mosaicism for mutations. We are studying the conditions that activate gypsy and also how an active strain becomes stable. At the present time all movement of gypsy in these strains has stopped. Attempts to reinitiate the mobility through treatment with mutagens has met with limited success in somatic tissues and no movement in germ line.

Transposons are also known to mediate gross chromosomal rearrangements through a process of asymmetrical pairing and exchange. This process occurs both as intrachromosomal and interchromosomal exchanges to produce, in the former case, deletions or inversions depending on the relative orientations of the transposons and in the latter case, duplications and deficiencies and possibly translocations. We are studying a large collection of such rearrangements and characterizing the breakpoints at the molecular level to establish the role of the transposons and investigate the mechanism of exchange.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65038-04 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	LMG NIEHS
Others:	D. C. Thomas	Senior Staff Fellow	LMG NIEHS
	A. Sugino	Visiting Scientist	LMG NIEHS
	R. K. Hamatake	Senior Staff Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*

Myron F. Goodman, University of Southern California, Los Angeles, CA
Robert A. Bambara, University of Rochester, Rochester, NY
Dale W. Mosbaugh, University of Texas, Austin, TX

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.15	0.85	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.)*

Replication and maintenance of the stability of genetic information requires the accurate synthesis of DNA. In animal cells, DNA synthesis is performed by four distinct classes of DNA polymerases, α, β, δ and γ. Our objective has been to characterize the accuracy of DNA synthesis by each of these enzymes and to analyze the errors committed by each in an attempt to understand how mutation rates are controlled. Having found that the high fidelity of the mitochondrial replicative DNA polymerase γ from chick results from exonucleolytic proofreading, we searched for and found similar activity in mammalian gamma polymerases from two sources. This generalizes the discovery of proofreading activity associated with this class of polymerase, and establishes that two of the four classes of higher eukaryotic DNA polymerases achieve high fidelity by a proofreading mechanism. To determine the mechanisms by which eukaryotic DNA polymerases discriminate between correct and incorrect nucleotides during polymerization, we have analyzed base substitutions produced by DNA polymerase-β by both classical miscoding and transient misalignment mechanisms, using steady state enzyme kinetic analyses. A major focus during the past year has been a continuing examination of the fidelity of the two putative replicative DNA polyerases, α and δ. We have established that the four-subunit DNA polymerase α-DNA primase complex purified from four different sources by immunoaffinity chromatography is no more accurate than the conventionally purified polymerase. Studies to examine the relationship between the processivity and fidelity of polymerization have begun.. Unlike Pol α, DNA polymerase δ is highly accurate, which is at least in part due to exonucleolytic proofreading. Two forms of the enzyme are being studied, a PCNA-stimulable and a PCNA-independent form. The latter enzyme is highly accurate for base substitution errors and is currently being tested in a forward mutation assay to examine error specificity. The former enzyme is being similarly tested, and the involvement of PCNA and its contribution to fidelity, if any, are under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65041-03 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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 LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

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.)*

DNA polymerases, enzymes that participate in the replication of DNA, play a major role in the generation of spontaneous mutations by making errors during DNA synthesis. These enzymes normally require a template to provide the information necessary for duplication of the genetic material. Recently, a novel, non-templated nucleotide addition reaction catalyzed by DNA polymerases from both procaryotic and eucaryotic sources was characterized. Reactions of this type may represent a new mechanism to generate spontaneous mutations. More recently, a second unusual reaction catalyzed by DNA polymerase I from E. coli was observed. Under normal circumstances the DNA template which is copied must have physical continuity. However, the requirement for template continuity can sometimes be circumvented, allowing information from physically unlinked pieces of DNA to be combined. This type of "recombinational synthesis" could be used in dividing cells to overcome potential blocks to replication represented by ionizing radiation-induced breaks in the DNA template.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65042-03 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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, Mutagenesis Section	LMG NIEHS
Others:	L. K. Derr	Guest Worker	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	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. uvsW is a cruical but mysterious gene in the bacteriophage T4 EPR system. Mutations in uvsW depress recombination, increase killing and abolish mutagenesis by agents acting through EPR. Temperature-sensitive mutations of uvsW have been generated and characterized by mapping and complementation tests and their effects on survival, recombination and mutagenesis have been determined. A deletion mutation of uvsW has been engineered, providing a rigorously defined null allele. The expression and regulation of uvsW is being explored by a combination of DNA-sequencing, northern-blot, primer-runnoff and RNA-sequencing methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65043-03 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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, Mutagenesis Section	LMG NIEHS
Others:	M. O. Rosario	IRTA Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	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, mutations are being introduced into early parts of the gene and the resulting mutants are being examined for phenotype, including viability. Second, tests are being performed for a correlation between recombination and mutagenesis: in a cross employing outside markers, newly induced mutations are 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 65045-03 LMG

PERIOD COVERED

October 1, 1988 to September 30, 1989

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, Mutagenesis Section	LMG NIEHS
Others:	D. C. Nguyen	Chemist	LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.05	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.)*

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 the rI mutants, we have investigated their general properties. Mutations that produce the characteristic rI phenotype arise at two loci, one the classically described locus at about 60 kb on the standard map and another a locus at about 1600 kb. Point mutations at the 60-kb locus recombine inter se at low frequencies, suggesting a small gene; several are suppressed by unlinked but as yet unmapped suppressor mutations. The 160-kb locus is being cloned and more closely mapped.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65046-03 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

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	LMG NIEHS
Others:	J. D. Roberts	Senior Staff Fellow	LMG NIEHS
	D. C. Thomas	Senior Staff Fellow	LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.55	1.55	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are interested in determining the mechanisms by which human cells control spontaneous and induced mutation rates. While DNA synthesis by purified DNA polymerases in vitro is not accurate enough to account for low spontaneous mutation rates in vivo, actual DNA replication involves the concerted action of a number of proteins. We have therefore been examining the fidelity of semiconservative bidirectional DNA replication by a human HeLa cell protein complex. The data obtained using mutagenesis vectors that monitor the base substitution and frameshift fidelity of replication indicate that this human cell replication complex is highly accurate. Since this implies that additional fidelity components enhance fidelity during replication, we are dissecting the replication system into its component parts and testing the effects of individual proteins on the error rates of the two DNA polymerases known to be involved, for specific types of base substitution and frameshift errors. We have demonstrated that one essential replication factor (Replication Factor A a class of DNA binding proteins required for initiation) has a slight effect on the frameshift fidelity of DNA polymerase-α. In examining the mechanisms that contribute to fidelity, we have demonstrated that efficient repair of mismatched base pairs occurs in the extract. Finally, the current model for the structure of the eukaryotic replication fork posits that the DNA polymerase-δ is the leading-strand polymerase, while DNA polymerase-α replicates the lagging-strand. Since we have shown that these two polymerases have very different fidelities, we are examining the fidelity of leading- versus lagging-strand DNA replication, for base-substitution and frameshift errors, including a determination of whether exonculeolytic proofreading is occurring during replication. These last two issues are important for understanding how DNA damage encountered by a human cell replication fork may be mutagenic and/or lethal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65047-03 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMG NIEHS
Others:	J. D. Roberts	Senior Staff Fellow	LMG NIEHS
	K. Bebenek	Visiting Fellow	LMG NIEHS
	K. Eckert	IRTA Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*

Samuel Wilson, Research Biochemist, LB, NCI

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	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.)*

A critical feature of the life cycle the human immunodeficiency virus (HIV-1) that causes Aquired Immunodeficiency Syndrome (AIDS) is its ability to generate diversity. HIV-1 has exceptionally high mutation rates within certain portions of its genome, permitting rapid evolution of new forms of the virus that are able to evade the host's immune response. In order to determine if errors committed by the viral reverse transcriptase could account for diversity in vivo, we have examined the accuracy of HIV-1 reverse transcriptase (RT) using in vitro fidelity assays. DNA-dependent DNA synthesis by this enzyme is exceptionally error-prone. The enzyme, whether recombinant or from virus particles, produces errors while replicating M13mp2 DNA at a rate that, if operative in vivo, would produce about five mutations per genome per round of replication. Sequence analysis of mutants resulting from in vitro synthesis demonstrates that the enzyme has unusual error specificity. Base substitution and one-base frameshift mutational hotspots are observed. The specificity and position of errors suggest that most of the frameshifts and many of the base substitutions are initiated by template-primer slippage. Processivity analysis for the enzyme on the M13mp2 DNA template reveals strong termination at specific sites. Termination sites within homopolymer sequences correlate with frameshift mutational hot spots. The results suggest that the formation and/or utilization of misaligned template-primers is increased during the dissociation-reinitiation phase of the reaction. Our future work will focus on elucidating the mechanisms responsible for the error-proness of HIV-1 RT. These studies may provide insights into the interaction of the enzyme's active site with its substrates and may be useful in designing RT-targeted drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65048-03 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMG NIEHS
Others:	K. Bebenek	Visiting Fellow	LMG NIEHS
	K. Eckert	IRTA Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*

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

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.65	1.35	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.)*

We are using two DNA polymerases obtained by recombinant DNA technology, the large (Klenow) fragement of E. coli polymerase I and the thermostable DNA polymerase from Thermus aquaticus, as model polymerases to examine the mechanisms and protein-DNA interactions that are important for the fidelity of DNA synthesis. The determination of the structure of the Klenow polymerase by X-ray crystallography enabled engineering of the protein by site-directed mutagenesis. We have determined the fidelity of DNA synthesis catalyzed by the wild-type Klenow polymerase, by two mutant derivatives lacking proofreading exonculease activity but having a normal protein structure, and by a protein that contains only one of two domains, the large polymerase domain. The fidelity results have permitted: 1) a determination of the contribution of base selectivity by the polymerase and proofreading by the exonuclease to both base substitution and frameshift fidelity, 2) an examination of the effects of the small domain on the fidelity of polymerization by the large domain, 3) the examination of a model for the production of minus-one base frameshift errors at non-reiterated base sequences.

We are also examining the fidelity of the thermostable Taq polymerase used in polymerase chain reactions (PCR). This enzyme, which polymerizes at high temperature, is highly homologous to the Klenow polymerase but lacks the proofreading exonuclease. The results, which are the same with natural or recombinant Taq polymerase preparations and similar to those obtained with the exonuclease deficient form of Klenow polymerase, demonstrate that the enzyme has a base-substitution error rate of 1/10,000. The effects of variations in reaction condition, including temperature, relative and absolute dNTP concentration and $MgCl_2$ concentration, have been determined for both base-substitution and frame-shift error rates. The results provide insights into the interactions important for fidelity and also have implications for the interpretation of data from individual clones obtained from DNA amplified by PCR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65049-03 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Mutagenesis Wtih 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	LMG NIEHS
Others:	A. Sugino	Visiting Scientist	LMG NIEHS
	R. K. Hamatake	Senior Staff Fellow	LMG NIEHS
	M. P. Smith	Biologist	LMG NIEHS

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Genetics

SECTION
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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 Number Z01 ES 65048-03 LMG.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65050-03 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

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 Senior Staff Fellow LMG NIEHS

Others:

COOPERATING UNITS (if any)

Dr. Leon F. Stankowski, Jr. Pharmakon Research Intern'l., Inc., Waverly, PA
Dr. William D. Caspary, Cellular & Genetic Toxicology Branch, DTRT, NIEHS

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	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 Chinese hamster overy (CHO) cell line, AS52, carries a single functional copy of the bacterial gpt gene stably integrated into the CHO genome. The site of integration of the gpt locus appears to allow the recovery of viable multilocus deletions (i.e., deletions affecting all or part of the gpt locus and adjacent essential DNA sequences), whereas multilocus deletions will be conditionally lethal at the analogous but hemizygous X-linked hprt locus. The ability to recover most deletions as viable mutants makes the AS52 cell line particularly useful for mechanistic studies. Presently, we are studying the molecular nature of deletions induced by three agents, mitomycin C(MMC), formaldehyde (FA) and 5-azacytidine (5AC). MMC induces mostly large deletions at high doses and mostly point mutations at lower doses. This change in spectrum with dose may reflect the nature of MMC adduction of DNA. MMC is a bifunctional alkylating agent that readily binds DNA to form monoadducts and DNA crosslinks. We propose that the MMC-induced point mutations are the result of the monoadduct (and perhaps the intrastrand crosslink) while the interstrand crosslink may be involved in the generation of deletions. To assess this hypothesis, we have begun to generate a collection of independently derived mutants induced with decarbamoyl-MMC (DCMMC), a derivative of MMC that is capable only of monoadduction. This agent is highly mutagenic in AS52 cells and the prediction is that DCMMC will induce mostly point mutations. In addition, FA has been demonstrated to be a potent mutagen in AS52 cells inducing mostly deletions as characterized by Southern blotting. 5AC is not mutagenic at the hprt locus but is highly mutagenic in AS52 cells. This observation suggests that 5AC may be generating deletions at gpt and a collection of independent mutants has been generated for further study. Finally, we continue to use the πvx-recombinational recovery system using E. coli hosts that carry mutations in the mcrA,B genes to isolate deletion endpoints for molecular characterization from genomic lambda libraries derived from selected mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65051-03 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Senior Staff Fellow	LMG NIEHS
Others:	R. W. Tuveson	IPA	LMG NIEHS
	C. A. Cheng	Guest Worker	LMG NIEHS

COOPERATING UNITS (if any)
Dr. Leon F. Stankowski, Jr., Pharmakon Research International, Inc., Waverly, PA

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.6	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.)

We are using a Chinese hamster ovary (CHO) cell line (AS52) with a single copy of the bacterial gpt gene stably integrated into the genome to study point mutational changes in mammalian cells. Mutations at the gpt locus can be readily isolated as 6-thioguanine resistant ($6TG^r$) colonies and mutant sequences can be efficiently recovered using the polymerase chain reaction (PCR). Direct sequence analysis of the PCR generated product allows rapid characterization of mutational spectra derived at gpt. We have developed reaction conditions to allow PCR amplification of mutant gpt sequences following lysis of a small number of mutant cells. Using this protocol, clonal expansion and DNA isolation procedures are eliminated which significantly shortens the time required to generate a mutant DNA sequence. Using these techniques, we have defined a spectrum of spontaneous and Mitomycin C(MMC) point mutnations. Among the spontaneous mutants, we find a 3-base deletion at a specific site in approximately 30% of the mutants analyzed. We are pursing studies that include reversion analyses, targeted gene conversion and sequence analysis of a collection of camptothecin-induced mutants to evaluate the mechanistic basis of this high frequency mutational event. MMC-induced mutants are predominantly GC ≥ TA transversions. A striking proportion of the mutants (25%) contain multiple base-pair substitutions, often a transition and a transversion, at adjacent bases in the gpt structural gene. It is likely that these mutations arise as a result of known MMC DNA adducts and we are investigating the possibility that the adjacent double mutations may result from MMC-intrastrand crosslinks. Finally, we have developed a selection system for the evaluation of gpt mutations in E. coli allowing direct comparison of spectra generated in bacteria and in mammalian cells. Comparative data should provide insights regarding DNA damage processing and the influence of mammalian higher order chromosome structure on the frequency and types of mutations observed at gpt.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65052-03 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

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 Senior Staff Fellow LMG NIEHS

Others:

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis 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.)*

We are interested in the effect of chromosomal position on the frequency and types of mutations observed using a target gene (gpt) integrated at different chromosomal sites in human cells. For these studies, we are using retroviral vectors that carry and express both the bacterial gpt and neo genes to construct single copy $gpt^{+}neo^{+}$ derivatives of human HT1080 cells. Appropriate derivatives are readily selected as colonies resistant to both mycophenolic acid and the aminoglycoside, G418. Among the numerous retroviral vectors available, there are substantial differences with regard to the efficiency of retroviral transcript packaging as well as in the efficiency of insert gene expression. Previous efforts in this laboratory have resulted packaged retroviral transcripts that carried either gpt or neo, but rarely both genes. Most likely, these data reflect rearranged packaged retroviral transcripts. Therefore, we are now using one of the direct orientation (DO) retroviral vectors which efficiently expresses both the gpt and neo genes. In addition, the DO vector we have chosen allows the regulation of gpt gene expression using the human metallothionein (MTII) promoter. Recently, several labs have demonstrated that DNA repair is more rapid in transcriptionally active regions of the genome and that the transcribed DNA strand is repaired more rapidly than the nontranscribed strand. Thus using the DO vector constructions, we can assess both the influence of DNA repair using a transcriptionally active or inactive target gene as well as global effects of chromatin structure on mutagenesis using gpt integrated at various genomic sites. These studies should provide a data base for using DOgpt retroviral vectors to assess the influence of human DNA repair pathways on mutational spectra generated in human repair deficient cell lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65053-02 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: DNA Sequence Characterization of Bacteriophage T4 rII Mutations

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

PI:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS
Others:	M. C. Kricker	Staff Fellow	LMG NIEHS

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Mutagenesis Section

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

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are using the bacteriophage T4 rII system as a model to explore mechanisms of DNA damage and mutagenesis. Because traditional DNA sequencing methods for analyzing the molecular nature of rII mutations are laborious and slow, we are developing methods based on genomic sequencing. With this method, important classes of mutations will be examined for their sequence changes. For instance, even mild heat damages DNA and could, if not repaired, produce on the order of 100 mutations per diploid human cell per day. Earlier studies showed that heat induces both transitions and transversions at G:C base pairs in phage T4. Genetic studies suggested that the main heat-induced transversion pathway is G:C to C:G but did not exclude G:C to T:A, and the distinction can be easily resolved by sequencing studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65054-02 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Invariant Per-Genome Mutation Rates

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

PI: J. W. Drake Head, Mutgenesis Section LMG NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Mutagenesis 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.)*

In the late 1960s the then-available data from several laboratories suggested that microbes exhibited a constant forward mutation rate of about 0.003 per genome per replication; genome sizes varied by about 1000-fold, and so, inversely, did mutation rates per base pair per replication. This observation suggested that mutation rates had evolved to an optimum that was surprisingly constant among diverse organisms. Since then, the published data base has improved for many organisms and the invariance of per-genome mutation rates appears not only still to be the norm, but to extend all the way from a bacteriophage containing single-stranded DNA to a lower multicellular eukaryote. (The RNA viruses and a DNA plasmid constitute the presently known exceptions.) This data base is being reexamined and analyzed to test the generality of the relationship.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65055-01 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Bacteriophage T4 Antimutator Mutations

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

PI:	J. W. Drake	Head, Mutgenesis Section	LMG NIEHS
Others:	D. C. Nguyen	Chemist	LMG NIEHS

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Mutagenesis 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.)*

Antimutator mutations reduce spontaneous (and sometimes induced) mutation rates and are therefore of interest both for understanding mechanisms of spontaneous mutation and for finding ways to reduce mutation rates generally, and thus to reduce the incidence of diseases of mutational origin. We long ago discovered several antimutator alleles among temperature-sensitive mutations in the DNA polymerase gene of bacteriophage T4. These antimutators strongly reduced mutation rates along certain pathways, such as A:T to G:C, but were then found to have little effect on other pathways and even to act as mutator mutations on yet other pathways. We have therefore initiated a search for generalized antimutator mutations, defined as mutations that reduce mutation rates measured in a large target representative of the genome as a whole.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65056-01 LMG

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Evolution of the T-Even Bacteriophage tRNA Genes

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

PI:	M. C. Kricker	Staff Fellow	LMG NIEHS
Others:	J. W. Drake	Head, Mutagenesis Section	LMG NIEHS

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Mutagenesis Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.45	0.45	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Transfer RNAs (tRNAs) are used as primers for reverse transcription during retroviral infection and may incorporate into the retroviral genome by illegitimate recombination. We are studying the tRNA gene cluster of the T-even bacteriophages as a model to investigate how they were incorporated into the viral genome. The tRNA genes vary widely among the T-even bacteriophages and have some features resembling reverse-transcribed mobile genetic elements. Additionally, each T-even phage expresses a unique set of tRNAs. The following questions will be explored. Are the tRNA genes mobile elements? Do they transpose via RNA intermediates? Is there sequence specificity at sites of loss or acquisition of tRNA genes? Is there illegitimate transfer of tRNA genes among the T-even bacteriophages or between them and other species? These questions will be approached by sequencing the tRNA gene clusters of phages T2, T4, and T6 in order to determine whether the tRNA genes are processed versions of tRNAs and to identify sites of loss or aquisition of tRNA genes. Genetic methods will be used to ask if these tRNA genes can transpose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 65057-01 LMG

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Mismatch Repair in Mutagenesis and Recombination

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

PI:	M. Radman	Visiting Scientist	LMG NIEHS
Others:	R. M. Schaaper	Visiting Scientist	LMG NIEHS
	K. R. Tindall	Senior Staff Fellow	LMG NIEHS
	M. A. Resnick	Head, YG/MB Group	CTGB NIEHS

COOPERATING UNITS *(if any)*
Dr. Paul L. Modrick, Professor of Biochemistry, Duke University, Durham, NC

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Laboratory of Molecular Genetics

SECTION
Mutagenesis 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.)*

Mismatch repair is a set of enzymological systems, encoded by numerous genes, that detect DNA base pair mismatches and convert them to standard base pairs. In one mode, mismatch repair examines freshly replicated DNA, detects mismatches of mutational origin, determines which is the wrong (progeny strand) base, and converts it to the correct base. In another guise, mismatch repair examines the hybrid regions of newly recombined DNA molecules, detects mismatches and acts to homogenize them in ways as yet poorly understood. Mismatch repair occurs in at least several bacteria, yeast, and mammalian cells, and is probably ubiquitous. It constitutes a major barrier to spontaneous and induced mutation and to certain harmful modes of genetic recombination. In this project, mechansms of mismatch repair are being investigated at the genetic and enzymological levels in bacteria, yeast and mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70090-06 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Research Physiologist	LMIN	NIEHS
Others:	I. Merchenthaler	Visiting Scientist	LMIN	NIEHS
	W. C. Wetsel	Senior Staff Fellow	LMIN	NIEHS
	F. Lopez	Visiting Fellow	LMIN	NIEHS
	M. Ching	Expert	LMIN	NIEHS

COOPERATING UNITS *(if any)*
University of North Carolina, Department of Anatomy, Chapel Hill, NC; University of Pécs, Department of Anatomy, Pécs, Hungary

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Reproductive Neuroendocrinology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.9	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.)*

The neuropeptide luteinizing hormone-releasing hormone (LHRH) is the prime regulator of gonadal function in vertebrates. Studies on the distribution of neurons containing pro-LHRH peptides have provided very useful information about the anatomical and functional arrangement of the LHRH network. Additional studies evaluating the expression, distribution and secretion of pro-LHRH peptides indicated that gonadal steroids can profoundly affect these parameters and thereby influence the overall activity of LHRH neurons. We also presented direct evidence that the LHRH neuronal system can "auto-regulate" its own activity, providing a functional correlate to the anatomical studies describing recurrent axon collaterals in LHRH neurons. This auto-regulatory mechanism may play a key role in determining a coordinated pulsatile or rhythmic LHRH neuronal activity. Using an in vitro system developed in our laboratory, we have performed an extensive characterization of the major neurotransmitters (norepinephrine, dopamine opioid peptides, GABA, etc.) regulating LHRH secretion, and of important internal (gonadal steroids and peptides, lactation, etc.) and environmental (stress, neurotoxins) factors affecting the interaction between neurotransmitters and the LHRH neurons. In many cases, these in vitro studies were conducted in parallel with in vivo paradigms, to obtain a direct estimation of changes in LHRH secretion and function in vivo. Steroids play a major role in maintaining the secretory capacity of the LHRH neuron, an effect which appears to be mediated by interneurons rather than by direct actions at the LHRH neuron. The in vitro model allowed us to characterize the role of CA^{2+}, arachidonate metabolites (PGE_2 and different lipoxygenase metabolites) and protein kinase C activation on the regulation of pro-LHRH peptide(s) secretion from nerve terminals. These studies should advance our understanding of the complex interactions between central neurotransmitter systems and internal or external environmental factors influencing reproductive functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70092-06 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Research Physiologist	LMIN	NIEHS
Others:	M. D. Culler	Senior Staff Fellow	LMIN	NIEHS
	W. Wetsel	Senior Staff Fellow	LMIN	NIEHS
	M. Ching	Expert	LMIN	NIEHS
	T. Inoue	Visiting Fellow	LMIN	NIEHS
	F. Lopez	Guest Researcher	LMIN	NIEHS
	I. Wanderley	Guest Researcher	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
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SECTION
Reproductive Endocrinology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.6	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.)

It is now well recognized that hypothalamic and pituitary hormones are secreted in a pulsatile pattern which is unique for each hormone and which may vary according to the physiological status of the subject. The evidence we have obtained supports the concept that the pulsatile secretory pattern contains encoded messages that convey the required inputs to elicit secretory responses and other important biological events, such as cell differentiation and even enhanced gene expression. It seems evident, therefore, that pulsatile hormone secretion represents a sophisticated, carefully regulated means of intracellular communication. We have evaluated the characteristics of the pulsatile pattern of secretion of most pituitary hormones and of some hypothalamic peptides as well. These studies indicate that several parameters of the pulsatile pattern can change during different physiological situations or after specific pharmacological interventions. Secretion of the neuropeptide LHRH into the hypophysial portal blood in intact animals occurs in a pulsatile fashion. Evaluation of the total amount (mass) of hormone secreted in each pulse (measuring area under the pulse) reveals that at least two distinct populations of pulses can be separated, i.e., "small" and "big" mass pulses. Orchidectomy results in an almost complete disappearance of "big mass" pulses. Testosterone replacement reestablishes the presence of large mass pulses. These observations are helping to re-define the established dogma of negative steroid feedback, into a new concept in which the steroids interact with neural structures to modify the pulse pattern of peptide release. This may be accomplished by establishing a functional neuronal network capable of generating a pulsatile pattern of LHRH secretion which can appropriately maintain pituitary-gonadal function. Additional studies on the pulsatile pattern of hormones under dual (stimulatory/inhibitory) control (such as prolactin) or under multifactorial neural regulation (ACTH) also provided very useful information about the encoding of signals on the pulsatile pattern which may contribute to the pleiotropic actions of these hormones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70096-05 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	Michael D. Culler	Senior Staff Fellow	LMIN	NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMIN	NIEHS
	Carl Paschall	Biologist	LMIN	NIEHS

COOPERATING UNITS (if any)
Department of Anatomy, University of North Carolina, Chapel Hill, NC
Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Reproductive Endocrinology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.0	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.)

Previous studies within this project have provided new insights into the manner in which the brain regulates gonadotropin (LH and FSH) secretion. Recent efforts have concentrated on the modulatory role of gonadal factors. The recent structural elucidation of the long sought inhibin molecule has allowed the generation of antiserum against this gonadal factor. Using this antiserum to passively immunoneutralize endogenous inhibin, a dramatic elevation of plasma FSH was observed in the female rat but, surprisingly, not in the adult male. By taking frequent, sequential blood samples from conscious, unrestrained rats coupled with detailed analysis of secretion parameters, it was determined that endogenous inhibin selectively suppresses the basal parameters of FSH secretion in the female without affecting pulsatile FSH secretion. In contrast to the long held dogma that inhibin selectively suppresses FSH secretion, it was demonstrated that endogenous inhibin also suppresses all parameters of pulsatile LH secretion, acting generally to suppress pituitary sensitivity to the brain factor, LHRH. The role of testosterone (T) in the male was also examined using the selective Leydig cell toxin, ethane dimethane sulfonate (EDS). From these studies, T was found to be the major inhibitor of gonadotropin secretion in the male, selectively affecting the same parameters of gonadotropin secretion as inhibin in the female. In the T deficient, EDS-treated male rat, endogenous inhibin can also be demonstrated to selectively affect the basal parameters of FSH secretion, suggesting that in the adult male, the inhibin system is either masked by T or quiescent until normal Leydig cell function is impaired. In addition, studies with cultured Sertoli cells have elucidated an inhibitory role of the adenosine system on inhibin secretion. The results from these and ongoing studies are dissecting the mechanisms by which the brain and gonads interact to control gonadotropin secretion and reproductive function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90033-07 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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

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

PI:	William E. Wilson	Research Chemist	LMIN	NIEHS
Others:	L. H. Lazarus	Research Chemist	LMIN	NIEHS
	K. B. Tomer	Head, Mass Spectrometry	LMB	NIEHS

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

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Peptide Neurochemistry Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.95	0.9	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.)*

Tissue functional involvement of bradykinin and/or related kinins is suggested by observations that (a) gonadal steroids regulate pituitary kallkrein levels and (b) bradykinin and related peptides, placed in the CNS, exert behavioral effects which could reflect physiological or pharmacological regulation. Discovery of bradykinin and a second, highly specific activity kinin in milk led us to attempt recovery of precursor kininogen(s) for the following reasons: (a) in the neonate, bradykinin may modulate physiological changes in the gastrointestinal tract and/or regulate the release of intestinal hormones into the blood; (b) a general scientific interest exists for elucidation of the nature of tissue kininogens, whose existence is implied by the wide occurrence of kallikreins; (c) investigation of milk kininogens may permit identification of new kinins; and (d) should they arise from mammary, tissue, milk kininogens may prove very useful in evaluation of our current understanding of the nature of tissue kallikrein digestion products, tissue kinin regulation, differential regulation of tissue kallikreins and kininogens, and related phenomena. We devised a simple fractionation scheme to recover bradykininogen from bovine milk; however, final purification is incomplete. Two species, high Mr (<68 kDa) and low Mr (about 16 kDa) kininogens have been resolved by gel filtration; the high Mr kininogen appears to be bradykinin, while the kinin in the low Mr form is not known. Future studies should permit us to determine whether milk kininogens are derived from mammary tissue or liver (the source of plasma kininogens).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90034-06 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	L. H. Lazarus	Research Chemist	LMIN	NIEHS

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

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Peptide Neurochemistry Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Efforts were undertaken to raise polyclonal rabbit antisera to PHLIP-8, the octapeptide recovered from rabbit stomach with the aid of an antiseum to the amphibian peptide physalaemin, in order to reexamine previous data which indicated that phasalaemin immunoreactive material occurs in the brain stem and other parts of the central nervous system. We were able to obtain only a trace amount of polyclonal antisera from 1 of 8 injected animals; a nonimmunized rabbit also had trace quantities of antisera to PHLIP-8. The latter result would appear to indicate that the rabbit immune system may be continually exposed to PHLIP-8, while the former result would appear to indicate that the rabbit immune system is not able to respond well to the amino acid sequence in PHLIP-8. Current efforts have subsided until we can obtain monoclonal antibodies using mouse spleen cells hybridized to myeloma cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90039-06 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Modulation of Brain Opioids and Tachykinins by Neurotransmitter Systems

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

PI:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
Others:	H.K. Jiang	Guest Worker	LMIN	NIEHS
	P. Hudson	Biologist	LMIN	NIEHS
	M. Stachowiak	Senior Staff Fellow	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The major goal was to characterize dopaminergic control over neuropeptide homeostasis in the basal ganglia, by manipulating dopaminergic tone. Early studies from our laboratory demonstrated that long-term blockade of dopaminergic transmission by daily injections of haloperidol, an antipsychotic drug which selectively blocks dopamine receptors, caused a large increase in the level of enkephalin in those brain areas enriched with dopamine innervation, such as the striatum and nucleus accumbens. Subsequently, we undertook studies which showed that chronic haloperidol treatment also elevated the levels of precursor and mRNA encoding proenkephalin in the striatum. Based on these results, we concluded that long-term blockade of dopaminergic transmission by haloperidol accelerated the biosynthesis of enkephalin. This conclusion was further supported by our findings in rats treated with 6-hydroxydopamine. Changes of enkephalin and its mRNA after 6-hydroxydopamine lesion were identical to those obtained from the haloperidol experiment. Results from these two experiments present strong evidence for a tonic inhibitory influence of DA on the biosynthesis of striatal enkephalin. Studies were extended to characterize dopaminergic control over striatal dynorphin and substance P systems. In contrast to enkephalin, our data showed that dopamine exerted a tonic excitatory influence on the biosynthesis of both peptides, as dopaminergic blockade reduced the levels of these peptides and their respective mRNA. Another series of experiments examined the influence of enhancement of dopaminergic transmission (phasic control) on the turnover of opioid peptides and substance P. Results indicated that dopamine exerted phasic excitatory influence on the turnover of dynorphin and substance P, but not on enkephalin. The phasic regulation of dopamine on dynorphin and substance P may have some relevance to conditions where animals are under stress and nigrostriatal dopaminergic transmission is enhanced. This project will be terminated after September 30, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90042-04 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	B. Rogers	Biologist	LMIN	NIEHS
	W. Zhang	Visiting Fellow	LMIN	NIEHS
	P. Tandon	Visiting Fellow	LMIN	NIEHS
	K. Nanry	Psychologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS
	L. Williams	Stay-in-Schooler	LMIN	NIEHS

COOPERATING UNITS *(if any)*
Duke University

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurobehavioral Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.55	2.60	2.95

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this research program is to study the conditions under which compensation and recovery of function occurs following experimentally induced neurodegeneration in the central nervous system (CNS). These studies employ neurotoxicants such as colchicine and excitotoxicants, such as N-methyl-D-aspartate, to destroy specific neuronal populations in the CNS. Studies focus on the hippocampus because of its well known cytoarchitecture and the fact that damage to this area evokes compensatory functional and anatomical changes. Research has found that intrahippocampal administration of neurotoxicants evokes a series of pre- and postsynaptic changes in cholinergic systems in the hippocampus. These changes depend on the integrity of the septohippocampal pathway and are indicative of injury-induced reactive synaptogenesis in the hippocampus. Subsequent work has found injury-related changes in the signal transduction step for the cholinergic system. Future work will focus on the specificity of injury-related effects on the signal transduction mechanism in the hippocampus and other regions of the CNS. The interaction between trophic factors and neurotransmitter-mediated turnover of phosphoinositides (PI) will also be studied, as well as the possible compensatory changes in PI turnover in other models of neurodegenerative disease. Research in this area aims to understand the more general process of synaptic plasticity that occurs following injury to the nervous system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90043-04 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	J. S. Hong	Pharmacologist	LMIN	NIEHS
	J. McGinty	Assoc. Professor	East Carolina University	

COOPERATING UNITS (if any)

Department of Anatomy, East Carolina University

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurophysiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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. 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 Zn Cl_2 prevents KA induced seizures in rats. Instead, we found no effect of Zn Cl_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 mgkg) or diethyldithiocarbamate (100-400 mg/kg) has a profound and dose related effect on the effects of KA. When given 15 minutes after the subcutaneous injection of KA, they markedly potentiate KA activity. They also produce a transient decrease in hippocampal levels of enkephalin and dynorphin. They also produce transient increases in the hippocampal levels of a number of amino acids (viz., taurine, glutamate, glutamine, and GABA). These effects are associated with reduced levels of hippocampal zinc (as measured by Timm staining of the hippocampus). It appears, then, that dithizone and diethyldithiocarbamate may prove to be useful tools 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 these compounds, and (2) examination of their electrophysiological effects on the hippocampus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90044-04 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	J. S. Hong	Pharmacologist	LMIN	NIEHS
	C. W. Xie	Visiting Fellow	LMIN	NIEHS
	P. Lee	Visiting Associate	LMIN	NIEHS
	J. McGinty	Assoc. Professor	East Carolina University	

COOPERATING UNITS (if any)
Department of Anatomy, East Carolina University

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurophysiology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	0.55	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.)

Work in this laboratory has focused on the role of enkephalin and dynorphin in seizure activity and related sequelae. 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 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. Current studies have demonstrated that the opioid receptor antagonist, naltrexone, when injected directly into the ventral hippocampus, produces an elevation in the threshold for eliciting wet dog shakes. We have also demonstrated that destruction of dentate granule cells in the ventral, but not dorsal, hippocampal formation abolishes wet dog shaking induced by perforant path or intrahippocampal stimulation or by systemic administration of kainic acid. It has also been found that slices obtained from the ventral portion of the hippocampus have a lower threshold for epileptiform bursting induced by an opioid mu receptor than slices from the dorsal end. Thus, these studies clearly demonstrate differences between the ventral and dorsal portions of the hippocampus. This is of importance since most previous studies have viewed the hippocampus as being functionally homogeneous.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90045-04 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	P. Lee	Visiting Associate	LMIN	NIEHS
	T. Xie	Visiting Fellow	LMIN	NIEHS
	C.L. Mitchell	Pharmacologist	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neuropharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purposes of this project were: 1) to determine alterations in the metabolism of enkephalins and dynorphins in the limbic-basal ganglia regions after electroconvulsive shock (ECS) or after electrical kindling-induced seizures; 2) to study the possible roles of brain opioid peptides in seizure-induced changes in hippocampal excitability. Previous studies showed that both repeated ECS and electrical kindling to full behavioral convulsions produced striking differences in the hippocampal levels of certain opioid peptides: an increase in enkephalin level, but a drastic decrease in dynorphin level. This project was aimed to determine if ECS-and electrical kindling-induced alterations in opioid peptides are mediated through the activation of perforant path which innervates dentate granule cells in the hippocampus. The perforant path was electrically stimulated at the angular bundle under conditions which elicit wet dog shakes but no motor seizures. Rats were given either an acute stimulation composed of several consecutive stimulation trials, or daily stimulations with a single daily trial for 6 days. A decrease in dynorphin mRNA level was found on both sides of the hippocampus one day after both acute and daily stimulation. Hippocampal dynorphin was also reduced at 24 h, and persisted for at least 6 days. In contrast, bilateral increases in enkephalin mRNA level were observed in the hippocampus and entorhinal cortex 24 h after the acute stimulation. Also, enkephalin immunoreactivity in the hippocampus tended to be increased at this time. These results indicate that activation of the perforant path inhibits the gene expression of prodynorphin, but enhances that of proenkephalin in the entorhinal cortex-hippocampal region. This study suggests that the activation of perforant path mediates both ECS- and electrical kindling-induced alterations in hippocampal opioid peptides. Since enkephalins and dynorphins have been shown to be potent in modulating hippocampal excitability, the differential regulation of these two opioid peptides may play important roles in mediating the postictal behaviors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90049-03 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	J. S. Hong	Pharmacologist	LMIN	NIEHS
	P. Hudson	Biologist	LMIN	NIEHS
	R. Tuominen	Guest Worker	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neuropharmacology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.0	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 goals of this project were: 1) to examine the nature of extracellular and intracellular signals controlling expression of tyrosine hydroxylase (TH), phenylethanolamine-N-methyl-transferase (PNMT), and proenkephalin (pEK) genes; 2) to determine whether these genes are differentially regulated; 3) to determine roles of transcription and post-transcriptional mechanisms in such regulations; and 4) to examine the possible role of nuclear oncogenes in coordinating the regulation of TH, PNMT, and pEK genes. Results from the hypophysectomy studies have demonstrated that TH, PNMT, and pEK mRNA levels were regulated by the pituitary-adrenocortical axis. This regulation was mediated by direct action of glucocorticoids on adrenal medullary cells. Angiotensin produced long-term increases in the activity of TH and PNMT and increased enkephalin levels. These effects were mediated through increases in TH, PNMT, and pEK mRNA levels. Expression of TH, PNMT, and pEK genes was also controlled by neural inputs to the adrenal medulla. Enhanced impulse activity of the splanchnic nerve produced frequency dependent increases in mRNA levels of TH and PNMT. Stimulation of the expression of TH, PNMT, and pEK genes by angiotensin or depolarization required voltage-dependent influx of calcium and protein kinase C activity. Effects of angiotensin, but not depolarization, were also mediated through the mobilization of intracellular calcium, calmodulin, and prostaglandins. Stimuli which elicted coordinate increases in the expression of TH, PNMT, and pEK genes (nicotine, angiotensin and increased neural input) also evoked rapid and transient increases in the expression of c-fos oncogene, which may play an important role in the signal transduction pathway to mediate the gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90050-03 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	P. Lee	Visiting Associate	LMIN	NIEHS
Others:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
	P.M. Hudson	Biologist	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neuropharmacology 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.)

Roles of opioid peptides in the regulation of hippocampal excitability are under intensive study after the discovery of endogenous opiates in the brain. Intraventricular administration of opioid peptides elicited epileptiform discharges and wet dog shakes (WDS) in rats, however, no behavioral convulsion was observed. We have shown that a single unilateral injection of specific mu opioid receptor agonists into the ventral hippocampus, but not into the dorsal hippocampus or other brain regions, resulted in a dose-dependent increase in the frequency of convulsions and wet dog shakes. We also demonstrated that these opioid-induced behavioral changes were mediated exclusively by mu but not delta or kappa opioid receptors in the ventral hippocampus. The disparity between the ventral and dorsal hippocampus in seizure sensitivity to mu opioid receptor agonists could be due to differences either extrinsic or intrinsic to the hippocampus. The latter possibility was tested in this study with an in vitro method using dorsal and ventral hippocampal slices from the same rat. Paired dorsal and ventral hippocampal slices were perfused with [NMe-Phe^3-D-Pro^4]morphiceptin (PL017), a specific mu opioid receptor agonist. A stimulating electrode was placed in the stratum radiatum of CA_3 and electrical activity was recorded from the pyramidal cell body layer of the CA_{3b} region. Application of 0.05 μM PL017 produced triggered and spontaneous bursting in 20% of ventral hippocampal slices, but no such effect was observed in dorsal hippocampal slices. At 0.5 μM PL017, 80% of ventral slices developed spontaneous bursting, whereas only 10% of dorsal slices had spontaneous bursting. The addition of 0.1 μM naloxone prior to or after PL017 inhibited the triggered response and reduced the frequency of the spontaneous bursting. These results suggest that the ventral hippocampus has a higher susceptibility to PL07-induced epileptiform bursting, and this effect is mediated, at least in part, through mu opioid receptors. Further studies are planned, by using hippocampal primary cell culture as a tool, to determine molecular mechanisms of opiate-induced excitability in the hippocampus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90051-03 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	LMIN	NIEHS
Others:	K. Nanry	Psychologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurobehavioral Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.60	0.25	0.35

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

DDT is believed to produce hyperexcitability and tremor, beginning in the head region and progressing caudally with an increase in intensity. DDT produces these neurotoxic signs through actions at axonal sodium and potassium channels. The minimal anatomical structures required for the expression of DDT-induced tremor and myoclonus appears to be contained within the brainstem and spinal cord. Neurochemical changes occur which may be related to the repetitive neuronal firing induced by DDT. For example, increases in norepinephrine concentration has been found in brainstem as well as hypothalamus while a decrease in brainstem norepinephrine has been found in DDT-treated rats. These data suggest that NE neurotransmission mediated via α_1 adrenoceptors may facilitate the expression of these signs. To further study the involvement of α_1 adrenoceptors in DDT-induced motor function, male Fischer-344N rats were chronically implanted with an intrathecal cannula, and gavaged with p,p'-DDT or corn oil. Seven hours later animals were infused with vehicle and several doses of prazosin. Prazosin reduced the spectral profiles of spontaneous movements in control rats. Tremulous movements induced by DDT were unaffected by intrathecal prazosin at lower doses while higher doses significantly reduced the spectral profiles of rats pretreated with 45 mg/kg DDT. Cortical and spinal tissues were used in *ex vivo* binding assays utilizing [^{3}H]-prazosin. Intrathecal prazosin occupied similar percentages of spinal [^{3}H]-prazosin binding sites, and produced a dose-related increase in cortical prazosin equivalents. These data indicate that while intrathecal prazosin will attenuate DDT-induced motor dysfunction, this effect requires blockade of α_1 adrenoceptors in regions other than solely the spinal cord. This project was terminated as of June, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90052-02 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Compensation and Recovery of Function in the Central Nervous System

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

PI:	William R. Mundy	Staff Fellow	LMIN	NIEHS
Others:	H. Tilson	Pharmacologist	LMIN	NIEHS
	C. Watters	Stay-in-Schooler	LMIN	NIEHS
	K. McDaniel	Biologist	LMIN	NIEHS
	R. McLamb	Biologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS
	S. Barone	Guest Worker	LMIN	NIEHS
	M. Bonner	Guest Worker	LMIN	NIEHS

COOPERATING UNITS *(if any)*

East Carolina University

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurobehavioral Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.60	2.15	3.45

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research program is to study the behavioral and neurochemical responses to experimentally-induced neurodegeneration in the basal forebrain cholinergic system of young and aged rats. Neurodegeneration in this area is associated with the cognitive deficits observed in aging and Alzheimer's disease. Lesions were made using the neurotoxicant colchicine, which binds to tubulin and blocks mitosis and axoplasmic transport. Initial studies examined the neurobiological effects of colchicine in the nucleus basalis. Stereotaxic infusion of colchicine resulted in damage limited to the site of injection, and decreased the number of cholinergic cells in the nucleus basalis. Histochemical and neurochemical analysis showed that colchicine lesions reduced presynaptic cholinergic markers including acetylcholinesterase, choline acetyltransferase and muscarinc receptor binding in the cortex. Nucleus basalis lesions had no effect on cholinergic markers in the hippocampus or striatum. At long post-lesion time points, recovery of cholinergic function in the cortex was observed. Behaviorally, colchicine lesions of the nucleus basalis resulted in impaired acquisition of both short-term and long-term memory tasks. Further studies characterized the effects of colchicine lesions of the medial septum. Intracerebroventricular administration of colchicine produced selective destruction of cholinergic cells in the medial septum, resulting in a decrease in cholinergic markers restricted to the hippocampus and an impairment of long-term memory. Future studies are planned to study the compensatory changes which occur after basal forebrain lesions including recovery of function and cholinergic activity, changes in cholinergic receptor mediated phosphoinositide turnover, and synaptic sprouting in response to neurotrophic factors. An important factor in all of these studies will be the comparison of young and aged animals, since compensation may be comprised with age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90053-02 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neuropeptides: Molecular Mechanism of Action

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

PI:	Lawrence H. Lazarus	Research Chemist	LMIN	NIEHS
Others:	William E. Wilson	Research Chemist	LMIN	NIEHS

COOPERATING UNITS (if any)
University of Torino, Italy; University of North Carolina, Chapel Hill, NC; Farmitalia, Milan, Italy; University of Kyoto, Japan; University of Ferrara, Ferrara, Italy

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Peptide Neurochemistry Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Structure-activity studies on the interaction of dermorphin, a natural D-amino acid containing heptapeptide, and a variety of synthetic analogues with rat brain opioid receptor preparations indicated that substitutions of amino acids, addition of hydrophobic protecting groups to existing residues, or deletions modified receptor selectivity for both the μ- and δ-type binding sites. Amino acid substitutions in the amino terminal pentapeptide domain generally decrease binding to μ-receptors and enhance that for δ-receptors. In particular, changes in the tripeptide sequence, Phe^3-Gly^4-Tyr^5, substantially diminish peptide affinnity, perhaps as a consequence of disruptions to the normal folding of the known tertiary structure of dermorphin. The D-configuration about the α-carbon of residue 2 is essential for biological activity and opioid receptor binding. A second naturally occurring dermorphin, [Hyp^6]dermorphin, exhibits twice the μ-selectivity of dermorphin. Central mediation of gastric acid secretion is positively correlated with opioid receptor affinities and selectivities; a similar correlation was also found for the pharmacological activity of these peptides. Deltorphin, or dermorphin gene-associated peptide, another D-amino acid containing heptapeptide predicted from the analysis of dermorphin cDNA transcripts, has high affinity for δ-receptors and is the most selective δ-ligand known with a δ-selectivity ratio >1300. Our studies led to the recognition that a single gene contained two very high affinity and selective opioid receptor peptide ligands: dermorphin for μ-receptors and deltorphin for δ-receptors. The significance of these results provide information on (1) the molecular mechanism of opioid-receptor interactions, (2) the ability to produce more selective opioid antagonists or agonists, and (3) to eventually obtain an understanding of the nature of opiate addiction in animal models and humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90054-02 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Biosynthesis, Processing and Secretion of Neuropeptides

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

PI:	William C. Wetsel	Senior Staff Fellow	LMIN	NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMIN	NIEHS
	I. Wanderley	Guest Researcher	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Reproductive Neuroendocrinology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	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.)*

Peptides represent a unique class of chemical messengers in the nervous and endocrine systems. These transmitters undergo precursor biosynthesis, processing, and secretion where the chemical nature of the secreted product determines the biological activity of the peptide. Studies from this group have shown that the LHRH precursor is processed into two major products: LHRH and GAP-(1-56). Orchidectomy for one month depresses biosynthesis of all pro-LHRH peptides in the hypothalamus of the rat, while the processing pathway and the molar ratios of the peptide products are unchanged. In vitro secretion of LHRH and GAP-(1-56) under basal and [K+]- and phorbol ester-(PDBu) stimulated conditions is also reduced by testis removal. When corrections are made for tissue stores, secretion is still reduced but only under PDBu stimulation. In a companion experiment, protein kinase C (PKC) activity is also depressed in ME of ORDX rats. Interestingly, testosterone-replacement therapy restored tissue and secreted levels of LHRH and GAP-(1-56) and PKC activity to the levels of unoperated controls. Results from these chronic studies revealed that gonadal steroids affect biosynthesis and secretion of the pro-LHRH-derived peptides. When changes in the molar ratio of GAP/LHRH were examined during the estrous cycle, rapid fluctuations occurred in the processing of the pro-LHRH in the cell body and fiber regions of LHRH neurons. These results are significant because they describe the pathway for processing the pro-LHRH, they demonstrate both that biosynthesis, processing and secretion of the pro-LHRH-derived peptides, and PKC activity, are regulated by gonadal steroids. Future studies will examine in detail: a) whether steroids influence mRNA and peptide levels of pro-LHRH in rat brain and in a neuronal LHRH cell line, b) which pro-LHRH-derived peptides are produced during different stages of the estrous cycle or in response to different secretagogues administered in vitro, c) whether any of these peptide products possess biological activity, and d) whether different isoforms of PKC in the ME are differentially regulated by gonadal steroids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90055-01 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Hypothalamic Control of the Anterior Pituitary, Morphological Aspects

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

PI:	Istvan Merchenthaler	Visiting Scientist	LMIN	NIEHS
Others:	Andres Negro-Vilar	Research Physiologist	LMIN	NIEHS

COOPERATING UNITS (if any)
University of North Carolina, Department of Cell Biology and Anatomy, Chapel Hill, NC; University of Pecs, Department of Anatomy, Pecs, Hungary

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Functional Morphology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

During the last decade studies from our laboratories and others have described the general distribution of peptidergic neurons in the hypothalamus. Certain neurons (hypophysiotropic neurons) form axon-terminals on capillaries of the median eminence releasing their product to the portal circulation, through which they reach the anterior pituitary. Other neurons, however, project to hypothalamic and extrahypothalamic regions where they affect the activity of other neuronal systems. The neuropeptides in the latter group serve as neurotransmitters or modulators. The two groups of neurons are combined in the brain. By using retrograde labelling from the median eminence combined with endogenous peptide immunocytochemistry, we have identified the hypophysiotropic LHRH and somatostatin neurons. We have shown that approximately 70% of LHRH neurons in the septum and the hypothalamus and 70% of somatostatin neurons in the anterior hypothalamus project to the median eminence. Coexistence of neurotransmitters and neuropeptides in the same neuron is commonplace in the nervous system. We have demonstrated for the first time that approximately 15% of LHRH cells in the preoptic area of the hypothalamus also produce galanin, a widely distributed brain-gut peptide. The presence of neuronal perikarya in the median eminence has been known for two decades; however, the chemical nature of these neurons is yet unknown. We have recently demonstrated the presence of several peptides, including neuropeptide Y, β-endorphin, galanin, neurotensin, substance-P, enkephalins and dynorphins in these neurons. In rats treated neonatally with monosodium glutamate (MSG), these neurons are absent from the medial basal hypothalamus, including the median eminence. The results should help explain the disorders in neuroendocrine function after neurotoxin damage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90056-01 LMIN

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Excitatory Amino Acids and Opioid Peptides in Hippocampus

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

PI:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
Others:	P. Lee	Visiting Associate	LMIN	NIEHS
	C.L. Mitchell	Pharmacologist	LMIN	NIEHS
	L. Thai	Lab.Technician	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology 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 x

☐ (a2) Interviews

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

Kainic acid (KA) is an excitatory amino acid which causes hippocampal epileptiform discharge and elicits wet dog shakes (WDS) and motor seizures. We and others have reported that systemic injection of KA produced a large release of both enkephalin and dynorphin from the hippocampus. A series of experiments was carried out to examine the possible roles of released opioid peptides and their relation to KA-induced WDS. First, we have shown that pretreatment with naloxone attenuates KA-elicited WDS. To determine which opioid peptide participates in KA-induced WDS, we directly injected antisera against either [Met^5]-enkephalin or dynorphin A(1-8) into the ventricle before KA was administered subcutaneously. Antisera against [Met^5]-enkephalin, but not dynorphin A(1-8), significantly attenuated WDS. These data indicate that enkephalin, but not dynorphin, may be associated with KA-induced shaking behavior. The notion that enkephalin is important in mediating KA-induced WDS was further supported by intrahippocampal injection of different opioid receptor agonists. Direct microinjection of analogues of enkephalin to the ventral, but not dorsal, hippocampus produced numerous WDS. In contrast, microinjection of dynorphin or its analogues elicited no WDS. Furthermore, it was found that the granular-mossy fiber pathway of the ventral, but not the dorsal, hippocampus was essential for the expression of this shaking behavior. However, destruction of the granular-mossy fiber pathway potentiated the seizures and the hipppocampal cell loss induced by KA. This unexpected, yet extremely interesting, finding not only distinguished the roles of the granular-mossy fiber pathway in mediating wet dog shakes vs. convulsive seizures, but also challenged the dogma that this granular-mossy fiber pathway is essential for the expression of limbic seizures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90057-01 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Modulation of Epileptiform Activity by Various Excitatory Amino Acid Inhibitors

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

PI:	Clifford L. Mitchell	Pharmacologist	LMIN	NIEHS
Others:	J. S. Hong	Pharmacologist	LMIN	NIEHS
	C. W. Xie	Visiting Fellow	LMIN	NIEHS
	P. Lee	Visiting Associate	LMIN	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Neurophysiology

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	0.55	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.)

Considerable work in this laboratory has focused on the role of excitatory amino acids in seizure activity and related sequelae. The major objective of this project is to determine the relative contributions of the kainate, quisqualate and NMDA receptors in the generation of epileptiform activity along the trisynaptic pathway of the hippocampal formation. Sustained stimulation of the perforant path activates this entire pathway. The NMDA antagonist, MK-801, prevents status epilepticus and loss of the CA1 and CA3 pyramidal cells associated with this stimulation. However, paroxysmal shaking of the head, neck and trunk, a phenomenon known as wet dog shaking (WDS) is exacerbated by MK-801. Since WDS are associated with epileptiform activity of the dentate granule cells these results suggest that NMDA receptors may be of little importance in the generation of epileptiform activity at the perforant path - dentate granule cell synapse. However, NMDA receptors appear to be critical for establishment of status epilepticus and subsequent death of the CA1 and CA3 pyramidal cells. Current efforts are examining in greater detail the relative abilities of kainate, quisqualate and NMDA receptor antagonists to block epileptiform activity generated by perforant path stimulation at each point along the trisynaptic pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 90058-01 LMIN

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Excitotoxins on Brain-Endocrine Functions

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

PI:	A. Negro-Vilar	Research Physiologist	LMIN	NIEHS
Others:	F. Lopez	Visiting Fellow	LMIN	NIEHS
	T. Iuone	Visiting Fellow	LMIN	NIEHS
	I. Merchenthaler	Visiting Scientist	LMIN	NIEHS
	A. O. Donoso	Guest Researcher	LMIN	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular and Integrative Neuroscience

SECTION
Reproductive Neuroendocrinology

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

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

CHECK APPROPRIATE BOX(ES)

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

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

Neonatal administration of the excitatory amino acid glutamate (monosodium salt) to rodents and non-human primates results in the selective destruction of certain neuronal populations in brain regions related to neuroendocrine regulation of important body functions. As a result of these MSG-induced lesions, impairment of growth, obesity, infertility and behavioral disturbances have been shown to occur. Researchers at LMIN have evaluated extensively the neurochemical and neuroendocrine damage as well as the consequences of the glutamate-induced lesions on endocrine, reproductive and neural activities during adult life. Recent studies have uncovered evidence indicating that a hyperresponsiveness to different stimuli is observed as a result of the neurotoxin treatment, and that opiate neurons in the brain play a role in this phenomenon. In addition, a disrupted pattern of pulsatile or episodic gonadotropin and prolactin secretion has been found to be responsible for the infertility associated with the MSG-induced brain damage. The impact of neonatal exposure to neurotoxic amino acids on the expression of specific neuropeptides in certain hypothalamic regions was evaluated by utilizing modern immunocytochemical approaches to localize and co-localize specific brain peptides in defined nuclei and pathways. A selective deficit in the expression of several key peptidergic systems (β-endorphin, enkephalin, neuropeptide Y, galanin, neurotensin, etc.) was observed in the median eminence region of rats exposed neonatally to high doses of monosodium glutamate. These findings may help to establish a link between the neurotoxic damage and the observed neuroendocrine and behavioral disorders. Additional studies have explored the pharmacological characteristics of glutamate receptors in the hypothalamus, establishing a dose-effect relationship for the different subtypes (NMDA, kainate, quisqualate) of glutamate receptors. These studies provide a useful in vitro model for the assessment of specific and non-specific actions of excitatory amino acids in neuroendocrine neurons known to be particularly susceptible to excitotoxin exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01ES 25020-07 LPP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	W. E. Bakewell	Graduate Student	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:
2.00	2.00	

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.)

Pulmonary surfactant is a heterogeneous complex consisting primarily of surface active phospholipids and apoproteins synthesized and secreted by Type II cells in the alveoli of the lungs. The major lipid component of surfactant is dipalmitoyl phosphatidylcholine and the major protein component is surfactant apoprotein A (SPA). SPA is a specific surfactantassociated protein found only in the lungs. The function of DPPC is to lower surface tension at the air/cell interface and the function of SPA is to assist in this process. Biosynthesis and secretion of these substances by alveolar Type II cells is critical for the prolonged stabilization and function of the lungs.

Silica dust causes massive increases in the surfactant content of the lungs but the mechanisms through which this occurs are not precisely known. In response to intratracheal injection of silica an inflammatory condition develops in the lungs and as a consequence some, but not all, Type II cells within the alveoli become hypertrophic. We have developed methods for the isolation of these hypertrophic Type II cells and for their separation from normal Type II cells and demonstrated that these hypertrophic cells exist in an activated state insofar as synthesis of critical surfactant components are concerned. The appearance of these hypertrophic Type II cells underlies the massive increases in surfactant found in the lungs of silica exposed rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01ES 25021-06 LPP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Visting Fellow	LPP, NIEHS
	T. Vollberg	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:
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.)

Our studies have been focussing on the mechanism that regulate proliferation and differentiation in normal and transformed tracheobronchial epithelial cells. Human bronchial and rabbit tracheal epithelial cells are used as in vitro model systems. Based on our findings we proposed a multi-stage model of squamous cell differen tiation. Most cells in the normal tracheobronchial epithelium are withdrawn from the cell cycle and are quiescent. Under conditions that induce hyperplasia, including vitamin A-deficiency, mechanical or toxic injury or culturing the cells in vitro, cells in culture are recruited to reenter the cell cycle (first stage of the differentiation process). We have shown that epidermal growth factor, transforming growth factor a, and insulin typeI are factors that regulate growth of these cells in a positive manner whereas transforming growth factor b (TGFB) act as negative growth regulator. TGFB regulates the synthesis of several gene products such as transglutaminase type II and fibronectin in normal tracheobronchial cells: however, transformed cells are resistant to TGFB. TGFB regulates these gene products at the level of mRNA synthesis. In the following stages cells undergo irreversible growth arrest and expression of the squamous differentiated phenotype. Several squamous cell markers have been identified, cloned and sequenced including the typeI (epidermal) transglutaminase. Retinoids, analogs øf vitamin A, inhibit the expression of squamous cell differentiation as indicated by the inhibition of squamous cell markers. Retinoids act by inhibiting the increase in the levels of mRNA of transglutaminase typeI, keratins and other markers. Both cytosolic retinoic acid binding protein (CRABP) as well as nuclear retinoic acid receptors (RAR) have been identified in these cells. Based on the comparison of the biological activity of retinoids with their ability to bind to CRABP or RAR, it was concluded that the modulation of gene expression during squamous differentiation by retinoids is mediated via nuclear retinoic acid receptor(s). These receptors regulate gene expression by binding to specific response elements of responsive genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01ES 25023-06 LPP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	A. Robertson	Senior Staff Fellow	LPP, NIEHS
	R. Steigerwalt	Senior Staff Fellow	LPP, NIEHS
	P. Ferriola	Staff Fellow	LPP, NIEHS
	S. Randell	Staff Fellow	LPP, NIEHS
	Z. Duniec	Visiting Fellow	LPP, NIEHS
	T. Gray	Biologist	LPP, NIEHS
	D. Rusnak	Technician	LPP, NIEHS

COOPERATING UNITS (if any)

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:
8	6	2

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of our studies is to elucidate mechanisms of multistage transformation of the epithelial cells of the conducting airways. Rat tracheal epithelial (RTE) cell cultures are used as experimental models. Studies were conducted 1) to determine the role of oncogene activation in transformation of RTE cells and 2) to determine whether the rate of progression and the pattern of oncogene activation are carcinogen specific. DNA isolated from 5 neoplastic RTE cell variants independently transformed with either MNNG or gamma irradiation failed to exhibit transforming activity in repeated NIH 3T3 transfection assays, suggesting that ras gene mutations were probably not involved. However, Northern-analysis of RNA with a dozen oncogene probes revealed 3-5 fold over-expression of c-fos, c-abl, p53, c-Hras and c-Kras but not c-myc. We then examined spontaneous transformants, transformants induced by MNNG or 5-azacytidine for carcinogen specific differences in the rate of progression and pattern of oncogene expression. The results indicated, that the rate of conversion from the pre-neoplastic to the neoplastic phenotype as well as the pattern of oncogene expression (Hras, Kras and raf were overexpressed in some of the transformants) was independent of the nature of the initiating insult. Studies on the possible role of growth factors in transformation of RTE cells showed, that in contrast to normal RTE cells most neoplatic transformants are insulin, EGF and BPE (pituitary extract) independent. Northern analysis showed marked overexpression of TGFalpha and TGFbeta transcripts. Presence of TGFalpha in media conditioned by transformed cell lines was demonstrated by RIA, Western blotting and radio-immune assays. TGFalpha probably acts as an autocrine growth factor in transformed RTE cells. The role of TGFbeta is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01ES 25027-06 LPP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	D. Dixon	Expert	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:
1.0	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 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) (CCSP) 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. Amino acid sequences of the purified protein indicate that the protein is probably identical to uteroglobin, the major secretory portein of the rabbit uterine epithelium. In addition, we have shown that CCSP and uteroglobin are immunochemically similar proteins. Recent studies indicate that CCSP exists in three immunochemically similar isoforms and that all isoforms are found in lavage effluents from the lungs of rabbits and in Clara cells and their secretions. We have also found CCSP to be secreted by cells within the trachea of rabbits and although it seems reasonable to assume that the cells responsible are Clara cells, the morphological appearance of nonciliated tracheal epithelial cells do not correspond exactly with the morphological appearance of Clara cells from the distal airways. In future studies we will try to identify those cells of the trachea that synthesize and secrete CCSP.

The origins of adenomas and adenocarcinomas in the lungs are not known. Examination of the literature identifies the bronchiolar Clara cell as a possible source. Using marker proteins, such as CCSP, we have begun to examine the hypothesis that the Clara cell is the cell of origin of the spontaneous pulmonary adenomas and carcinomas in strain A mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01ES 25030-03 LPP

PERIOD COVERED
October 1, 1988 to September 30, 1989

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

PI:	Arnold R. Brody	Research Biologist	LPP, NIEHS
Others:	V. Kalter	Staff Fellow	LPP, NIEHS
	A. Osornio	Visiting Fellow	LPP, NIEHS
	J. Bonner	IRTA Fellow	LPP, NIEHS
	L. Moore	Biologist	LPP, NIEHS
	A. Badgett	Biologist	LPP, NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pulmonary of Pathobiology

SECTION
Pulmonary Pathology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	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.)

Research in the pulmonary pathology laboratory has been focused upon the basic biological mechanisms through which inhaled particles cause lung disease. We have developed models of asbestosis and silicosis using rats and mice and have shown that the disease process is initiated at junctions of bronchioles and alveolar ducts. One hour of exposure to chrysotile asbestos is sufficient to cause progressive fibrogenesis at alveolar duct bifurcations. The process is initiated by a complement-dependent chemoattraction of lung macrophages to the sites of particle deposition. The central working hypothesis in our laboratory is that these macrophages synthesize and secrete an array of products which mediate the pathogenesis of lung fibrosis. To test this hypothesis our work over the past year has focused on two major classes of pulmonary macrophage (PM) products, arachidonic acid (AA) metabolites and mesenchymal cell growth factors. We showed that alveolar PMs produced at least five AA metabolites including prostaglandin (PG) $F_2\alpha$. HHT, 5, 12 and 15-HETE; whereas intravascular PMs produced three additional vasoactive metabolites, thromboxane B_2, PGD_2 and PGE_2. Varying amounts and combinations of these metabolites are produced under the influences of inorganic particles, infectious agents and soluble mediators. Concerning the growth factors, we had shown that rat PMs produced a homologue of human platelet-derived growth factor (PDGF). This past year we have demonstrated that the PMs also synthesize and secrete α2-macroglobulin (αM) which serves as a binding protein for the macrophage-derived PDGF. The αM competes for the PDGF receptors on rat lung fibroblasts and could play a significant role in modulating the growth promoting and chemotactic activities of PDGF. Ongoing studies are designed to study the biology biochemistry and molecular characteristics of these products in vitro and in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70060-16 LRDT

PERIOD COVERED: October 1, 1988 to September 30, 1989

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
	K. G. Nelson	Senior Staff Fellow	LRDT NIEHS
	R. Santti	Visiting Scientist	LRDT NIEHS
	T. Takahashi	Visiting Scientist	LRDT NIEHS
	C. T. Teng	Senior Staff Fellow	LRDT NEIHS
	N. Bossert	Staff Fellow	LRDT NIEHS
	C. Burroughs	Staff Fellow	LRDT NIEHS

COOPERATING UNITS (if any)
Bowman-Gray School of Medicine
Duke University Medical Center
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	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.)

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. Neoplasias in the female structures derived from the Müllerian duct (e.g., uterus) were demonstrated to be hormonally dependent, transplantable tumors and cell lines were recently established from them. The developing Müllerian duct has been further studied at the cellular and molecular levels. 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. During this developmental period, estrogen was shown to transiently inhibit uterine epithelial cell proliferation; the possibility of a cellular toxicity response to estrogen in the newborn mouse uterus is supported by finding an enzyme, peroxidase, which is known to bioactivate estrogen, in the neonatal uterine epithelium. The ontogeny and tissue specificity of estrogen and androgen metabolism was also studied in the male genital tract. Studies on the formation of estrogens by mouse prostatic tissue separated into different embryological or anatomical zones failed to detect aromatase activity; on the other hand, formation of dihydrotestosterone from testosterone and conversion to diols was regionally distributed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70062-02 LRDT

PERIOD COVERED: October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Role of Growth Factors in Growth and Differentiation of the Reproductive Tract*

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

PI:	K. G. Nelson	Senior Staff Fellow	LRDT NIEHS
Others:	T. Takahashi	Visiting Scientist	LRDT NIEHS
	N. L. Bossert	Staff Fellow	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS

COOPERATING UNITS (if any)
None
*Formerly: The Role of Growth Factors and Inhibitors in Estrogen-Induced Uterine Growth

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:
3.5	3.2	0.3

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

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

Our goal is to understand the cellular mechanism by which prenatal and neonatal DES exposure permanently alters the cell growth and differentiation of male and female reproductive tracts. Recent studies have shown that estrogen-induced growth of various target tissues is mediated in part by the production of polypeptide growth factors that act in an autocrine or paracrine fashion. Our current research involves the identification of polypeptide factors associated with estrogen-induced growth of the female mouse reproductive tract. Our data suggest that several peptide growth factors may act as positive mediators of estrogen-induced growth including insulin-like growth factor 1, epidermal growth factor-like peptides (EGF and transforming growth factor-alpha TGFα), transforming growth factor-beta TGFβ, and lactoferrin. Our recent studies directed at the elucidation of the role of EGF-like peptides as possible autocrine factors have shown that estrogen treatment induces both uterine EGF and TGFα (a polypeptide structural related to EGF), antibodies against these peptides inhibit estrogen-stimulated proliferation, EGF is a potent in vivo mitogen for vagina and uterus, and EGF (like estrogen) stimulates the appearance of uterine lactoferrin. This data suggest that EGF has definite estrogen-like effects in the promotion of cell growth, in vivo, and that EGF and TGFα may serve as important mediators of estrogen action. Our future plans are to continue to characterize and define the role of peptide mediators of estrogen-induced growth, determine the cell type responsible for the synthesis of these factors, locate the cellular target where these factors act, elucidate whether these factors act alone, synergistically or temporally, and investigate the second messenger systems that tranduce the growth factor signal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70065-13 LRDT

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	V. Davis	IRTA Fellow	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS
	M. Ikeda	Visiting Associate	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.)

Induction of certain genes by estrogens involves the interaction of the hormone with a receptor protein. The specificity and responsiveness of tissues to hormonal stimulation are governed in most part by the presence and biochemical action of this receptor protein. We have purified and characterized the receptor protein and its intracellular site(s) of action. Earlier observations had indicated during uterine estrogen stimulation a bimodal nuclear receptor occupancy. New findings have shown a change in chromatin receptor acceptor sites and nuclear matrix binding coordinately with the receptor pattern indicating a possible alteration in the pattern of gene expression at the different times. The estrogen receptor protein has been purified from mouse uterus by steroid affinity and oligonucleotide chromatography. Molecular properties of the protein have been analyzed by epitope specific antibodies to understand the mechanism of receptor activation and conformation. Characterization of the receptor has indicated multiple forms which are proteolytic fragments and not separate gene products. The protease action results in a receptor form which has lost its ability to interact with DNA and, consequently, its biological activity. We have recently shown that the nuclear estrogen receptor specifically exhibits a doublet form when bound by biologically active estrogens. Studies of receptor DNA interactions have indicated multiple complexes by band shift assays. The specificity and stability of these complexes varies depending on the biological potency of the ligands. The higher molecular weight component of the doublet is phosphorylated and associated with tightly bound chromatin sites. Weak estrogens or antiestrogens did not produce the doublet form. These findings suggest that this form of the estrogen receptor may be involved in gene activation and hormone responsiveness. Cell culture studies have indicated the production of stable transfectant clones of the estrogen receptor and reporter gene constructs. These systems are being used as in vitro test systems for studies of estrogen receptor protein structure and gene regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70067-06 LRDT

PERIOD COVERED: October 1, 1988 to September 30, 1989

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	Senior Staff Fellow	LRDT NIEHS
Others:	Y. H. Liu	Visiting Fellow	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 action since it contains estrogen receptors and depends on estrogen stimulation for normal physiological functions. We have previously purified an estrogen-induced secretory protein from mouse uterine luminal fluid. 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. We have mapped lactotransferrin gene to human chromosome 3 (q21-q23) and mouse chromosome 9 and shown that it was induced by estrogen in a time and dose-dependent fashion in the uterus but not the mammary gland. A high level of lactotransferrin was detected by immunocytochemistry in uterine epithelial cells 1 day after parturition, but immunoreactivity disappeared quickly thereafter. Lactotransferrin message was, however, relatively abundant in the mammary gland at the end of the lactation period. The presence of lactotransferrin in various tissues also was investigated, and two forms of immunoreactive material were detected. A 70kDa band was found in uterine luminal fluid from the estrogen-stimulated immature mouse and in homogenates of lung, vagina, mammary gland, oviduct, spleen, lymph node, and uterus of the adult female mouse. In addition, a 65kDa band was detected in submaxillary gland, kidney, ovary, and all of the above tissues. Brain and duodenum had no detectable immunoreactive material. We isolated ten genomic clones from a mouse genomic library which contained the entire lactotransferrin gene and several Kb of 5' flanking sequences. We are presently sequencing a 8 Kb EcoR1/PstI fragment which contains the 5' flanking sequence, the first 5 exons and the introns. We hope to identify the cis-acting positive and negative regulatory elements of the lactotransferrin gene and gain some understanding of the trans-acting factors leading to lactotransferrin expression in response to different hormonal signals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 70076-05 LRDT

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	J. E. Welch	IRTA Fellow	LRDT NIEHS
	R. S. McGee	IRTA Fellow	LRDT NIEHS
	D. R. Joseph	IPA	LRDT NIEHS

COOPERATING UNITS (if any)
U. of Pennsylvania School of Medicine
U. of Washington School of Medicine
U. of North Carolina, Chapel Hill
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The Biomembrane Institute, Seattle

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:
5.4	3.9	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.)

The developmental process of spermatogenesis in mammals produces a cell highly specialized in structure and function. Our goals are to define the intrinsic and extrinsic mechanisms that regulate gene expression during male germ cell development. We use monoclonal antibodies, immunofluorescence, protein purification, and gel electrophoresis methods to identify, localize, and isolate proteins, and antibody screening of expression vector libraries prepared from germ cell mRNA to clone the genes for these proteins. We have prepared antibodies to key proteins of the sperm flagellum and shown that the antibodies recognize germ cell-specific cytoskeletal proteins, that specific proteins are synthesized at different times during male germ cell development, and that these proteins may be products of unique intermediate filament genes expressed during spermatogenesis. Other studies have examined the influence of somatic cells on germ cell function and gene expression. In the embryo, primordial germ cells (PGC) migrate from the hindgut to the developing genital ridges. An *in vitro* assay was developed to show that PGCs migrate in response to a chemotactic signal from the genital ridges. We also found that PGCs lose two surface markers after they enter the genital ridges or when they are cultured with somatic cells from the genital ridges, but not when they are incubated alone, indicating that an extrinsic signal influences expression of these markers. In addition, sperm cannot fertilize ova when they leave the testis and are modified during epididymal maturation to gain this ability. Binding of epididymal glycoproteins to the sperm surface are an important part of the maturation process, and we have identified a factor produced by Sertoli cells that stabilizes attachment of an acidic epididymal glycoprotein to sperm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70077-03 LRDT

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	Senior Staff Fellow	LRDT NIEHS
Others:	E. M. Eddy	Head, Gamete Biology	LRDT NIEHS

COOPERATING UNITS *(if any)*

Division of Toxicology Research and Testing, NIEHS
The University of North Carolina, Chapel Hill

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:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The synthesis of heat-shock proteins (hsp) in cells exposed to stress is one of the most highly conserved regulatory systems known and apparently protects cells against the effects of adverse environmental conditions. The process of spermatogenesis is unusually sensitive to slight elevations in temperature and to many toxic agents. However, we have shown that one of the most abundant proteins (P70) in mouse spermatogenic cells is related closely to hsp70, the major inducible hsp. P70 and hsp70 have almost identical mass and isoelectric points. P70 reacts strongly with a monoclonal antibody that is specific for products of the hsp70 gene family. Both P70 and hsp70 are ATP-binding proteins and are purified by using ATP-affinity chromatography. However, P70 and hsp70 are unique proteins on the basis of peptide map analysis and are regulated differently in germ cells. By examining purified preparations of spermatogenic cells, we have shown that preleptotene and leptotene-zygotene spermatocytes contain little P70, while relatively large amounts of P70 are present in pachytene spermatocytes and round spermatids. Labeling studies show that P70 is synthesized primarily in pachytene spermatocytes with little synthesis occurring in other stages of spermatogenesis. The synthesis of hsp70 is not detectable in unstressed cells but is induced in all stages of isolated germ cells following heat stress. These results indicate that P70 is expressed in a stage-specific manner during cell differentiation, whereas hsp70 is only synthesized in germ cells in response to stress. Specific antibodies have been prepared to P70 and hsp70 and are being used to examine the function of these proteins in the testis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 ES 70078-06 LRDT

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

COOPERATING UNITS *(if any)*

The University of North Carolina, Chapel Hill
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:
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.)*

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 process is the appearance of several germ cell-specific constituents in a precise temporal sequence. Three areas of research have been pursued to further characterize these unique constituents, both in the acrosome and on the cell surface. (a) Monoclonal antibodies have been used to identify and characterize germ cell components expressed during restricted periods of spermatogenesis. Antibody 1D4 reacts with multiple glycoconjugates that appear in the acrosome of early spermatids but are modified during the late haploid stages so that the determinant is no longer detected. In contrast, this antibody recognizes acrosomal glycoconjugates that are retained in guinea pig spermatozoa. Additional monoclonal antibodies that recognize cell surface constituents with stage- and tissue-specificity have been prepared against proteins excised from two-dimensional gels. (b) The two mannose 6-phosphate (M6P) receptors, which may have roles in intercellular communication and in targeting hydrolases to the acrosome, are synthesized in distinct proportions in pachytene spermatocytes, round spermatids, and Sertoli cells. We have shown that these cell types have functional M6P receptors on their cell surfaces and that Sertoli cells secrete M6P-bearing glycoproteins. (c) Interactions between Sertoli cells and germ cells at defined stages of spermatogenesis have been examined in short-term cultures. When cultured in the presence of Sertoli cell-conditioned medium (SCM), pachytene spermatocytes and round spermatids maintain elevated viabilities and ATP levels. SCM contains multiple glycoproteins and its active fraction has stability characteristics that distinguish it from Sertoli cell growth factors described previously. Germ cell and Sertoli cell constituents, particularly those exhibiting stage and tissue specificity, are candidates for further studies exploring gene regulation and cell interactions during spermatogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 80040-06 LRDT

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Pharmacogenetics of Microsomal Steroid Hydroxylases

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

PI:	M. Negishi	Head, Pharmacogenetics	LRDT NIEHS
Others:	M. Lang	Visiting Scientist	LRDT NIEHS
	K. Aida	Visiting Scientist	LRDT NIEHS
	R. Lindberg	Visiting Fellow	LRDT NIEHS
	H. Yoshioka	Visiting Fellow	LRDT NIEHS
	B. Burkhart	Biologist	LRDT NIEHS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Reproductive and Developmental Toxicology

SECTION
Pharmacogenetics

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	4.5	3.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We characterized the gene structures of male- and female-specific steroid 16α-hydroxylase (C-P-450$_{16\alpha}$ and P-450$_{16\alpha}$OH-A, respectively) and of female-specific 15α-hydroxylase (P-450$_{15\alpha}$). Each gene is a member within a large, different family, and the regulation mechanisms and catalytic activities within each P-450 family diverged. Of five genes in the P-450$_{16\alpha}$ family, gene ca is expressed specifically in male liver and kidney, while both sexes express gene cb only in liver. The nucleotide sequence identity and expression of the cDNAs in COS-1 cells revealed that C-P-450$_{16\alpha}$ is encoded by gene ca. We placed the 5'-flanking regions of gene ca and cb at the front of hGH gene (a reporter) and transfected them into various cells, which resulted in finding the presence of gene ca-specific and tissue-specific positive and negative cis-acting elements. Furthermore, footprinting assay showed the presence of a gene ca-specific nuclear protein. The P-450$_{15\alpha}$ family consists of at least two members, steroid 15α-hydroxylase and coumarin 7-hydroxylase (P450coh). In spite of the divergent catalytic activities, however, these enzymes differ only 11 amino acids within their 494 residues. Site-directed mutagenesis of each 11 residue and transfection of the mutated cytochromes into COS-1 cells, indicated that the activities of both cytochromes depend critically on the identities of the amino acids at position 117, 209 and 365; and, moreover, that a single mutation in which Phe209 is substituted by Leu is sufficient to convert the specificity of P-450coh from coumarin to steroids. Genetic and hormonal regulation of the gene expressions and drug-inductions are differentiated between the genes within the P-450$_{15\alpha}$ family. A regulatory and functional divergence between the genes within each P-450 family might be the reason explaining why P-450 is so polymorphic, has evolved, and perhaps still evolving, so rapidly and to such a great extent, which are reflecting directly to sex- and tissue-specific toxicity and carcinogenicity of chemicals and drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 43010-04 DBRA

PERIOD COVERED
October 1, 1988 to September 30, 1989

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/LMT	NIEHS
	Lee G. Pedersen	Research Chemist	DBRA/OD	NIEHS
	Charles Foley	Staff Fellow	DBRA/LMT	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:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

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

This project is concerned with determining theoretical factors involved in mutagenesis and in the initial steps of carcinogenesis. The proliferation of new experimental techniques in genetic engineering is providing innovative pathways for studying the dependence of chemically induced mutational events on DNA sequence. Computer modeling is being used to examine the physical chemical factors (charge distributions, binding energies, stereo-chemistry, activation energies, solvation, counterions) contributing to site specificity of DNA damage by chemical agents. The same techniques are also being employed to determine changes in molecular properties of oncogene proteins as a consequence of specific mutations.

Specifically, computer intensive quantum mechanical calculations are employed to determine the properties of small molecules. These results are then used to paramaterize empirical force fields that can in turn be used to model the mechanical properties of large molecules such as meaningful segments of DNA and proteins with molecular mechanics/dynamics and computer graphics.

Research issues of ongoing interest include the characterization of local structures of DNA sequences (native and chemically modified) that contain known mutational hotspots from mammalian oncogenes and bacterial systems, the examination of the molecular details of the initial attack by mutational metabolites, sequence dependent DNA bending, DNA-protein interactions, and the understanding of the consequences of single amino acid changes on the function of critical proteins such as the p21 ras oncogene protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 40004-12 SBB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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)*

PI:	Beth Gladen	Statistician	SBB	NIEHS
	Clarice Weinberg	Mathematical Statistician	SBB	NIEHS
OTHERS:	Allen Wilcox	Medical Officer	EB	NIEHS

COOPERATING UNITS *(if any)*
Department of Biomathematical Sciences, Mt. Sinai Medical Center
Epidemiology Methods Section, NCI

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION
Statistics 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 involves the development and evaluation of statistical methods which are appropriate for various types of epidemiologic research. This year, work has concentrated in two main areas. Mathematical modelling in reproduction has continued, with the focus being on the estimation of the probability of conception. In addition, new methodologies for the design and analysis of case-control studies have been developed and are being applied Specific developments are as follows. (1) An improved algorithm for identifying the day of ovulation in a menstrual cycle using urinary estrogen and progesterone levels was developed and refined. This is a useful preliminary to other work, as it allows the detection of anovulatory cycles and gives a well-defined time point in ovulatory cycles. (2) The development of models for determining the probability of conception following intercourse on different days of the menstrual cycle continued. One potential long-term application of this would be the development of methods for estimating the effects of putative environmental reproductive toxins on ovum viability and sperm survival. (3) A new design for case-control studies was developed which offers notable advantages over current methods. This approach allows for non-uniform sampling of subjects so that study efficiency can be optimized. Unlike matched designs currently in use, the design permits a flexible analysis that includes estimation of effects associated with factors which were allowed to influence the selection of study subjects. This design is being implemented in a large case-control study of residential radon exposure and lung cancer. (4) In collaboration with Dr. Sholom Wacholder at the National Cancer Institute, methods are being developed for the analysis of case-control studies where data are missing; the data may be missing either unavoidably or by design. (5) In ongoing work in collaboration with Dr. Sylvan Wallenstein of Mt. Sinai Medical Center, methods for testing for the existence of seasonal patterns in event data were refined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 44002-13 SBB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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)*

PI:	Norman Kaplan	Research Mathematician	SBB	NIEHS
OTHERS:	Charles H. Langely	Research Chemist	LG	NIEHS

COOPERATING UNITS *(if any)*

Laboratory of Animal Genetics. LRDT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biomathematics Section

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Research has continued on the coalescent process for a random sample of genes from a population that is undergoing selection. This process describes the genealogical history of the sample. Using the coalescent process for a sample of genes from a selectively neutral locus that is linked to a locus at which selection is taking place, a population genetic model was analyzed that describes the steady state effects of strongly selective ancestral substitutions on the number of selectively neutral polymorphic sites in the sample. The model predicts that in a region of low crossing over, strongly selective substitutions in the history of a sample can substantially reduce the number of polymorphic sites in the sample from that expected under neutrality. Thus by ignoring this effect, one can markedly underestimate the rate of substitution. Work has also continued on developing the analytic properties of the coalescent process for a sample from a geographically subdivided population undergoing selection. The results show that including migration in the analysis of recent Adh data for Drosophila Melanogaster has negligible effect when compared to the previous analysis which assumes a panmictic population. New research has begun to model the evolution of the P-element in Drosophila. This work is motivated by recent findings suggesting that P-element regulation results from defective copies generating defective transposase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 45001-9 SBB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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)*

PI:	Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
OTHERS:	Christopher Portier	Mathematical Statistican	SBB	NIEHS
	Gregg E. Dinse	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Statistics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	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.)*

This project is concerned primarily with statistical issues in the design, analysis, and interpretation of laboratory animal experiments. A statistical analysis of carcinogenicity and genetic toxicity data for 41 chemicals evaluated by the NTP confirmed an earlier finding that no combination of the four short term tests studied improved the ability of Salmonella alone for predicting rodent carcinogenicity. An evaluation of the NTP historical control database indicated a significant time-related increase in the incidence of a number of different tumor types. A re-evaluation of tumor diagnoses in untreated animals from selected early NCI and recent NTP studies indicated that differences in pathology terminology could not totally explain this effect. Possible additional factors influencing tumor incidence include increasing amounts of tissue examined in the current studies and the associated increased body weight of control animals in more recent studies. In a related project, viral infections did not appear to significantly influence tumor incidence in F344 rats or B6C3F1 mice when inter-laboratory differences and time-related trends were taken into account. Statistical methodology has been developed that allows an assessment of differences in tumor incidence without determining cause of death and requiring at most one sacrifice time. The key assumption of this method is that the difference between the death rates for tumor-free and tumor-bearing animals (i.e., the risk difference) is constant. Furthermore, the estimate of the risk difference provides a summary measure of tumor lethality. Statistical methods have also been developed for extracting information on disease incidence from data on disease mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 48001-02 SBB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Analysis of Data from Genotoxicity Experiments

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

PI: Walter Piegorsch, Mathematical Statistician, SBB, NIEHS

OTHERS: Michael A. Resnick, Supervisory Research Geneticist, CGTB, NIEHS
Errol Zeiger, Supervisory Microbiologist, CGTB, NIEHS

COOPERATING UNITS *(if any)*
Cellular Genetics and Toxicology Branch, DTRT

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION
Biomathematics Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

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

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

The focus of this project is the development of appropriate statistical methodologies for analysis of genotoxicity data from a variety of animal and microbial systems. Investigations continued into the statistical analysis of data on aneuploidy induction (chromosomal loss or gain) in yeast. The results identified possible differences in the nature of the aneugenic response between the gain and loss systems, particularly in the patterns of variability exhibited by data from each system. Additional analyses examined the level of qualitative agreement among contract laboratories conducting the NTP Salmonella mutagenicity assay. Evidence of significant departure from purely chance agreement was seen in all categorizations and classifications of interest. An associated study was begun to examine measures of association between test systems in bioassays with specified endpoints. Attention was also directed at average concordance as a measure of inter-assay agreement. It was shown that average concordance is, in general, a difficult measure of agreement to interpret, since it inherently depends upon the potency/toxicity of the compounds under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 48002-2 SBB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Models in Toxicology and Biochemistry

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

PI:	Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
OTHERS:	Norman L. Kaplan	Research Mathematician	SBB	NIEHS
	Annette Kopp	Visiting Fellow	SBB	NIEHS

COOPERATING UNITS *(if any)*
Department of Mathematics and Statistics, Miami University of Ohio, Oxford, OH
Department of Biostatistics, University of North Carolina, Chapel Hill, NC
Department of Biostatistics, German Cancer Research Center, Heidelberg, WG

LAB/BRANCH
Statistics and Biomathematics Branch

SECTION
Biomathematics Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0.0

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

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

The goal of this project is to increase our understanding of the use and application of existing models in toxicology and biochemistry and to develop and implement new statistical models to aid in explaining current research findings. One research effort concerns the utilization of individual-to-individual variability of biologically interpretable parameters in the risk estimation process. A computer-intensive method was developed for assessing the overall impact of the variability of biologically interpretable parameters on the variability of estimates of safe exposure levels. Another research effort concerns the development and use of multistage models of carcinogenesis which incorporate clonal expansion of sub-populations of cells. Recent work is concerned with the development of simple approximations of the cumulative tumor incidence function. These approximations were compared to the exact values obtained using discrete event simulations. We have also examined whether tumor incidence data can be used to estimate parameters in multistage models of carcinogenesis both with and without clonal expansion. This research included a study of changes in the design of carcinogenicity experiments with the objective of improving our ability to illucidate mechanism. Research on this topic is continuing with a focus on utilizing biochemical information on altered cell populations when estimating model parameters. Multistage models that incorporate DNA damage and repair are also under consideration. In teratology, the relationship between maternal toxicity and developmental defects is being studied using a database of teratological studies developed by the NTP. In addition, we are studying the statistical characteristics of several models proposed for estimating risks of developmental defects from exposure to chemicals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-43002-13 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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, NIEHS

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

Polychlorinated biphenyls (PCBs) and DDT/DDE (DDE is the stored metabolite of DDT) family are toxic, widespread pollutants. Both 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 both compounds in North Carolina, a study in Mexico where levels of DDE are two to five times higher, and a study of children poisoned in utero by PCBs in Taiwan. In North Carolina, we measured PCBs and DDE in breast milk and followed about 800 children. Most have now completed second grade. Since evaluation in early life showed subtle changes in development related to transplacental exposure to PCBs, we are now gathering data on conduct, behavior, and school performance. We also observed earlier weaning among women with higher DDE levels in North Carolina. In Mexico, we have completed enrollment for a study of 200 women and their children, who will be followed to see if the high levels of DDE to which the mother was exposed interfere with lactation. In Taiwan, an epidemic of 2000 cases of PCB poisoning occurred in 1979. In 1985, we did a survey of 117 children who were born to mothers who were poisoned. We had previously reported the general findings in the children, and have now completed a detailed evaluation of the dermatologic findings. This showed that the results of transplacental exposure differ from direct exposure - the children had less acne and more hyperpigmentation. Preliminary analyses of blood samples from the children show that PCB levels are not high, implying that the clinical and developmental changes are due to early life exposure rather than continued release of stored chemical.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-43004-11 EB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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
	Gwen W. Collman	Senior Staff Fellow	EB	NIEHS

COOPERATING UNITS *(if any)*
Bowman Gray School of Medicine/Baptist Hospital, Duke University Medical Center, University of North Carolina Medical School, Charlotte Memorial Hospital

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.)*

Environmental exposures may play an important role in a number of poorly understood chronic diseases. To identify potentially preventable causes of one such disease, chronic renal failure, a multi-center case-control study was carried out. Increased risk was associated with the use of non-aspirin nonsteroidal anti-inflammatory drugs such as ibuprofen and indomethacin. Excess risk was, however, confined to older men or to those with underlying conditions that result in compromised renal circulation, a finding that is consistent with at least one proposed mechanism for renal injury from these drugs. Occupational and environmental factors were also found to play a role. Patients with glomerulonephritis were twice as likely as other renal disease patients or controls to report exposure to a variety of solvents and to silica. Analysis of this and a related case-control study of risk factors for IgA nephropathy is ongoing.

Two other studies explored health risks from asbestos and from passive smoking. A 3-fold increase in lung cancer mortality was found among persons identified during the First National Health and Nutrition Examination Survey with x-ray evidence of asbestos exposure who were not necessarily career asbestos workers. In a 12-year follow-up study of over 40,000 adults from western Maryland, overall death rates were found to be elevated among nonsmokers who lived with smokers. Of special note were increased risks of heart and lung disease, and an increased risk of smoking-related cancers, particularly among those exposed at a younger age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-44003-12 EB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)* Statistics and Biomathematics Branch, NIEHS, 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:
1.45	1.45	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

The purpose of the reproductive epidemiology project is to develop and apply methods for measuring damage to human reproduction. Reproductive damage can include infertility, endocrine dysfunction and menstrual disorders, subclinical pregnancy loss, clinically-recognized pregnancy loss (spontaneous abortion), impaired fetal growth, preterm delivery, and perinatal death. Any of these can be caused by environmental factors, and each represents a possible endpoint for detecting effects of toxins on human health. This year we have used data from our prospective study of early pregnancy to explore factors that affect fertility. Women who were exposed prenatally their mother's cigarette smoking were found to be substantially less fertile as adults. We also found a strong relation between current consumption of caffeinated beverages and lower fertility - to our knowledge, the first time such an association has been investigated. Preliminary data from our study of dental technicians shows an association between nitrous oxide exposure and decreased fertility. These several observations suggest that the methods we have developed for measuring fertility may provide a sensitive means for detecting environmental hazards. In further analysis of data from our early pregnancy study, we have looked for factors that affect the risk of very early pregnancy loss. While the study has limited power for this purpose, it is the first study to be able to ask the question at all. The associations we observe between risk of early pregnancy loss and plausible hazards provide hypotheses for future studies. We are starting a new study in collaboration with the University of Chicago to look at reproductive outcomes among DES daughters and sons. This study of adults who were prenatally exposed to synthetic estrogen is part of a broader Institute initiative in environmental estrogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 46002-05 EB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Senior 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:
0.6	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.)*

Environmental exposures may play an important role in the etiology of the acute leukemias, although past studies have had only limited success in identifying risk factors. Immunologic, cytogenetic, and molecular studies indicate that acute lymphocytic and nonlymphocytic leukemia are each comprised of different diseases with similar appearance but with differing prognoses, treatment responses and possibly etiologies. The failure of past studies to identify important risk factors may have been due, in part, to a lack of precision in definition of leukemia subtypes.

Data collection is nearly complete in a study of risk factors for the acute leukemias in adults in which patients will be grouped according to clonal chromosome characteristics, immunologic phenotype, and other biochemical markers, as well as according to more widely available classification systems, to determine if risk factors differ for distinct subgroups of patients. The study was motivated by reports that 50 % of leukemia patients have chromosome abnormalities in bone marrow, and that these patients are likely to have had prior chemotherapy or occupational exposure to solvents. To that end, patients who are enrolled in cancer treatment protocols sponsored by Cancer and Leukemia group B, a cooperative cancer study group, are invited to participate in a telephone survey covering exposure to solvents and chemicals, smoking, irradiation, use of potentially toxic medications, and family medical history. More than 700 leukemia patients have been studied, including 560 with acute nonlymphocytic leukemia and 150 with acute lymphocytic leukemia. Population controls who are demographically similar to cases have been selected by random telephone screening and are also interviewed by telephone. Approximately 350 controls have been interviewed to date. Preliminary analyses suggest that solvent exposure, certain medications, and life-style factors may play an etiologic role, and that risk factors vary by cytogenetic group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-47001-03 EB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Exposure to Radon and Cancer Risk

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

PI:	Dale P. Sandler, Ph.D.	Epidemiologist	EB	NIEHS
Others:	Clarice R. Weinberg, Ph.D.	Mathematical Statistician	SBB	NIEHS
	Gwen W. Collman, Ph.D.	Senior Staff Fellow	EB	NIEHS

COOPERATING UNITS *(if any)*

Yale University, New Haven, Connecticut, University of Utah, Salt Lake City, Utah and 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:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

Recent surveys indicate that between 10 and 20 percent of homes in the United States have indoor radon levels that exceed EPA's guideline level for remedial action, resulting in an estimated 5,000 to 20,000 lung cancer deaths each year. This estimate is based on extrapolating from results of studies of miners with very high radon exposures; findings from these studies may not be generalizable to the population at large.

The relationship between cumulative residential exposure to radon and lung cancer risk is being evaluated in a collaborative study involving Yale University and the University of Utah. The study will include 1000 smokers with lung cancer, 750 nonsmokers with lung cancer, and over 2100 population controls from Connecticut, Utah, and Southern Idaho. Because smoking may enhance the effects of radon exposure, the study is specifically designed to evaluate the potential interaction between radon and cigarette smoke exposure. Controls and a fraction of the available lung cancer cases who smoke will be selected using an individual probability sampling method that will maximize statistical power and allow for the evaluation of different interaction models. Detailed residential histories will be obtained and measurements will be made in past homes using year-long alpha track etch detectors, in order to estimate cumulative radon exposure since age 25 for each subject. Complete lifetime exposure assessments (including childhood) will be made for a subset of participants. A companion study in Connecticut will evaluate the potential childhood cancer risk associated with residential radon exposure. Cumulative radon exposure will be determined for approximately 125 childhood cancer cases and 250 healthy comparison subjects. The project is expected to take at least 5 years to complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-47002-03 EB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:
1.10	1.10	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, as well as influence bone metabolism and cardiovascular health. Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides. Health effects of these environmental estrogens are not known. As a first step, biological effects of plant estrogens will be measured in postmenopausal women. Ninety-four volunteers were recruited for the study, 68 of whom ate a diet rich in plant estrogens, and 26 of whom ate their usual diet. Urine, blood, and vaginal cells were collected to examine effects on the pituitary, the liver and vagina. Blood samples (two prediet and two during the diet) have been analyzed for luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, estradiol, estrone, total cholesterol, high density lipoprotein cholesterol, and apolipoprotein A. Slides of the vaginal smears have been read. Urinary plant-derived estrogens (daidzien, genistein, and equol) and endogenous estrogens in the urine are now being measured. Preliminary examination of the data suggests that the soy diet may lower follicle stimulating hormone as hypothesized, but does not increase sex hormone binding globulin levels. We will begin detailed analyses when we can incorporate an estimate of phytoestrogen dose (derived from the measures of urinary plant estrogens).

If plant estrogens are found to be biologically active in postmenopausal women, other questions to be addressed include: (1) What effects do these chemicals have on other segments of the population, especially 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?

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-48004-03 EB

PERIOD COVERED

October 1, 1988 to September 30, 1989 TERMINATED 10/01/88

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

COOPERATING UNITS *(if any)*

The Johns Hopkins University, Training Center for Public Health Research, Hagerstown, MD

LAB/BRANCH

Epidemiology Branch

SECTION

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 terminated October 1, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-49001-01 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Studies of Occupational Populations Exposed to Carcinogenic Agents

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

PI:	Eric S. Johnson	Visiting Scientist	EB	NIEHS
Others:	Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)* Statistics and Biomathematics Branch, NIEHS, Poultry Science Department, North Carolina State University Regional Poultry Research Laboratories, USDA, East Lansing, Michigan

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	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.)*

Several studies of workers exposed to potential carcinogens are currently ongoing. A chort mortality study of workers engaged in slaughtering and processing of chickens with exposure to the oncogenic viruses of chickens (ALV, MDV, & REV) is being conducted to examine if they are at increased risk of developing certain cancers. The data for over 20,000 poultry workers and similar number of controls, are currently being extracted and edited. The edited file will be sent to Social Security Administration, the National Death Index and State Motor Vehicle Departments for the determination of vital status.

We plan to collect blood from 400 current poultry workers and similar number of controls, and test for antibodies to chicken oncogenic viruses using the ELISA test, and for viral proteins using Western Blot. White blood cells from these samples will be tested for presence of integrated viral genome using the PCR technique. These studies will investigate whether humans are infected with these viruses, and will be complemented by studies of occurrence of these viruses in eggs and chicken products from supermarkets, and in vitro testing of the infectivity of these viruses for human cells.

Blood from 40 sprayers of phenoxy herbicides, and 40 controls is being collected from individuals in Australia, for the determination of serum levels of dioxins and furans, to see if persons who use these herbicides are significantly exposed to these compounds. Participants are being identified and blood collection will follow shortly.

Analysis of data from a case-control study of lung cancer (occurring in excess) in the meat industry is being completed. It is hoped to identify the exposure(s) within the industry responsible for the excess.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-49002-01 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Molecular Epidemiologic Studies of Cancer Susceptibility and Oncogene Activation

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

PI:	Jack A. Taylor	Sr. Staff Fellow	EB NIEHS
Others:	Marshall W. Anderson	Chief	LMT NIEHS
	Rachel Patterson	Technician	LMT NIEHS

COOPERATING UNITS *(if any)* Laboratory of Molecular Toxicology, NIEHS, University of North Carolina, Duke University, Roswell Park Memorial Institute, University of Georgia, Fox Chase, Telemark Sentralsjukehus (Norway), The Finsen Institute (Denmark)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	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.)*

This is an expanded effort of Project Number Z01-ES-48003-02 EB.
Studies in the branch have been established to investigate the role of proto-oncogene alleles in cancer susceptibility, and the role of oncogenes in carcinogen-induced human tumors. A case control study of bladder cancer has been initiated to investigate whether restriction fragment length polymorphisms of proto-oncogenes correlate with cancer susceptibility. Exposure information, along with blood, urine, and tumor tissue, are being collected on 200 bladder cancer cases and 200 controls. Southern blots will be used to determine whether rare alleles of H-*ras* and other proto-oncogenes correlate with cancer susceptibility. The interaction between genotype and exposure will also be explored.

To investigate the role of oncogenes in chemical carcinogenesis, fixed tissue blocks have been obtained from approximately 50 cases of benzidine or beta-napthylamine associated bladder cancer, and 100 bladder cancer cases without such exposures. In addition, a small number of cyclophosphamide associated bladder tumors have been obtained. The polymerase chain reaction (PCR) is being used to amplify H- K- and N-*ras* genes followed by oligonucleotide probing for oncogene activating mutations at codons 12 and 61. The pattern and mutational spectra of oncogene activation will be compared between benzidine/beta-napthylamine associated tumors, cyclophosphamide associated tumors and those which arose spontaneously or were smoking-associated.

In a similar study, fixed tissue samples of lung tumors will be obtained from individuals with primary lung cancers who had high dose occupational exposure to one of a variety of known lung carcinogens, including radon, asbestos, nickel, chromate, and vinyl chloride. PCR with oligonucleotide probing will be used to characterize *ras* family mutations which will then be correlated with exposure information.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-21024-08 LBRA

PERIOD COVERED: October 1, 1988 to September 30, 1989

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
Others:	H. Yeowell	Visiting Associate	LBRA	NIEHS
	M. Faletto	Staff Fellow	LBRA	NIEHS
	P. McClellan-Green	Biologist	LBRA	NIEHS
	M. Romkes	IRTA Fellow	LBRA	NIEHS
	P. Linko	Chemist	LBRA	NIEHS
	G. Lucier	Chief	LBRA	NIEHS

COOPERATING UNITS (if any): M. Negishi, LRDT, NIEHS
Judy Raucy, University of New Mexico
Jack Taylor, Epidemiology Branch, NIEHS
Charles Lieber, Mt. Sinai School of Medicine, Bronx, NY

LAB/BRANCH: Laboratory of Biochemical Risk Analysis

SECTION: Metabolism and Receptors

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 cytochrome P-450 system is the principal monooxygenase system which metabolizes foreign chemicals to inactive compounds and activates them to mutagens and carcinogens. Some of these enzymes are polymorphic in both man and rodents. A polymorphism in a human P-450 (IIC8) has recently been reported. This P-450 is high in 50% of the human population and is much lower in the remainder. The rat may be a good model for the human since P-450g (IIC13) is also present in 50% of the population and absent in the remainder. We recently cloned P450g and found the mRNA for P-450g to be present in equal amounts in both phenotypes suggesting that the mRNA in the (-g) rats might be defective. In contrast, P-450g mRNA is absent in female rats showing that the sex difference is regulated pretranslationally. The sex difference appears to be mediated by the continuous growth hormone pattern seen in the female. We have now prepared a cDNA library from a (-g) rat and have shown that the cDNA for the (-g) phenotype differs from that of the (+g) phenotype by only 9 single base changes. These base changes would result in 7 amino acid differences between the phenotypes. Two specific probes for the (+g) and (-g) cDNAs have been hybridized differentially to mRNA from the two phenotypes, demonstrating that the low phenotype is the result of a defective mRNA. Intermediate phenotype animals have both mRNAs. We are presently performing breeding studies to demonstrate that these are heterozygotes. We have also constructed a library from a human liver and are now screening it with a the P-450g cDNA to select related human isozymes to study human polymorphisms. Additional libraries from selected human individuals will be constructed. cDNAs for P-450g and human P-450s related to this isozyme will be expressed in an expression system to allow us to study their substrate specificity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-46003-05 LBRA

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	Senior Staff Fellow	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	D. DiAugustine	Research Chemist	LBRA	NIEHS
	G. Jahnke	IRTA Fellow	LBRA	NIEHS
	Y. Liu	Biologist	LBRA	NIEHS
	Z. McCoy	Bio. Lab Tech.	LBRA	NIEHS
	C. Miller	Biologist	LBRA	NIEHS
	J. Goldring	Biologist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, DBRA

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Cellular Epidemiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	2.2	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The long range objective is to evaluate the possible use of human lymphocytes as potential markers of human exposure and susceptibility. The relationship between chemical exposure and changes in biochemical or cytogenetic parameters will be investigated in both defined human populations and animal models. The effect of activation/deactivation pathways on the formation of DNA adducts and the resulting consequences on biological endpoints such as SCEs or DNA damage (assessed by nucleoid sedimentation or microelectrophoresis) will be evaluated in human lymphocytes following in vitro exposure to chemicals. We have shown that for benzo(a)pyrene (BP) metabolism, BP-derived DNA adducts, SCE induction by BP and ethoxyresorufin-O-deethylase (EROD) activity there was an 8 to 10-fold variation between individuals and this was not related to smoking status. For BP-derived DNA adducts and EROD activity mixed model analysis for variance showed that the variance between individuals significantly outweighed the variance within suggesting true interindividual differences. The relationship between BP metabolism and DNA adduct formation has been evaluated. Human lymphocytes are polymorphic in one isozyme of glutathione S-transferase (GST). This family of enzymes plays a key role in the metabolic detoxicaton of polycyclic aromatic hydrocarbons. In contrast, GST appears to be involved in the metabolism of ethylene dibromide and perhaps methylene chloride to DNA reactive species. We have shown that the GST activity measured in human lymphocytes correlates very strongly with human liver activity. We are currently studying the effects of ethylene dibromide on human lymphocytes following in vitro exposure and initial studies have shown that there are marked differences between individuals in the sensitivity towards this chemical and it appears that there is a correlation between metabolism and DNA adduct formation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-46004-05 LBRA

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptor Interactions for TCDD and Its Structural Analogs: Species Comparisons

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

PI:	George Lucier	Chief	LBRA	NIEHS
	Joyce Goldstein	Pharmacologist	LBRA	NIEHS
Others:	F.-H. Lin	IRTA Fellow	LBRA	NIEHS
	G. Clark	IRTA Fellow	LBRA	NIEHS

COOPERATING UNITS *(if any)*
Chemical Pathology Branch, NIEHS
Statistics and Biomathematics Branch, NIEHS; Systemic Toxicology Branch, NIEHS
Chemical Industries Institute for Toxicology; Baylor University

LAB/BRANCH
Laboratory of Biochemical Risk Analysis

SECTION
Metabolism and Receptors

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

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

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

The long-range plan of this project is to evaluate the actions of receptors for halogenated aromatic chemicals in various species including humans. There are enormous species differences in the acute toxicity for TCDD and its structural analogs such as the polychlorinated dibenzofurans (PCDFs). These compounds appear to exert their effects in in vivo and in vitro systems through a mechanism requiring the Ah receptor. TCDD and its structural analogs are also potent carcinogens in chronic bioassays. However, the role of the Ah receptor in the carcinogenic process remains unclear. In our studies, we are attempting to determine the mechanism whereby TCDD and PCDFs alter the action of hepatic epidermal growth factor receptor (EGFR), glucocorticoid receptor (GCR) and estrogen receptor (ER). These receptor systems are important in the regulation of mitotic activity and perhaps the carcinogenic actions of TCDD. We have shown that the Ah receptor is essential for the effects of TCDD on EGFR and GCR which is consistent with previous reports on the requirement of this receptor system for the induction of cytochrome P-450 dependent monooxygenases. Another key issue in the risk assessment for the toxic halogenated aromatics is dose-response relationships for TCDD carcinogenicity including biochemical/molecular changes which are likely associated with the carcinogenic process. We have shown that dose-response relationships for the generation of hepatic preneoplastic lesions, TCDD liver concentrations and effects on EGFR and ER are similar. However, dose-response relationships for the induction of AHH are different than those for the above parameters. In order to address the issue of human sensitivity to the effects of TCDD and PCDFs, we have examined placentas from humans exposed to PCDFs in Taiwan and compared biochemical changes in human placenta to those occurring in rats. Our data reveal that humans are a sensitive species to PCDFs based on enzyme induction and effects on EGFR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-48005-02 LBRA

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Biochemical Mechanisms Related to Risk Factors of Mammary Carcinogenesis

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

PI:	Richard DiAugustine	Research Chemist	LBRA	NIEHS
Others:	S. Snedeker	Senior Staff Fellow	LBRA	NIEHS
	G. Jahnke	IRTA Fellow	LBRA	NIEHS
	C. Brown	Biologist	LBRA	NIEHS
	G. Lucier	Chief	LBRA	NIEHS

COOPERATING UNITS *(if any)*

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

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Hormones and Growth Factors

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.)*

Approximately one out of every eleven females born today in the U.S. will develop breast cancer. This high incidence has prompted the need to understand the biochemical basis for susceptibility to this disease. Ovarian estrogens are known to have an essential role in this disease but it is not understood how these steroids function in the progressive development or maintenance of this disease. Since estrogens are essential for the ductal growth of the mammary gland that occurs near puberty, we investigated this phase of development of the gland for a potential role epidermal growth factor (EGF) related hormones in the mediation of estrogen-promoted growth. Gene expression of both EGF and transforming growth factor-α (TGF-α) have been detected during ductal growth of the mouse by primer-directed enzyme amplification. Transcripts were not detected in the mammary gland of adult or pregnant animals. Immunolocalization of EGF and TGF-α was detected in epithelial cells of the mammary gland during ductal morphogenesis. Slow-release pellets containing either of these growth factors were able to stimulate growth of the epithelium when placed in mammary glands of ovariectomized mice. These studies suggest that one or more EGF-like peptides might function as local mitogens for ductal growth but not for the phase of growth observed during pregnancy. The capacity of regressed ducts of ovariectomized mice to respond to EGF or TGF-α suggests that it is unlikely that estrogens are required for EGF receptor synthesis. Estrogens may stimulate growth by increasing the local availability of appropriate growth factors. Experiments are in progress to examine whether inhibition of the EGF-receptor tyrosine kinase pathway can affect estrogen-stimulated mammary gland growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES-70069-07 LBRA

PERIOD COVERED
October 1, 1988 to September 30, 1989

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 DiAugustine	Research Chemist	LBRA	NIEHS
	George Lucier	Chief	LBRA	NIEHS
Others:	C. Thompson	Senior Staff Fellow	LBRA	NIEHS
	G. Jahnke	IRTA Fellow	LBRA	NIEHS
	M. Walker	Chemist	LBRA	NIEHS

COOPERATING UNITS *(if any)*

Epidemiology Branch, DBRA
Genetic Toxicology Division, Enviromental Protection Agency

LAB/BRANCH
Laboratory of Biochemical Risk Analysis

SECTION
Hormones and Growth Factors

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.)*

Many chemical carcinogens must first undergo metabolism to compounds that react covalently with DNA before they can evoke neoplasias. The formation of these DNA adducts is considered to be a common mechanism by which structurally diverse chemicals ultimately produce mutations and cancer. We had shown earlier that when DNA of human lymphocytes are analyzed by the P_1-nuclease-modified ^{32}P-postlabeling method, multiple lipophilic adducts were detected on thin-layer maps. The level of adducts varied among individuals from 1 per 10^7 - 10^8 nucleotides. The level and pattern of adducts observed did not appear to be influenced by smoking. Thus, the ^{32}P-postlabeling method appears to be a sensitive means of detecting lipophilic adducts that accumulate in tissues. We have extended the application of the ^{32}P-postlabeling method to mammary gland. In one study, we are examining the influence of pregnancy/lactation on the repair of mouse mammary DNA adducts produced by treatment of animals with benzo(a)pyrene. This study will also evaluate whether the mammary gland that has undergone differentiation exhibits a different profile of adducts when exposed to polycyclic aromatic carcinogens known to yield multiple adducts. These studies are designed to evaluate dose-response relationships for mammary gland DNA adducts in relation to risk factors for breast cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-35005-10 LMT

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters 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, and institute affiliation)*

PI:	Marshall Anderson	Research Chemist	LMT	NIEHS
	Steven Belinsky	Senior Staff Fellow	LMT	NIEHS
	Fred Tyson	Senior Staff Fellow	LMT	NIEHS
Others:	C. White	Biologist	LMT	NIEHS
	T. Devereux	Biologist	LMT	NIEHS

COOPERATING UNITS *(if any)*

Dr. Robert Maronpot, Chemical Pathology Branch, NIEHS

LAB/BRANCH

Laboratory of Molecular Toxicology

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.)*

The major focus of these studies is to identify critical target genes and alternations in biochemical pathways which are involved in cell transformation and progression to neoplasia. Some of the biological endpoints which are currently being investigated include DNA damage, cytotoxicity, cell turnover, gene expression and activation of proto-oncogenes in chemical induced rodent tumors. Results from studies with the nitrosamines 4-(N-methyl-N-nitrosamino)-1-3-pyridyl)-1-butanone (NNK) and N-nitrosodimethylamine (NDMA) indicate that lung tumors from both C3H and A/J mice induced by these nitrosamines contain a K-ras oncogene which had been activated primarily by a mutation in codon 12. This mutation was consistent with the activation of both nitrosamines by the formation of the O^6-methylguanine adduct (O^6MG). In contrast to the A/J and C3H mouse, tumor induction by NNK in the rat does not appear to occur via activation of the ras family of oncogenes. The nude mouse tumorigenicity assay is being employed to attempt the detection of novel transforming genes in NNK-induced rat pulmonary tumors. Factors involved in the promotion and progression to the neoplastic state are also being evaluated by the establishment and characterization of epithelial cell lines from benign and malignant lung tumors from A/J mice. Subtractive cDNA cloning will also be employed using cell lines and/or benign and malignant tumors to identify specific proteins whose expression or suppression may be involved in the progression from benign to malignant to a fully metastatic phenotype. The potential for using DNA adducts as an index of carcinogenic potential was examined in a dose response carcinogenicity study with NNK in the Fischer rat. The relationship between DNA methylation and pulmonary tumor induction over a dose range encompassing 3 orders of magnitude was compared. A linear relationship was observed when the dose response for O^6MG formation in Clara cells was plotted against tumor incidence as a function of dose. These data indicate that dosimetry for O^6MG in Clara cells following chronic treatment with NNK can be used to predict accurately the tumorigenic potential of this carcinogen in the rat lung.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-46005-05 LMT

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Oncogene Activation in Rodent and Human Tumors

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

PI:	Steve Reynolds	Expert	LMT	NIEHS
	Marshall Anderson	Chief	LMT	NIEHS
Others:	Colleen Hunnicutt	Biologist	LMT	NIEHS
	Rachel Patterson	Microbiologist	LMT	NIEHS
	Vicki Burnett	Biologist	LMT	NIEHS
	Katie Brown	Biologist	LMT	NIEHS
	Jonathan Wiest	IRTA Postdoctoral	LMT	NIEHS

COOPERATING UNITS *(if any)*

Dr. Robert Maronpot, National Toxicology Program, NIEHS

LAB/BRANCH

Laboratory of Molecular Toxicology

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	2.5	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.)*

Recent work from two independent lines of investigation has merged to suggest that neoplasia results, at least in part, from the abnormal activation of a relatively small number of cellular genes. These genes, termed proto-oncogenes, can be activated by genetic alterations which range from point mutations to gross DNA rearrangements such as translocation or amplification. Induction of tumors in rodents by genotoxic carcinogens results in activation of specific oncogenes with high frequency. We have investigated oncogene activation in chemical-induced and spontaneous rodent tumors as well as some types of human tumors. For example, we have shown K-ras activation in a very high percentage (>90%) of both spontaneously occurring and chemically induced lung tumors of the strain A mouse. In another study, activated H-ras oncogenes were detected in 100% (18/18) of rat esophageal papillomas induced by methylbenzylnitrosamine (a naturally occurring carcinogen associated with an increased incidence of human esophageal cancer). Finally, a moderate percentage (~30%) of human breast carcinomas appear to contain activated oncogenes when assayed by the NIH 3T3 - nude mouse tumorigenicity assay. Therefore, the actual percentage of human breast tumors which contain activated oncogenes may be much higher than previously estimated (i.e., ~2%). Characterization of oncogene activation in human and rodent tumors suggest that activation of a proto-oncogene is a common pathway for tumor induction for some carcinogens. 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

Z01-ES 21050-06 CTEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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)*

P.I.	C. W. Jameson	Head, Program Resources Group	CTEB	NIEHS
Others:	T. J. Goehl	Chemist	CTEB	NIEHS
	R. L. Melnick	Head, ETU	CTEB	NIEHS
	B. J. Collins	Chemist	CTEB	NIEHS
	J. H. Yuan	Visiting Fellow	CTEB	NIEHS
	A. Greenwell	Technician	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Carcinogenesis and Toxicology 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, 1,1,1-trichloroethane and 2-ethylhexanol compared to the neat test material indicates no significant difference in absorption after oral administration. Palatability studies using the microencapsulated trichloroethylene, 1,1,1-trichloroethane and 2-ethylhexanol have been successfully completed. Current studies include the demonstration of bioequivalence of microencapsulated citral, cis-dichloroethylene, trans-dichloroethylene and cis/trans-dichloroethylene and 1,1,2,2-tetrachloroethane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21076-06 CTEB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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. D. Shelby	Head, Mammalian Mutagenesis	DTRT/CGTB	NIEHS
	G. A. Boorman	Pathologist	DTRT/CPB	NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

CTEB

SECTION

N.A.

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC

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 constituents in anticancer and antihelminthic 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. Metal salts that have been studied include mercuric chloride, nickel sulfate, and titanocene dichloride. Further studies are underway with sodium chromate, zinc potassium chromate, and chromium carbonyl in female mice to compare the genotoxic and myelotoxic effects of the different hexavalent salts. The effects of 20-day i.p. injections on micronuclei, on bone marrow stem cell proliferation rates, and on cellular biochemistry are being conducted. The absorption, distribution, accumulation, and retention of antimony potassium tartrate in blood, spleen, heart, liver, and kidney are being investigated in both sexes of rats and mice after i.p. injections. Studies of the absorption and inhalation toxicity of ferrocene in rats and mice are planned. In addition, similar inhalation toxicity studies of lead oxide and lead sulfide are being designed for prechronic testing, and these will include tissue lead analyses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21078-06 CTEB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Palatability/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	Toxicologist	DTRT/CTEB	NIEHS
	Thomas Goehl	Chemist	DTRT/CTEB	NIEHS
	C.W. Jameson	Chemist	DTRT/CTEB	NIEHS
Others	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
	Brad Collins	Chemist	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Carcinogenesis and Toxicology 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:
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.)*

The National Toxicology Program has been exploring the feasibility of adopting microencapsulation for toxicology studies as a more practical and natural method of exposing laboratory animals to volatile, reactive, and/or unpalatable chemicals. In a previous dosed feed study of microencapsulated 2-ethylhexanol in Fischer 344 rats, the compound was stable in feed, and consumed at doses which are sufficient for toxicologic evaluations. Because the microencapsulation of 2-ethylhexanol did not interfere with its absorption in rats, it was concluded that this technique would be a suitable alternative for studying the oral toxicological properties of volatile chemicals in laboratory animals. A feed study of microencapsulated 2-ethylhexanol in B6C3F_1 mice was performed this year. Although feed spillage by mice (probably due to the palatability of the feed mixture) prevented a determination of the actual dose received, a treatment-related increase in the activity of peroxisomal acyl CoA oxidase activity was observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21079-06 CTEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Toxicologist	DTRT/CTEB	NIEHS
Others:	Walter Jenkins	Biologist	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

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:
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.)*

In a 2-year study conducted by the National Toxicology Program, the industrial plasticizer, di(2-ethylhexyl)phthalate (DEHP), was found to be carcinogenic to the liver of F344 rats and B6C3F$_1$ mice. Because DEHP induces peroxisome proliferation, but is not itself a mutagen, it has been suggested that the carcinogenicity of this chemical may be due to excessive peroxisomal production of hydrogen peroxide. Peroxisomal enzyme activities were also found to increase in primary hepatocyte cultures incubated with mono(2-ethylhexyl)phthalate (the primary metabolite of DEHP), and with nafenopin or clofibric acid, two hypolipidemic drugs which are potent peroxisome proliferators in rats. Conjugated dienes, an indicator of lipid peroxidation, were also found to increase in concentration in hepatocytes incubated with peroxisome proliferators. The latter increases were sensitive to the antioxidant, N,N'-diphenyl-p-phenylenediamine (DPPD). Furthermore, the extent of peroxisome proliferation by nafenopin was increased in the presence of DPPD. 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

Z01 ES 21095-03 CTEB

PERIOD COVERED
October 1, 1988 to September 30, 1989 Terminated March 31, 1989

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 Lines

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.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.

To date several hybridoma cell lines have been developed that have been determined to secrete antibodies that may be unique to these leukemic cells. Cell separations have been developed that allow separation of different leukemia cell types. These different subsets of leukemia cells have been propagated in vivo through several passages and have been cryopreserved for future characterization, (including monoclonal antibody analysis), and in vitro culture as the methods for diffusion chamber, soft agar, and Dexter type cultures and culture conditions are refined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21096-03 CTEB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	S.A. Stefansky	Pathologist	DBRA/LMT	NIEHS
	C. Walker	Molecular Biologist	CIIT	

COOPERATING UNITS *(if any)*

Laboratory of Molecular Toxicology, DBRA, 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.)*

Leukemia cells isolated from spontaneously occurring tumors in aging rats and transplanted cells maintained by serial propagation in 8 to 12 wk old male F344/N rats were examined for the presence of the oncogene, c-fms, surface antigens, and karyotype. The origin and biology of this leukemia are not well understood. Karyotype analysis indicates that both spontaneous and serially transplanted leukemia cells have a normal complement of chromosomes (2N=42) with a variant X subterminal chromosomes. Examination of cell surface markers indicate variable expression of differentiation antigens consistent with myeloid origin. The oncogene, fms, is homologous to the gene that encodes for hematopoietic growth factor receptor, CSF-1. Expression of CSF-1 receptor coincides with the commitment of multipotential hematopoietic precursors to the monocyte lineage. Expression of the fms/CSF-1 receptor in total cellular RNA was examined in leukemia cells by the guanidine thiocyanate method. Samples (30 µg total RNA) from Fischer 344/N rat spleen, peripheral blood leukemia cells, or alveolar macrophages (positive control) were separated by electrophoresis and sequentially hybridized to a 3'v-fms probe or gamma-actin probe to control for variations in amounts of RNA loaded onto the gel. Results indicate that the leukemia cells express the fms/CSF-1 receptor gene as a 3.8 kb RNA transcript identical in size to that expressed by normal rat macrophages. The expression of c-fms in both spontaneous leukemia and a transplanted cell line indicates that these pleomorphic leukemia cells are committed to a myelomonocytic lineage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21097-03 CTEB

PERIOD COVERED

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:	C. W. Jameson	Chemist	DTRT/CTEB	NIEHS
	R. R. Maronpot	Pathologist	DTRT/CPB	NIEHS
	R. Langenbach	Genetic Toxicologist	DTRT/CGTB	NIEHS
	J. E. French	Physiologist	DTRT/CTEB	NIEHS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

CTEB

SECTION

N.A.

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

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.)*

Spontaneous mononuclear cell leukemia is a confounding factor in evaluating chemical leukemogenicity in the NTP 2-year carcinogenicity studies. A short-term assay for F344 rat leukemia was developed to better discriminate between age-induced and chemically-enhanced leukemia. The accuracy and sensitivity of the transplant model for predicting the leukemogenic potency of chemicals was confirmed with seven chemicals that had increased or decreased the prevalence of leukemia in previous 2-year carcinogenicity studies. Additional studies with the short-term assay revealed structure-activity relationships for chemicals that were either negative or positive for leukemic trends. Nine glycol ethers were evaluated in the short-term assay for anti-leukemic activity. Ethylene glycol monomethyl ether and the monoethyl ether exhibited chemotherapeutic potential. None of the other seven glycol ethers (ethylene glycol and the monopropyl, monobutyl, and monophenyl ethers; diethylene glycol and the monomethyl and monoethyl ethers) affected the expression of leukemia. Ethylene glycol monomethyl ether was a more potent anti-leukemic agent than the monoethyl ether, and at non-toxic doses completely eliminated the manifestations of leukemia at 60 days post-transplant, when mortality of non-chemically treated rats began to occur. The tumor latency period was doubled, and mortality was prevented for over 120 days post-transplant. In vitro, ethylene glycol monomethyl ether also caused a dose-dependent and progressive reduction in the number of suspended leukemic cells over a 5-day period after a single exposure of 1 - 100 to micromoles. Two chemicals containing dimethyl esters of phosphoric acid (dichlorvos and trichlorfon) enhanced the expression of leukemia in the short-term assay and in 2-year carcinogenicity tests; there were three other chemicals with the same structural relationship (dimethyl hydrogen phosphite, dimethyl methylphosphonate, and dimethylmorpholinophosphoramidate) that were also shown to increase the incidence of leukemia in recently completed 2-year studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21102-02 CTEB

PERIOD COVERED

October 1, 1988 to September 30, 1989 TERMINATED September 1988

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21108-02 CTEB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cellular and Subcellular Effects of a Mixture of Groundwater Contaminants

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

PI:	Ronald L. Melnick	Toxicologist	DTRT/CTEB,	NIEHS
	Raymond Yang	Chemist	DTRT/CTEB	NIEHS
	Arnold Greenwell	Biologist	DTRT/CTEB	NIEHS
Other:	Brenda Ferguson	SIS	DTRT/CTEB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION
Experimental Toxicology Unit

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.1	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.)

A chemical mixture of 25 groundwater contaminants, including heavy metals, aromatic hydrocarbons, and halogenated solvents is being studied by the National Toxicology Program for potential toxicologic effects in rats and mice by the dosed water route of exposure. Studies were initiated to examine the effect of this chemical mixture on oxidative phosphorylation in isolated rat liver mitochondria. The total chemical mixture inhibited state-4 (oxidation) and state-3 (phosphorylation) respiration rates at 1/500 the concentration used in the dosed water study. Cadmium was by far the most potent inhibitor of mitochondrial oxidative phosphorylation of any component in the mixture. Neither a mixture of the organic components nor the individual heavy metals inhibited the mitochondrial state-3 or state-4 respiration rates at the effective concentration of the total mixture. Thus, the inhibition of mitochondrial respiration by the chemical mixture is probably due to a synergistic effect of some of its components. The chemical mixture was also toxic to isolated rat hepatocytes, causing extensive leakage of lactate dehydrogenase after 4 hours of incubation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21120-01 CTEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Phospholipid Changes in Animals Exposed to Diethanolamine

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

PI:	Ronald L. Melnick	Toxicologist	DTRT/CTEB	NIEHS
Others:	Walter Jenkins	Biologist	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:
0.7	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.)*

Diethanolamine, a chemical widely used in industrial processes (e.g. cutting fluids) and consumer products (e.g. cosmetics), has been reported to alter hepatic phospholipid composition in rats by competing with choline and ethanolamine incorporation. The present study was initiated to determine if an association exists between hepatic phospholipid changes and toxicity after dermal and drinking water exposure to diethanolamine. Livers of F344 rats and $B6C3F_1$ mice exposed to diethanolamine for 14 or 90 days were analyzed for phospholipid composition by high performance liquid chromatography after extraction with chloroform/methanol. In rats, phosphatidylethanolamine and phosphatidylcholine were decreased after treatment with diethanolamine; whereas in mice, multiple unresolved peak were present in the regions of phosphatidylethanolamine and phosphatidylcholine elution. These new lipid substances may be involved in the hepatotoxicity of diethanolamine in mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-ES 21123-01 CTEB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Investigation of Absorption of Chemicals Physically Bound to Rodent Feed by Rats

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

P.I.	C. W. Jameson	Head, Program Resources Group	CTEB	NIEHS
Others:	T. J. Goehl	Chemist	CTEB	NIEHS
	B. J. Collins	Chemist	CTEB	NIEHS
	J. H. Yuan	Visiting Fellow	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Carcinogenesis and Toxicology Evaluation Branch

SECTION
Program Resources Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.8

CHECK APPROPRIATE BOX(ES)

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

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

Some chemicals physically bind to rodent feed. This binding usually increases with time. This phenomenon may also have an effect on the absorption of the chemical after ingestion by rodents. The objective of these studies is to determine if there is a difference in absorption of study chemicals in rats given freshly prepared vs aged chemical/feed mixes for a series of chemicals known to physically bind to feed with time. Current studies include the investigation of the absorption of o-nitroanisole which has been found to physically bind to NIH-07 rodent feed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21124-01 CTEB

PERIOD COVERED

March 1, 1989 to September 30, 1989

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

Development of A Model To Study The Influence of Nutrition on 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:	M.P. Dieter	Physiologist	DTRT/CTEB	NIEHS
	F.W. Kari	Chemist	DTRT/CPB	NIEHS
	S. Hursting	Student	Nutrition	UNC-CH
	B. Switzer	Professor	Nutrition	UNC-CH

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.)*

Understanding the occurrence of leukemia in NTP two-year chronic toxicology and carcinogenesis studies is complicated by a high background rate of leukemia in the Fischer 344 rat. A transplant model has been developed to characterize the biology of this rodent leukemia and to investigate the relationship between age-induced and chemically-induced or modulated (promoted) leukemia. In male F344 rats the historical control incidence of MNCL in untreated controls (feed studies) is 636/1,936 or ~33.0. The incidence in historical vehicle control male rats receiving water by gavage is 118/300 or ~40%. In contrast, historical vehicle control male rats receiving corn oil vehicle by gavage exhibited a rate of 321/1,949 or ~17%, which is approximately one-half that of the other routes of administration. Once clinical symptoms of this rat leukemia present the time course of the disease is characterized by rapid weight loss (anorexia) to an emaciated state within 4 to 6 weeks. Food consumption is less and the rate of weight loss in untreated controls (feed studies) and gavage (water vehicle) with leukemia is greater when compared to animals within those same control groups without leukemia or animals with leukemia force fed with corn oil (vehicle control) at the termination of two year experiments.

By using a known inoculum of transplanted leukemia cells that results in an expected frequency of "takes" and latency period the effects of nutrition and/or caloric intake on the time course and expression, of this disease may be determined (diet restriction, force feeding, etc.) Pertinent end-points for study are: the number of transplanted versus number of sham transplanted animals that leukemia occurs, latency period, clinical pathology (hematology and serum chemistry), cytogenetics, rate of weight loss, clinical observations, etc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21012-08 CGTB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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 profiles and genetic toxicities of the chemicals with human tissue activation are then being compared to the results from rodent tissues. Human and rodent liver and kidney cell metabolism of the model carcinogens, benzo(a)pyrene and acetylaminofluorene, have been studied. For human liver, nine individual tissue specimens have been investigated and for acetylaminofluorene eight of the nine human samples were more active metabolizers than the rat hepatocytes. The interindividual variation in the overall human metabolism was about 3-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. For benzo(a)pyrene, the differences in total metabolism between human hepatocytes and rat hepatocytes were less. Studies with kidney tissues have also indicated that human cells are more active than rat kidney cells in producing acetylaminofluorene metabolites; but kidney cells from both species are less active than hepatocytes. Again, about a 3-fold interindividual variation in human kidney metabolism was observed. Again with benzo(a)pyrene, differences in total metabolism between human and rat kidney cells were less than for acetylaminofluorene. The findings indicate that the extent of interindividual variations can vary with the chemical being studied. Furthermore, differences between human and rodent metabolism of chemical carcinogens can also vary with the chemical class, and understanding these species differences will be necessary in the extrapolation of rodent carcinogenesis data to humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21013-08-CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	Microbiologist	CGTB	NIEHS
Others:	R. W. Tennant	Supervisory Microbiologist	CGTB	NIEHS
	K. Borroto-Esoda	Biologist	CGTB	NIEHS
	C. L. Innes	Microbiologist	CGTB	NIEHS
	C. K. Heitman	IRTA Fellow	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:
3.6	1.6	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 regulation of retrotransposition/retrovirus integration has continued to be the primary focus of this laboratory. By using a genome packaging deficient retrovirus developed in this laboratory we have demonstrated that the provirus integration block due to Fv-1 restriction can be abrogated by genome deficient virions. This observation suggests that virus capsid target molecules specifically interact with the Fv-1 gene product and titrates out this activity, allowing additional virus to infect and integrate without restriction. This finding excludes the published model which involves a requirement for the viral RNA genome in abrogation. Future work will be focused on the identification of the Fv-1 gene product and the nature of its interaction with the virion target.

PHS 6040 (Rev. 1/84) GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21016-08 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
Other:	E. Perkins	NRC Fellow	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.)*

Nucleases play a major role in DNA repair and recombination. We had previously shown that the RAD52 gene product is essential in repair of double-strand breaks, mitotic recombination and during normal meiosis. A nuclease yNUCR had been identified which was shown to be under the control of this gene. Using an expression library and antibody to the nuclease, a cloned sequence has been identified that appears to contain the gene for the nuclease. The sequence has been mutagenized with transposons and the altered sequence has been transplaced into the genome of a diploid. Based on genetic analysis we have concluded that the gene is essential in normal growth. This is the first example of an essential nuclease in eukaryotic organisms. Transcription of this gene is under the control of the RAD52 gene. Control of expression is being examined during mitotic growth and development and following exposure to DNA damage.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21032-05 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	M. Hughes	Guest Worker	LMB	NIEHS
	T. Eling	Biologist	LMB	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. Prostaglandin H synthetase is being used to activate compounds in mammalian cell mutation assays. Initial experiments showed hydrogen peroxide with sodium pyruvate. Using 5-phenyl-4-pentenyl hydroperoxide as a substrate, we have observed the mutagenic response to several chemicals. The possible mechanisms responsible for the formation of mutagenic metabolites induced by prostaglandin H synthetase as well as the mutation spectrum are being elucidated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21035-05 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989 TERMINATED February 15, 1989

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, NC

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. In contrast, chromosome pairing and synapsis proceed apparently normally in a deletion mutant of the RAD52 gene of yeast. 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. An antigen associated with paired yeast chromosomes during meiosis has been identified.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21039-04 CGTB

PERIOD COVERED: October 1, 1988 to September 30, 1989 TERMINATED September 30, 1988

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

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:
0.3	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.)*

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21045-07 CGTB

PERIOD COVERED
October 1, 1988 to September 30, 1989 TERMINATED February 15, 1989

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. Specifically, the SPO11 gene is required for the assembly of the synaptonemal complex. The SPO11 gene product is being expressed by recombinant DNA methods in E. coli to facilitate its biomedical characterization.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21048-06 CGTB

PERIOD COVERED: October 1, 1988 to September 30, 1989 TERMINATED February 15, 1989

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 X 10^{-7} events per cell division. The distribution (or spectrum) of mutations occurring 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 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. This work is being extended to an examinatiion of the spectrum of spontaneous mutation in strains which are mutant for genes required for mutation avoidance and/or repair. The first such mutant to be examined is the rem1 hypermutator of yeast.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21049-07 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	E. Perkins	NRC 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:
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 recombination. 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. The RAD52 gene has control function. Its importance is being examined by domain mapping, i.e., using different rad52 mutants. The importance of chromosome pairing is also being examined using strains which undergo meiosis as haploids. The role of strand exchange protein in overcoming requirements for precise homology in recombination is also being examined.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21051-06 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

COOPERATING UNITS *(if any)*

University of California, Davis

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	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.)*

Mutants of the mei-9 and mei-41 genes of Drosophila melanogaster are sensitive to a wide variety of mutagenic agents, defective in excision and post replication repair respectively, and meiotic recombination, 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 relatively large. To confirm this hypothesis and to better understand the structure and regulation of genes controlling DNS repair, these two genes have been cloned and are being characterized molecularly. The mei-41 transcript is 2.2 kilobase pairs in length and distributed over 14-28 kilobase pairs of genomic DNA. The mei-9 has not yet been cloned because multiple repeated sequences in the immediate region make molecular walking difficult.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21053-06 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:
1.0	0.3	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.)*

This project is designed to determine the relationship between DNA repair, chromosome structure and mutagenesis in Drosophila melanogaster. A mutation that 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. DNA sequences are being lost from the deficient chromosomes, suggesting that the new telomeres on the broken ends are not as effective as the original telomeres at replicating the chromosomal ends. The terminal restriction fragments of several of these deletions have been cloned and sequenced. The vast majority of the cloned fragments have no DNA sequence distal to the genomic breakpoint. This observation suggests that proper replication of the chromosomal end may require the telomeric DNA sequence described in a number of species, but that chromosome viability is determined by a non-DNA component of the telomer. We are also developing a rapid assay for the mutator to facilitate genetic analysis of the mutator.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21054-06 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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 yeast cells: 1) the ability of cells to modify the number of centromeres; and 2) 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 and a gene for β-galactosidase. Increases in plasmid number lead to increased resistance and more β-galactosidase. We have observed that haploid 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. We have shown that toleration of extra centromeres is greatly reduced in cells of higher ploidy (i.e., diploids, triploids, and tetraploids), indicating a limitation of components for segregation. 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 21091-04 CGTB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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

PI:	M. A. Resnick	Supv. Research Geneticist	CGTB	NIEHS
Others:	C. Bennett	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:
1.1	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.)*

Recombinational repair mechanisms have been proposed that require the direct participation of the DSB lesions, which producces an invasive free duplex end(s), and a paired homologous chromosome or sister chromatid that templates a repair event. Inaccurate repair of DSBs can directly lead to such potentially lethal events as chromosomal rearrangements, deletions and possibly aneuploidy. Indirectly, unrepaired DSBs are also thought to be indirect inducers of recombination. We are utilizing the site specific endonuclease HO and the MAT switching locus (YZ junction) from the yeast Saccharomyces cerevisiae to address the following questions relating to the biological consequences of site specific DSBs: (1) What are the consequences of extrachromosomally induced DSBs? YZ junctions (24bp) have been placed in non-homologous plasmid target sequences in a nonswitching yeast strain. Induction of a second plasmid containing HO endonuclease under GAL control resulted in an increased plasmid loss rate; however, physical monitoring of the DSB cut site in vivo suggests that the 24bp YZ junction is being cut inefficiently. The target plasmid has now been reconstructed with a 45bp YZ junction to improve in vivo cutting by GAL induced HO. (2) Will a single site-specific DSB in a linear yeast chromosome result directly in aneuploidy (chromosome loss)? We are cloning a YZ junction near the telomere. Chromosome loss will be measured following GAL induction of HO. (3) Will specifically induced DSBs in an immortalized cell line (NIH 3T3) result in chromosomal damage such as deletions, rearrangements or loss? We have integrated the vector pSV2neoYZ (containing a 45bp YZ junction next to the neopromoter) into NIH 3T3 cells selected $G418^R$ colonies. These cells will be transformed with purified HO (using lipofectin) and cytogenetically analyzed for chromosome damage.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21094-03 CGTB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mutagenesis and Other Cellular Responses to Chemicals that Generate 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
	A.-A. Stark	Visiting Scientist	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:
1.7	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.)*

The mutagenicity of sodium bisulfite in Salmonella, is inversely related to its autoxidation. Decreased oxidation allows more bisulfite to be available. Experiments with mannitol and ethanol, known scavengers of bisulfite-generated, oxygen-centered radicals, and DMPO, a scavenger of the sulfur-centered radical, suggest that the sulfur-centered trioxide radical may be responsible for bisulfite mutagenicity. Mutagenicity and toxicity studies with specific generators of oxygen-centered bisulfite radicals support that conclusion. In mutagenesis studies with glutathione (GSH) and other thiols, we showed that thiol mutagenesis is oxidative and involves active oxygen species. The formation of oxygen radicals from GSH is dependent on the activity of γ-glutamyltranspeptidase (GSH) an enzyme frequently present in high amounts in preneoplastic cells. It was hypothesized that a free-radical rich microenvironment near GGT-rich preneoplastic cells, may increase their probability of becoming malignant, because, oxygen radicals can cause mutation by direct interaction with DNA, and oxygen radicals and lipid peroxidation products appear to be tumor promotors. We have shown that the GSH-GGT system can induce lipid peroxidation in vitro, using linolenic acid and linoleic acid as substrates, and in cultured human hepatoma cells. The reaction requires iron, an iron chelator, GSH, and GGT. Lipid peroxidation occurs in the presence of physiological concentrations of iron, and chelators (citrate, ADP, transferrin). Mutagenicity studies of bisulfite and glutathione have been extended to mammalian cells. The optimum conditions for exposure to bisulfite and GSH have been established by mutagenicity studies in Salmonella lipid peroxidation studies. Preliminary results suggest that both bisulfite and GSH-GGT are mutagenic in mammalian cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21103-03 CGTB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	Beth Anderson	Biologist	CGTB	NIEHS
	Walter Piegorsch	Mathematical Statistician	SBB	NIEHS
	Joe Haseman	Res. Mathematical Statistician	SBB	NIEHS

COOPERATING UNITS *(if any)*
Barry Margolin, Dept. of Biostatistics, UNC, Chapel Hill, NC
W. Kalsbeek, Dept. of Biostatistics, UNC, Chapel Hill, NC

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	0.4	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.)*

As a follow-up on an evaluation of the ability of STT (Salmonella (SAL) and L5178Y mouse lymphoma cell (MLA) mutagenicity; in vitro chromosome aberrations (ABS) and in vitro sister chromatid exchanges (SCE) in CHO cells) to predict carcinogenicity using 73 chemicals, an additional 41 chemicals have been examined. These results showed that the original 73 were representative of the database as a whole; MLA and SCE had the highest sensitivity and lowest specificity; SAL had the lowest sensitivity and highest specificity; and ABS was intermediate between the two groups. None of the tests complemented each other, and no combination of tests had a higher predictivity for carcinogens than SAL. A study has been initiated to examine the predictivity of the four STT as a function of the potencies of the tests and cancer test responses. Preliminary activities have made comparisons of different Salmonella potency measurements.

Approximately 300 coded chemicals have been tested for mutagenicity in Salmonella in more than one laboratory. The reproducibility of the assay has been examined. There was a high level of inter- and intra-lab agreement when equivocal responses were excluded. When the equivocal responses were included, the level of agreement was less; this was expected because of the uncertainty surrounding the original determinations of 'equivocal' and the fact that many chemicals were subjected to retest when the original test produced an equivocal result. Therefore, chemicals with equivocal responses were over-represented.

A study was initiated to estimate the proportion of mutagens among the chemicals to which humans have been exposed. A representative list of chemicals in seven use categories was developed, and the chemicals will be tested for mutagenicity in Salmonella.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21106-02 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

In situ Protocols for Mammalian Cell Mutagenesis 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)*

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.)*

An in situ protocol for mammalian cell mutagenesis assays, in which cells are fixed during the expression and selection phases, was developed. It allows for the calculation of the mutation frequency, the proportion of new mutations in a population of cells, and rather than the mutant fraction, the proportion of mutants in a population of cells. The mutant fraction can give misleading assessments of the mutagenic activity of chemicals when a large number of mutants grow more slowly than the rest of the population. This protocol permits the calculation of mutation rates and we anticipate that it will give a more accurate assessment of the mutagenic activity of chemcials than standard protocols.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES 21107-02 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Mutagenicity Studies of Hydrogen Peroxide and Hydrogen Peroxide Generating Systems

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

PI:	Amal Abu-Shakra	Visiting Fellow	CGTB	NIEHS
	Errol Zeiger	Supervisory Microbiologist	CGTB	NIEHS
Others:	Dennis Pagano	Microbiologist	CGTB	NIEHS
	A.-A. Stark	Visiting Scientist	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:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The mutagenicity of hydrogen peroxide (H_2O_2) in Salmonella occurs within a narrow dose range. The presence in the cells of error-prone repair capability protected them from the mutagenic and toxic effects of H_2O_2. The mutagenic response did not correlate with the levels of catalase in the cells. In Salmonella strain TA104, the increase in revertants obtained following H_2O_2 treatment was due, to a large extent, to the induction of deletion mutations.

The HPLC procedure for examining H_2O_2-induced DNA damage has been significantly improved with the use of an electrochemical detector. The detection system has been optimized to detect picomole levels of 8-hydroxyguanosine, one of the oxygen-damaged bases of interest. This system is being used to study oxidative damages induced in DNA by H_2O_2 and thiols that are be mutagenic through H_2O_2 formation.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21121-01 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Transfection of cDNAs for Drug Metabolism into Mammalian Cells

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

PI:	R. Langenbach	Microbiologist	CGTB	NIEHS
Others:	H. Tiano	Biologist	CGTB	NIEHS
	P. Smith	Visiting Scientist	CGTB	NIEHS

COOPERATING UNITS *(if any)*

Dr. S. Nesnow, U.S. EPA, Research Triangle Park, NC

LAB/BRANCH

Cellular and Genetic Toxicology Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

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

The metabolism capability of mammalian cells used in mutation and transformation assays is being increased by constructing vectors containing the cDNAs and transfecting them into the appropriate cells. The cDNAs for P450s IA2, IIA3, IIB1 and for a flavin monooxygenase have inserted into retroviral vectors. The mutable hamster cells, V79 and AS 52, and the transformable mouse cells, C3H10T½, are being transfected. To date, C3H 10T½ cells have been successfully transfected with P450 II B1 and the resultant cells show an increased cytotoxic response to dimethylnitrosamine, aflatoxin and acetylaminofluorene. The studies will continue with the goal of making these cells responsive to a wider variety of chemicals and mimicking individual steps in the carcinogen activation process as it occurs in vivo.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 ES 21122-01 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Genomic Stability and Recombinational Interactions

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)*

T. Nillsson-Tillgren, University of Copenhagen, Denmark

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	0.4	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.)*

Recombination is required for the repair of many types of lesions and it can be a source of genetic diversity. We are investigating the requirements for homology in recombination and the consequences of recombination between DNA divergent sequences. From this information we can determine the mechanisms of chromosome rearrangements, generation of novel genes and possible mechanisms of initiation in carcinogenesis. In addition we are developing a system for the genetic detection of double-strand damage after exposure to very low, nonlethal doses of an agent. We (PNAS, 1989) developed a method for examining the role of homology between a specific pair of homologoues in "protecting" chromosomes against DNA damage. A major conclusion was that nonlethal radiation doses to S. cerevisiae diploid cells containing a single pair of DNA divergent (80% homologous) but functionally homologous chromosomes greatly increased aneuploidy induction (chromosomes III from S. cerevisiae and S. carlsbergenesis). Using these approaches we are also investigating the fate of damaged human DNA contained in yeast vectors in yeast. Our results indicate that this DNA can be repaired provided there is an homologous vector. We have also concluded that some double-strand breaks can be recombinationally repaired from DNA of limited homology.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60102-11 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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
	Amal Abu-Shakra	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:
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.)*

Treosulphan: Studies on treosulphan mutagenicity have continued with an emphasis on mechanisms. Treosulphan mutagenicity is mediated through pH-dependent its hydrolysis to 1,2:3,4-diepoxybutane (DEB). As pH increases from 6 to 8, treosulphan is converted to DEB more rapidly, with a resulting increase in toxicity and decrease in mutagenicity; DEB mutagenicity is unaffected by pH. The differences in mutagenic potency of treosulphan and DEB may be related to the rates at which cells are exposed to DEB, and the growth stage of the cells during exposure. The high-dose, short-duration exposure of pure DEB or of treosulphan at pH8 (rapid hydrolysis) is expected to be more toxic and less mutagenic than low-dose, sustained exposure to growing cells, as seen with treosulphan at pH6. The other hydrolysis product, methanesulfonic acid, was not mutagenic. Phenobarbital: A series of phenobarbital metabolites and chemicals having similar structures to portions of the phenobarbital molecule are being tested in an attempt to elucidate its mutagenic mechanism in Salmonella.
HC blue 1: This is a direct-acting mutagen and a rodent carcinogen. The mutagenicity appears to be due to the presence of contaminants, the removal of which do not diminish the carcinogenicity. Studies are planned to isolate and identify the mutagenic contaminant(s). Imidazoazaarenes: IQ and MeIQ, which are formed in meats during cooking, are mutagenic in TA98 in the presence of an exogenous metabolic activation system (S-9). Mutagenicity can be modulated by other food-borne chemicals, such as the biogenic amines tryptamine, tyramine, and histamine. These amines inhibited or enhanced the mutagenic responses as a function of the amine, its concentration, the mutagen, and the source of S-9. There were strong rat strain differences in the ability of liver S-9 to activate these mutagens in the presence of the various amines.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60122-10 CGTB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

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 and these have been characterized. Based on genetic and biochemical changes, the order of gene function appears to be RAD50, RAD52, and RAD57. Given the important role RAD52 plays in repair and recombination, we have initiated studies to characterize its function in normal DNA metabolism and following treatment with DNA damaging agents. This is being done by "domain mapping" the functional regions of the RAD52 gene. Included among the processes affected by RAD52 are growth, recombination, mutagenesis, control of the essential yNUCR gene, control of strand exchange protein levels in meiosis, meiotic cellular viability, post replication repair, and DNA strand breaks in meisois. Mutations are being created in vitro and the altered gene is being transplaced into the genome. The consequences to the above gene functions are being examined. In addition, the role of the gene and mutants in relation to gene dosage is being examined using a system developed in this laboratory for isolating cell lines with different numbers of the gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21074-05 CPB

PERIOD COVERED: October 1, 1988 to September 30, 1989 TERMINATED December 15, 1988

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

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:
0.01	0.01	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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. Bone marrow parameters were examined on days 1, 5, and 14 after their final treatment. Exposure to EG produced hypocellularity and suppression of granulocyte-macrophage progenitor (CFU-C) 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 (CFU-E), 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 also 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 published in J. Environ. Path. Toxicol. Oncol., 8 (7), p. 27-38, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21080-C5 CPB

PERIOD COVERED
October 1, 1988 - September 30, 1989

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:	R. R. Maronpot	Veterinary Pathologist	CPB	NIEHS
Others:	G. A. Johnson	Radiologist	Dept. of Radiology	Duke Univ. Med. Center

COOPERATING UNITS *(if any)*
Duke University Medical Center
Durham, NC

LAB/BRANCH
Chemical Pathology Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, NC 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 renal papillary necrosis and subsequent repair 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 1989, a 7 Tesla magnetic imaging system will become operational and has provided resolution approximately 10 times greater than that previously available. Several scientific articles have been published thus far.

GPO 814-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21082-04 CPB

PERIOD COVERED: October 1, 1987 to September 30, 1988 TERMINATED November 1, 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	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.)

Ethylene glycol monomethyl ether (EGMME) has been reported to cause hematopoietic abnormalities in man. We have shown that mice exposed to EGMME postnatally have suppressed bone marrow cellularity and progenitor cells 8 weeks postexposure which returns 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 injected subcutaneously 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. Bone marrow functions were examined during the recovery period. 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. 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 published in Toxicol., 50, p. 107-115, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21083-04 CPB

PERIOD COVERED: October 1, 1987 to September 30, 1988 TERMINATED December 1, 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

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. 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	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 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 the bone marrow as evidenced by hypocellularity, suppression of pluripotent stem cells (CFU-S), granulocyte-macrophage progenitors (CFU-C) 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 published in Environ. Health Persp. 72, p. 143-148, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21098-03 CPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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 Prk, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	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.)*

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 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, 28 and 56 after the final treatment to study the histopathology, hematology and myelotoxicity of Lindane. In addition, additive and/or synergistic effects of chemical and radiation toxicity as a model for the complex events of exposure to Lindane have not received adequate attention. Therefore, we also examine the recovery of mice and the residual marrow effects following stress of multiple radiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21099-03 CPB

PERIOD COVERED
October 1, 1988 to September 30, 1989 TERMINATED October 31, 1988

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

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:
0.02	0.02	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 to determine the hematopoietic effects of prolonged exposure to this gas. Female B6C3F1 mice were exposed by inhalation to 0, 0.5, 2.5, and 5 ppm arsine, 6 hr/day for 14 days. Body weights of exposed mice were comparable to controls, but a marked, dose-related splenomegaly was observed. Arsine exposure produced significant decreases in red blood cells, hematocrit and hemoglobin, with increases in white blood cells counts 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/femur cells for all treated groups and on day 3 postexposure and only at the 5 ppm dose group on 24 days postexposure. There was no alteration in bone marrow cellularity and less significant effect on granulocyte-macrophage progenitors. A 12-week study or arsine at 0, 0.025, 0.5 and 2.5 ppm (6 hr/day) by inhalation showed similar effects on hematopoiesis in mice. In addition, a depression of CFU-E was seen 3 weeks postexposure at 2.5 ppm group. In conclusion, arsine exposure at low doses produces a stress on the hematopoietic system characterized by a hemolytic anemia. These results were published in Toxicol. Appl. Pharmacol. 97, p. 173-182, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21100-03 CPB

PERIOD COVERED
October 1, 1987 to September 30, 1988 TERMINATED July 1, 1988

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, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	

CHECK APPROPRIATE BOX(ES)

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

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

Ochratoxin A (OCT A) has the potential to cause myelotoxicity in addition to the well-known toxic effects on the liver and kidney. Experiments reported here were designed to determine whether mice would recover from the myelotoxic effects induced by OCT A injection and secondly whether mice previously injected to OCT A would be sensitive to radiation-induced myelotoxicity than vehicle controls. Six-week-old female B6C3F1 mice were injected intraperitoneally on alternate days over a week with a total dose of 0, 20 or 40 mg/kg of OCT A and bone marrow parameters monitored for up to 16 weeks. There was a suppression of marrow granulocyte-macrophage progenitors (CFU-C) in OCT A treated animals which returned to normal values by two weeks (20 mg/kg group) or by five weeks (40 mg/kg group) following the last treatment. Some of the OCT A treated mice were additionally irradiated with 200 rads whole body irradiation at 10 and 52 days following OCT A injections. Irradiation caused a significant reduction in CFU-C's in all mice but the effect were more pronounced in the mice that had received OCT A previously. The delayed recovery in bone marrow progenitors was also reflected in lower peripheral white blood counts after the second irradiation in 40 mg/kg OCT A that residual bone marrow effect of OCT A makes the mice more sensitive to subsequent irradiation induced injury. These results were published in Toxicol., 53, p. 57-67, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21111-02 CPB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stability and Tissue Reaction of an Implantable Identification Device

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

PI:	G. N. Rao	D.V.M., Ph.D.	CPB	NIEHS
Others:	H. L. Amyx	D.V.M., BS	CMB	NIEHS
	J. Edmondson	Biologist	CPB	NIEHS

COOPERATING UNITS *(if any)*
Comparative Medicine Branch, Division of Intramural Research

LAB/BRANCH
Chemical Pathology Branch

SECTION
Laboratory Animal Management

INSTITUTE AND LOCATION
NIEHS, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	0.4

CHECK APPROPRIATE BOX(ES)

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

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

Carcinogenicity studies require positive identification of test animals. Due to unreliability of ear notches and tags and inability to tattoo pigmented rodents, it is necessary to investigate more dependable and esthetically acceptable identification methods that can be read directly or by electronic means. The purpose of this study is to determine the stability, readability and tissue reaction of a microchip glass sealed device when implanted in the subcutaneous tissue of B6C3F1 mice for two years. Seventy B6C3F1 mice/sex were anesthetized, implanted, and housed individually in polycarbonate cages. The devices were read by a radio frequency scanner weekly and palpated at monthly intervals. Ten mice/sex were necropsied at 3 months and at 15 months with the remaining animals to be evaluated at 24 months. Two of the 140 devices were lost and 3 failed by 14 months. Devices were palpable and appear to be fixed at one location with no inflammation or palpable masses at 20 months. At the 3- and 15-month necropsies, implants were encapsulated by thin fibrous tissue, and a clear cyst was present around one implant. The transponder-detector system is working satisfactorily. However, alternately sized scanning/reading systems may be required based on various animal housing requirements. Procedures are in progress to convert the 10-digit random alpha-numeric identification of the implant to a more practical user number sequence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21112-02 CPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Growth Patterns of F344 Rats Fed NIH-07 and NTP-88 Diets

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

PI:	G. N. Rao	D.V.M., Ph.D.	CPB	NIEHS
Others:	J. Edmondson	Biologist	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Laboratory Animal Management

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.1	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.)*

The maximum mean body weights of rats attained during the course of two-year studies increased by about 20% from 1975 to 1985. Higher body weights will lead to increases in the incidences of mammary tumors, pituitary tumors, and possibly other tumors. Modification of diet and feeding procedures may slow the growth and lower the maximum body weight attained which in turn may decrease the incidences of spontaneous tumors. Lower protein diet may decrease the incidence and severity of kidney disease. The purpose of this study is to determine the feasibility of a 15% protein diet (NTP-88) with restricted feeding from 4 p.m. to 8 a.m. in lowering the maximum body weights and decreasing the severity of nephrosis of rats in comparison with Ad libitum feeding and 24% protein diet (NIH-07). Groups of 25M + 25F F344 rats housed 5/cage by sex are being fed NIH-07 or NTP-88 diet Ad libitum or 4 p.m. to 8 a.m. daily. Body weights and feed consumptions are being determined at one- to eight-week intervals. Water consumption and urine analysis will be done at selected intervals and tissues will be collected for histology at the end of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21113-02 CPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Myelotoxicity in Mice Caused by Drinking Mixture of Groundwater Contaminants

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
	R.S.H. Yang	Ph.D.	CTEB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Branch

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.47	0.47	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 concerning the health effects of groundwater contaminants have been focused primarily on cancer as an endpoint. In the present studies, bone marrow parameters were monitored in mice exposed to 0, 1, 5, and 10% of a chemical mixture in drinking water for 17 days or up to 32 weeks. The mixture consisted of 25 common groundwater contaminants frequently found near toxic waste dumps, as determined by EPA surveys. Mice exposed to 5 and 10% of stock solution for 15.5 weeks showed suppression of granulocyte-macrophage progenitor cells and erythroid precursors with few or no effects on body weight, histopathology and peripheral blood counts. Mice were allowed to recover for 10 weeks at which time they received whole body irradiation. Previously chemical-treated mice were more sensitive to irradiation than untreated controls. Furthermore, synergistic effects of chemical and irradiation were demonstrated by continuing chemical exposure during multiple irradiation. These effects became more pronounced following multiple irradiation and the recovery of progenitor cells occurred more slowly. Thus, chemical exposure caused a significant residual marrow damage that was not apparent with routine hematological or pathological techniques, but could be demonstrated by subsequent irradiation. These results suggest that long-term exposure to highly contaminated groundwater may present a subtle risk to the hematopoietic stem cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21114-02 CPB

PERIOD COVERED
October 1, 1988 to September 30, 1989 TERMINATED October 30, 1988

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

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:
0.15	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.)*

DTRT has three cooperative agreements with universities to study the effects of corn oil administration on the exocrine pancreas of the rat. Part of their studies would require that rats be gavaged with corn oil for up to two years to produce hyperplasia and adenomas in F344 rats. Since most universities do not have the capability for repeated long term gavage in rodents, DTRT set up a contract with EG&G Mason to gavage 150 F344 rats with 10 ml. corn oil/kg 5 days a week for 100 weeks. In order to determine the number, size and transplantability of exocrine pancreas lesions in male F344 rats that have received corn oil by gavage for 75-100 weeks, the rats were necropsied, the pancreas weighed, nodules counted and portions of nodules transplanted to young male F344 recipients (one nodule/four recipients). If lesions are found, they will be transplanted into four recipients for up to ten lesions and at three months the recipients will be killed and examined. If growth is seen, one more three-month transplant. The ability to transplant part of a nodule and have histology on the rest may help validate our current classification scheme. The results indicate there is little or no growth on transplantation to the kidney capsule.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21115-02 CPB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Effects of d-Limonene on Alpha 2U-Globulin in Rat Kidney

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

PI:	H.L. Hong	Biologist	CPB	NIEHS
Others:	S. Eustis	Ph.D.	CPB	NIEHS
	G.A. Boorman	D.V.M., Ph.D., Chief	CPB	NIEHS
	M. Elwell	Ph.D.	CPB	NIEHS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Chemical Pathology Branch

SECTION

Tumor Pathology Branch

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, N.C. 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

d-Limonene is a natural component of a variety of foods and beverages and is found in many fruits, vegetables, meats, spices and other food items. Recently NTP conducted chronic two-year studies of d-Limonene in rats and mice and found there was clear evidence of carcinogenic activity for male F344 rats only as shown by increased incidences of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney. The response observed in male rats may be linked to specific renal pertubation of alpha 2U-globulin, unique to the male rat kidney. This study was performed to evaluate the hyaline droplet formation and the presence of alpha 2U-globulin in the kidney of male and female F344 rats. The alpha 2U-globulin was determined by the enzyme linked immuno-sorbent assay (ELISA) in the kidney homogenates. Total protein were measured in the same aliquots for alpha 2U-globulin by the Lowry method. We have confirmed that d-Limonene produced significant dose-related increase of alpha 2U-globulin only in male rats. These results suggest that d-Limonene-associated nephrotoxicity in male rats may be related to altered catabolism of alpha 2U-globulin, a low molecular weight protein synthesized by the liver under androgenic control. Thus we developed the ELISA technique for alpha 2U-globulin and refined the procedures for our use in the future research projects at NTP and NIEHS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21003-09 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	Yolanda Banks-Case	Biologist	DTRT	NIEHS
	Janet Diliberto	Biologist	DTRT	NIEHS
	Lorrene Kedderis	Guest Researcher	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:
1.4	0.1	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.)

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are highly toxic environmental contaminants with no known industrial use which have been involved in several incidents of human poisoning. Polybrominated compounds such as the PBDDs and PBDFs have also been detected occupationally and environmentally. Metabolism of these compounds constitutes a detoxification process. The more highly halogenated congeners tend to be more persistent and resistant to metabolism. Dermal absorption constitutes a major route of exposure to these chemicals with as much as 40% of an applied dose being absorbed. Absorption through the skin, however, appears to be an extremely slow process, fitting a finite dose model. Our results with TCDD suggest that repeated exposure to low doses might allow for enough material to be absorbed to build up to a toxic body burden.

The pharmacokinetic behavior of 2,3,7,8-TBDD is currently under study. The oral absorption of this compound is very dose-dependent, with absorption increasing as the dose is decreased. TBDD appears to be metabolized and eliminated in the bile. Urinary excretion is minimal. Elimination appears biphasic, rapid clearance followed by redistribution and a slower terminal elimination phase with a half-life of approximately 10 days. The liver and adipose are the major tissue depots, with the relative amounts in these tissues being dependent on dose: at low doses, more TBDD is found in the adipose tissue and less in the liver than is observed as the dose is raised. This dependence of liver/adipose concentration has previously been reported for other chemicals in this class. Future studies will examine the pharmacokinetics of several related brominated compounds, such as 2,3,7,8-TBDF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21004-09 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	Yolanda Banks	Biologist	DTRT	NIEHS
	Timothy McMahon	IRTA Fellow	DTRT	NIEHS
	Janet Diliberto	Biologist	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:
1.4	1.1	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)

The aged may represent a population at special risk to environmental toxicants and drugs. The basis for this enhanced sensitivity may involve pharmacokinetic and/or pharmacodynamic factors. Current work in our laboratory focuses on changes which occur with age in the absorption, distribution, metabolism, and elimination of toxic chemicals in rodents. Since skin serves as a major route for exposure to many environmental compounds, changes in the percutaneous absorption of two highly toxic environmental contaminants, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,4,7,8-pentachlorodibenzofuran (4PeCDF), were examined in aging rats. Dermal absorption was greatest in young adult (3 month) rats, and declined after that. No changes in blood flow or in epidermal thickness were observed in rats and mice between 3-24 months of age. Dermal absorption in weanling and pubertal rats is currently under investigation.

Older rats have been reported to be more sensitive to the toxicity of salicylate. At high doses, old rats produce higher levels of oxidative metabolites as compared to 3 month old rats. Gentisic acid appears to be more nephrotoxic than the parent salicylate, as measured by elevated levels of glucose, AST, ALT, and LDH in urine within 4 hours of treatment. Measurement of urinary markers also appears to be an early and sensitive indicator of renal damage.

Many industrial toxicants and drugs are metabolized to cyanide. Old mice have been found to be more sensitive to cyanide toxicity than young mice. The major target for cyanide toxicity is the mitochondrial electron transport chain, specifically, inhibition of cytochrome oxidase. No differences in liver or brain cytochrome oxidase have been detected as a function of age, nor have any age-related differences been observed in rhodanase activity. The basis for the enhanced sensitivity to cyanide toxicity remains to be elucidated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21009-08 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Toxicologist	STB	NIEHS
Others:	Jerrold J. Heindel	Expert	STB	NIEHS
	Jacqueline Williams	Visiting Fellow	STB	NIEHS
	Kimberley Treinen	Staff Fellow	STB	NIEHS
	Leo T. Burka	Research Chemist	STB	NIEHS

COOPERATING UNITS (if any)
Program Resources Branch, NIEHS
Comparative Medicine Branch, NIEHS
NIOSH

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:
2.6	1.6	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.)

Numerous chemicals encountered in the environment alter male reproductive function. The rabbit studies are aimed at a better assessment of these changes after exposure to ethylene dibromide. Six semen samples will be collected weekly prior to exposure, then males will be dosed with EDB, and semen will be collected for 12 additional weeks. Females will be inseminated with semen from males before, and twice after, dosing to assess fertilizing capacity of the sperm.

The rat studies focus on the testicular lesion produced by boric acid. The rats consumed BA in the diet (9000 ppm), and were sacrificed by anesthetic overdose at 4, 7, 10, 14, 21, and 28 days after start of exposure. Histologic analysis revealed that the release of mature spermatids was the process initially affected. Future studies will examine the extent of possible hormone changes in male rats exposed to dietary BA.

Other rat studies have focused on the disposition of an active organophosphate intermediate in vivo. These studies (now complete) have found that the testis does not appear to accumulate this intermediate, which suggests that there is testicular production. We have shown that testicular cells in vitro can produce this active metabolite from tri-o-cresyl phosphate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21031-05 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Computer Simulation of Inhalation Exposures

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

P.I.:	M. P. Moorman	Engineer	DTRT	NIEHS
Others:	R. A. Sloane	Biologist	DTRT	NIEHS
	R. S. Yang	Research Chemist	DTRT	NIEHS
	H. S. Kermani	Visiting Fellow	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Northrop Services

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:
1.2	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.)*

Computer simulations of physiologically-based pharmacokinetic models are being used to study the uptake and metabolism of compounds administered by inhalation. The application of these models to specific compounds requires two types of compound specific data--tissue partition coefficients and metabolic constants. Systems for making these measurements have been developed by adapting the designs used by other laboratories. Tissue partition coefficients are determined by measuring the partitioning between tissue homogenates and the headspace in sealed vials. Metabolic rate constants are measured by monitoring the removal by the test animals of the compound from the atmosphere of a sealed recirculating exposure system. A computer simulation of the test animals and the exposure system is used to estimate the metabolic rates from these measurements. The values of metabolic parameters are determined by adjusting the metabolic constants of the simulation until the simulation results agree with the measured data. This method has been used to make in vivo measurements of the metabolism of test compounds in animals of different ages. Measurements have also been performed using animals which were dosed with a drinking water mixture or by inhalation exposure to the test compound.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21033-05 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	Janet Diliberto	Biologist	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:
0.5	0.1	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.)

An understanding of pharmacokinetic factors can assist greatly in both dose-setting after 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 route of exposure. These studies help to predict the results 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 focus has been on citral, "oil of lemon." This chemical is a common flavoring and fragrance. Previous work demonstrated that citral is rapidly metabolized and eliminated, primarily in the urine. Some of the citral is oxidatively decarboxylated and eliminated as $^{14}CO_2$. Glucuronide and sulfate conjugates were also identified. Identification and characterization of some of these metabolites is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21038-07 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	STB	NIEHS
Others:	L.T. Burka	Research Chemist	STB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.4	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 of chemical metabolism and disposition are designed to provide both applied knowledge of the fate of chemicals in intact animals in support of toxicity tests conducted by the National Toxicology Program and basic knowledge of mechanisms of chemical toxicity. These studies are designed to determine the absorption, tissue distribution, metabolism and clearance of chemicals and the effect of such factors as dose and route of exposure on each of these parameters. Recent and ongoing work in this group has addressed the species and sex dependent toxicity of an organophosphate flame retardant and plasticizer, tris(2-chloroethyl)phosphate (TRCP), which causes a lesion in the hippocampus of the brain. Toxicity to the hippocampus is limited, among the species tested, to rats and is not seen in mice receiving higher doses. Further, this unusual toxicity is much more pronounced in female than male rats. Work in this laboratory has investigated the regional distribution of TRCP in the brain of male and female rats, isolated and identified the major metabolites of TRCP from both rats and mice and determined the rates at which it is metabolized and cleared by both sexes of rats. Other studies have determined the absorption of a water soluble polymer used in birth control devises, polyvinyl alcohol (PVA), from the vagina and gastrointestinal tract of female rats. These studies indicate that the vagina may be more permeable than the gastrointestinal tract to this type of molecule. A study recently initiated in this program is designed to determine the absorption, tissue distribution and clearance of an anabolic steroid, oxymetholone. This work will determine the rate at which oxymetholone is metabolized and cleared and the major tissues, if any, in which it may accumulate.

PHS 6040 (Rev. 1/84) GPO 914-016

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21046-06 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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. To evaluate the rat as a model for human milk excretion of chemicals and drugs, several different types of drugs were evaluated for their excretion into rat milk, and the data were compared with that previously obtained in humans. Using gas chromatography and high pressure liquid chromatography, the amounts of several drugs in rat milk and plasma were determined, as were the effects on the quantity and composition of the milk of the mothers. Water soluble, basic drugs diphenhydramine, cimetidine, and ranitidine were studied. The antihistamine diphenhydramine was present in rat milk in concentrations higher than those in plasma. There were no effects on milk composition and milk synthesis. Cimetidine was found in very high concentrations in milk relative to plasma, and studies were performed to investigate the mechanism of secretion of high amounts of cimetidine into milk. These studies examined protein binding in milk and serum, active transport into mammary gland and kidney slices in vitro, and the kinetics of elimination of cimetidine from plasma and milk. The effects of exposure to ranitidine during lactation on milk production and composition, as well as on the suckling pups, were also determined. The amount of ranitidine present in rat milk after oral dosing was greater than the concentrations in plasma and was similar to results reported in humans. The excretion of nickel (Ni) into rat milk following sc doses of nickel chloride ($NiCl_2$) and the effects on the lactating rat and her suckling pups were determined. High doses of $NiCl_2$ led to the excretion of Ni into rat milk, changes in milk quality and production, and changes in liver weight in the suckling pup.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21070-06 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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	Toxicologist	DTRT	NIEHS
	Martha Harris	Biol. Lab. Technician	DTRT	NIEHS
	Eric Haskins	Biol. Lab. Technician	DTRT	NIEHS
	Janet Allen	Biol. Lab. Technician	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:
1.6	0.2	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.)*

TCDD (2,3,7,9-tetrachlorodibenzo-p-dioxin) is a potent developmental toxin in all species examined, causing fetal toxicity at doses well below the maternal LD_{50}. However, teratogenic effects have only been demonstrated in sensitive strains of mice where TCDD causes hydronephrosis and cleft palate at extremely low doses. Compounds which are approximate isostereomers of TCDD also cause these malformations and exhibit parallel dose response curves. Thus, the potency of four different polychlorinated dibenzofurans and at least one PCB can be expressed as dilutions of the toxicity of TCDD. Combination treatment with these chemicals also results in an additive response. However, a PCB such as 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) can antagonize the teratogenic effects of TCDD over a very narrow window of concentrations. HCB by itself can cause hydronephrosis, but it does not appear to cause cleft palate even at doses as high as 1g/kg. In combination with TCDD, it can block the induction of cleft palate and hydronephrosis, but the ratios for these antagonistic effects are different. The mechanism of this antagonism remains to be determined. The brominated dioxins and furans, which are also environmental and occupational hazards, are closely related in structure to the chlorinated congeners. The developmental toxicity and teratogenicity of 2,3,7,8-TBDD, 2,3,7,8-TBDF, 2,3,4,7,8-PeBDF, and 1,2,3,7,8-PeBDF were examined in C57BL/6N mice. These compounds produced the same spectrum of effects as observed with TCDD, specifically cleft palate and hydronephrosis. At high doses, fetal thymic atrophy was also noted. TBDD was approximately 1/5 as potent as TCDD, while TBDF was equipotent to TBDD. This is in contrast to the relative teratogenicity of TCDF which is 1/20-1/30 as potent as TCDD. The two PeBDFs are approximately equiteratogenic and are 1/20 as potent as TBDD. Thus, the presence of the larger bromine atom appears to enhance the toxicity of TBDF, relative to TCDF, but decrease the toxicity of 2,3,4,7,8-PeBDF relative to its chlorinated congener.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21075-06 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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
	Nik Mahmood	IRTA Fellow	DTRT	NIEHS
	Diane Overstreet	Chemist	DTRT	NIEHS
	Cornelis Kool	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:
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.)*

Understanding how a xenobiotic is metabolized, distributed, and eliminated is often critical to an appreciation of the toxic effect(s) of the compound. Further, extrapolation of results from animal testing to possible human health effects requires knowledge of metabolic pathways; the fidelity of the extrapolation is enhanced if the metabolism of a xenobiotic is known for both (all) species used in the extrapolation. Investigation of the mechanistic aspects of metabolic processes 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 predictions of the possible metabolic pathways for new compounds should be possible. This group has investigated the metabolism of 1,2,3-trichloropropane (TCP), citral, tris(chloroethyl)phosphate (TRCP), and 2,3,7,8-tetrachlorodibenzofuran (TCDF). The metabolism and disposition of ^{14}C-TCP was investigated in male and female rats and male mice. High concentrations of radioactivity were found in liver, forestomach and kidney after 24 hr. Two urinary metabolites, both from the mercapturic acid pathway, were identified. These metabolites, while major metabolites in rats, are present in only low concentration in male mice. Citral is a flavor and fragrance component found in citrus and other sources. Four metabolites of this terpenoid were identified from NMR and mass spectra and chemical synthesis; three other metabolites were tentatively identified from NMR spectra. The structure of the metabolites of TCDF, a highly toxic contaminant often found in PCB's, continues to be under investigation. Five possible metabolites which have hydroxyl groups substituted on the rings recently synthesized. The presence of these compounds in bile of rats treated with TCDF is under investigation. Three metabolites of TRCP were identified. Two of these result from oxidation of one of the chloroethyl groups; the third, a minor component, results from hydrolysis of the phosphate ester bond.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21084-04 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Toxicologist	STB	NIEHS
Others:	H.B. Matthews	Research Chemist	STB	NIEHS
	R.R. Maronpot	Pathologist	CPB	NIEHS

COOPERATING UNITS (if any)

Chemical Pathology Branch, DTRT

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

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

Chemically induced neoplasia is a major concern of the NTP. The mechanisms by which cancer is induced following exposure to chemicals are diverse and not well understood. Further, there are very few good models or test systems available to study chronic insult by chemicals. Present work in our laboratory has focused on forestomach carcinogenesis in rats. Ethyl acrylate (EA) has been selected as a model carcinogen, and current work is designed to: 1) examine the role of acutely and subchronically induced forestomach lesions in the development of carcinogenesis; 2) determine the reversibility or progression of forestomach lesions induced by subchronic administration of EA; and finally 3) investigate the mechanism(s) of forestomach carcinogenesis by studying forestomach and liver cell turnover and ^{3}H-thymidine uptake by autoradiography. Male F344/N rats were treated with EA for periods ranging from 1 to 13 weeks at doses shown by the NTP to be carcinogenic. Forestomach lesions were documented at the end of the treatment and after various recovery periods thereafter. Rats treated for 13 weeks were allowed a recovery period of up to 20 months in order to determine if forestomach lesions induced in 13 weeks would progress to tumors. These studies are still in progress; however, preliminary findings suggest that forestomachs of EA treated rats underwent a nearly complete recovery within 22 months after the last EA dose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21089-03 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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	Toxicologist	STB	NIEHS

COOPERATING UNITS (if any)

Program Resources Group, CTEB, DTRT
Comparative Medicine Branch, DIR

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Developmental and Reproductive Toxicology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.4	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.)

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 FY89, efforts have focused on effects of mono-2-ethylhexyl-phthalate, Δ9 tetrahydrocannabinol, 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 determined that MEHP and Δ9 tetrahydrocannabinol exert some of their effects by altering these second messenger systems in Sertoli cells.

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 21090-04 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	M. P. Moorman	Engineer	DTRT	NIEHS
Others:	B. A. Schwetz	Supervisory Toxicologist	DTRT	NIEHS
	R. E. Morrissey	Toxicologist	DTRT	NIEHS
	G. J. Rosenthal	Biologist	DTRT	NIEHS
	R.A. Sloane	Biologist	DTRT	NIEHS
	P. C. Blair	Supervisory Biologist	DTRT	NIEHS

COOPERATING UNITS *(if any)*

Northrop Services
University of Maryland

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Inhalation Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	.05	.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 been conducted in the two previous years to evaluate the acute and short-term toxicity of arsine gas. Fischer 344 rats, B6C3F$_1$ and C57BL/6 mice, and Syrian golden hamsters have been exposed to arsine gas at concentrations of 10 ppb to 50 ppm for periods ranging from .5 hr to 90 days. All groups exposed to a single 6 hr exposure of 25 ppm experienced 100% mortality, while those exposed to 5 ppm for four weeks or 2.5 ppm for 13 weeks showed no overt signs of toxicity. Urine samples from these studies showed increased levels of coproporphyrin and 7 and 8 carboxyl uroporphyrin. This data suggests that alterations in the heme biosynthetic pathway as reflected in increases of specific species of urinary porphyrins may be used as early biological indicators of ongoing arsine toxicity. A method has been developed to improve the measurement of specific porphyrin species in rodent urine to further refine this model. Tissues from these exposures are being analyzed for arsenic content to provide a measure of the tissue dose for specific exposure regimens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21093-03 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Mechanisms 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:	Charles Hebert	Biologist	DTRT	NIEHS
	Barbara Abbott	IRTA Fellow	DTRT	NIEHS
	Laurie Couture	Biologist	DTRT	NIEHS
	Cao Qun-li	Guest Researcher	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:
3.7	1.7	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

TCDD has a broad range of toxic effects which are both species and tissue specific and may involve interference with normal regulation of cell growth and differentiation. TCDD can modulate the levels of receptors for glucocorticoids, estrogens, and epidermal growth factor. In mice congenic at the Ah locus, these effects appear to segregate with the responsive allele encoding the wild-type TCDD receptor. During development, TCDD causes increases in the EGF receptor in both the medial epithelium of the palate and the ureteric epithelium, and causes the medial epithelium to differentiate into an oral epithelium rather than transform into mesenchyme and the ureteric epithelium undergoing hyperplasia. These effects, which result in cleft palate and hydronephrosis in vivo, can be achieved in organ culture of the developing palatal shelves and urinary tract, allowing for species comparison. The lack of cleft palate induction in the developing rat fetus following TCDD exposure is due to lower sensitivity of the target tissue as compared to the mouse since in culture, rat palatal shelves can be affected by high concentrations of TCDD. In vivo, these doses are maternally toxic. The relative sensitivity of human embryonic tissue can also be explored by this method. Using the organ culture model, TCDD effects can be blocked by the addition of TGFβ, a potent growth regulator. TGFβ also blocks the TCDD-induced proliferation of human squamous carcinoma cells.

TCDD induces hydronephrosis following prenatal and/or lactational exposure. The functional consequences of hydronephrosis are under investigation. The kidneys of mice exposed prenatally and lactationally are examined at weaning, puberty, and young adulthood to assess the persistance of the lesion. Urinalysis measurements, including urine concentrating ability and urinary enzymes, are being used as sensitive and non-invasive measures of altered renal function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21105-02 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Heat Shock Proteins in Testicular Somatic Cells

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

Co-PI:	Robert E. Chapin	Toxicologist	STB	NIEHS
Co-PI:	Randy L. Allen	Staff Fellow	LRDT	NIEHS

COOPERATING UNITS (if any)

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:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Cells exposed to elevated temperatures or chemical stressors respond by synthesizing heat shock proteins (hsp's). This protein expression is thought to represent an adaptive response to protect cells in stress. This project has evaluated the response of rat Sertoli cells in primary culture to the following stresses: cadmium, arsenic, A23187 (calcium ionophore), heat, and an amino acid analogue, in addition to two "industrial" toxicants known to adversely affect Sertoli cells in vivo: MEHP and saligenin cyclic-o-tolyl phosphate. The results indicate that Sertoli cells respond uniquely to each toxicant, and the response to MEHP and SCOTP is unlike the response to the other, "classic" stressors. Further work is needed to evaluate the number of chemicals to which these cells respond, and to develop a way to monitor this response in vivo.

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 21109-02 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanisms of 2-Butoxyethanol Induced Hematotoxicity

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

PI:	Burhan I. Ghanayem	Toxicologist	STB	NIEHS
Others:	H.B. Matthews	Research Chemist	STB	NIEHS
	S.M. Ward	Biol. Lab. Tech.	CPB	NIEHS

COOPERATING UNITS (if any)

Chemical Pathology Branch, DTRT

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

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

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

Our earlier reports indicated that 2-butoxyethanol (BE) causes acute hemolytic anemia in rats as evidenced by a time- and dose-dependent decrease in the number of red blood cells (RBC), hemoglobin concentration (HGB), and hematocrit (HCT). More recent in vitro studies showed an increase in HCT indicating that hemolysis is preceded by erythrocyte swelling. Since erythrocyte swelling (increased HCT) was not originally observed in vivo, the hematologic effects of BE were reinvestigated using the Ortho ELT-8/ds which was used in the early studies and a Coulter S-Plus IV hematology analyzer simultaneouly. Hematology profiles of BE-treated rats obtained from the Coulter analyzer showed an early dose- and time-dependent increase in HCT and mean cell volume (MCV). In contrast, analysis of the same blood samples using the Ortho analyzer showed a decrease in HCT with little or no change in MCV. Changes in spun HCT were consistent with the results obtained from the Coulter analyzer. Therefore, the Ortho ELT-8/ds analyzer was unable to detect increases in HCT and MCV in rats treated with BE and its use has resulted in reports with some spurious results. Microscopic examination of smears prepared from blood of BE-treated rats showed enlarged erythrocytes accompanied by a time- and dose-dependent increase in stomatocytes (erythrocytes with slit-like hopochromia), schistocytes (debris from massive erythrocyte destruction), and vesicle formation. Microscopic examination of smears from blood incubated with BAA showed similar effects to those observed in vivo. In contrast, incubation of human blood with BAA showed none of the effects observed in rat blood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21110-02 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Developmental Immunotoxicity Studies of Inbred Mice

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

PI:	Richard E. Morrissey	Toxicologist	STB	NIEHS
Other:	P. Lindstrom	IRTA	STB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systemic Toxicology Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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.)

Chemicals such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and diethylstilbestrol (DES) adversely affect immunologic function in offspring following treatment of pregnant mice late in gestation.

Studies are in progress to explore the relationship between developmental immunotoxicity and the induction of structural malformations. These studies are, in particular, addressing the following question: Are modulations in lymphocytic surface antigens, induced by prenatal chemical exposure, resulting in functional immunologic deficits later in life?

TCDD or DES are administered to pregnant C57Bl/6 mice at various times during gestation to establish the sensitive period for induction of immunologic deficits and to identify the initial lesion. Fetal T and B lymphocytes from the spleen and thymus, and subpopulations of these cells, are stained immunocytochemically to determine the morphologic effects of TCDD and DES on lymphocytic surface antigens and their development. In addition, these cell populations are evaluated by flow cytometry to determine quantitative changes. If changes in these surface markers persist beyond the age of 4 weeks in the prenatally exposed animal, functional tests for immunologic deficits are conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21116-01 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Primary Culture of Mixed Testicular Cells

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

PI:	Robert E. Chapin	Toxicologist	STB	NIEHS
Others:	Jerrold J. Heindel	Expert	STB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Developmental and Reproductive Toxicology Group

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

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

CHECK APPROPRIATE BOX(ES)

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

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

A need was identified for an in vitro system that would correctly identify testicular toxicants. This model would have present all the cell types found in the testis, and would be capable of metabolizing xenobiotics to active or inactive metabolites. Initial efforts are aimed at generating a reproducible culture, and characterizing the populations of cells therein. Specific secretory products have been identified as markers of cell function, and assays for these are being set up and validated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21117-01 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Role of Calcium in Chemical-Induced Toxicity

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

PI:	Burhan I. Ghanayem	Toxicologist	STB	NIEHS
Others:	R.E. Chapin	Toxicologist	STB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Chemical Disposition

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

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

Disruption of normal calcium homeostasis has been implicated in the development of cell injury by certain chemicals. Present work was designed to address the role of calcium in ethylene glycol monomethyl ether (EGME) testicular toxicity by investigating the effect of the calcium channel blockers, verapamil and diltiazem, on the pathogenesis of such lesions. Male F344/N rats were treated with EGME alone (po) or in combination with one, two, three or four i.p. doses of verapamil or diltiazem. Twenty-four hrs after administration of EGME, the animals were sacrificed, and a testis and epididymis were excised, fixed in Bouin's solution, embedded in paraffin, sectioned, and stained with PAS-H. The sections were evaluated "blind", and scored for the number of lesioned tubules. EGME at 200 mg/kg produced a moderately severe lesion as characterized by pachytene spermatocyte cell death in stage XIV seminiferous tubules. Verapamil protected against this lesion with this protection being directly proportional to the number of verapamil doses administered and was maximum in rats treated with 3 doses. At 300 mg/kg, EGME caused a severe lesion in the testis, and verapamil was not as effective in protecting against this lesion as that against the low dose of EGME. Diltiazem was not as effective as verapamil at either dose of EGME.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21118-01 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanism of Action of Ovarian Toxicants

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

PI:	Jerrold J. Heindel	Expert	STB	NIEHS
Others:	Kimberley A. Treinen	Staff Fellow	STB	NIEHS

COOPERATING UNITS (if any)

Program Resources Group, CTEB, DTRT
Comparative Medicine Branch, DIR

LAB/BRANCH
Systemic Toxicology Branch

SECTION
Developmental and Reproductive Toxicology Group

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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.)

Mono(2-ethylhexyl)phthalate (MEHP) is both a male and female reproductive toxicant as determined in the NTP Reproductive Assessment by Continuous Breeding protocol. In the male, MEHP has been shown in vivo and in vitro to be a Sertoli cell (SC) toxicant. In vitro MEHP inhibited FSH-stimulated cAMP accumulation in cultured SCs. This inhibition occurred after a 6 hr preincubation period, with maximal inhibition (50%) by 24 hrs. Half-maximal inhibition is seen at 12-15 μM MEHP. Since MEHP is also a female reproductive toxicant, and granulosa cells are thought to be the female counterpart to SCs, we examined the effect of MEHP on FSH-stimulated cAMP accumulation in cultured granulosa cells (GCs). GCs were harvested by ovarian puncture of DES-primed immature (19-22 d) F-344 rats and 300,000 viable cells were incubated in plastic tubes for up to four days. FSH, forskolin, and isoproterenol were shown to stimulate cAMP accumulation. MEHP inhibited FSH-stimulated cAMP accumulation in a dose- and time-dependent manner. Significant inhibition (30-50%) of GC cAMP accumulation occurred with 200 μM MEHP after a 15 hr exposure, with maximal inhibition at 30 hrs. These results indicate that, like SCs, the FSH-stimulated cAMP accumulation in GCs is inhibited by MEHP which may play a role in its reproductive toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 21119-01 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Metabolism and Genotoxicity of Mutagenic Noncarcinogens

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

PI:	Michael L. Cunningham	Senior Staff Fellow	DTRT	NIEHS
Others:	H.B. Matthews	Research Chemist	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, NC 27709

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Short-term mutagenicity tests are used to detect compounds which may also be genotoxic carcinogens in animals. However, some compounds are mutagenic in short-term tests in vitro, yet do not produce cancer in rodents in 2-year NTP bioassays. These "false positive" mutagens represent 23% of chemicals for which acceptable mutagenicity and carcinogenicity data exist. Such discordance between short-term mutation tests and bioassays decreases the confidence and therefore the value of the short-term tests to predict the carcinogenicity of chemicals. Therefore, the objective of this project is to determine and describe reasons for such apparent discordances between in vitro mutagenicity tests and two-year bioassays. The approach used in this study is to determine the metabolic fate and mechanisms of mutagenicity and carcinogenicity of selected chemicals in the Ames test and in the whole animal, respectively, in order to determine differences which might explain the observed discordance. Initial studies have focused on the carcinogen-noncarcinogen pair 2,4- and 2,6-diaminotoluene (DAT). Both these compounds are equally mutagenic in the Ames test, but only 2,4-DAT is carcinogenic in rodent bioassays. Both compounds are rapidly absorbed and extensively metabolized following oral dosing. By using strains of Salmonella with enhanced or deficient N-acetyltransferase activity and inhibitors of cytochrome P_{450} mixed function oxidase, it was demonstrated that 2,4-DAT but not 2,6-DAT requires N-oxidation followed by N,O-acetylation in the target bacterial cell to produce the ultimate mutagenic species, 4-acetoxy-2-aminotoluene. Both these metabolic activation systems are present in rodent liver and are assumed to contribute to the carcinogenicity exhibited by 2,4-DAT. Studies to further characterize the ultimate mutagenic species of 2,6-DAT produced by S9 in the Ames test are in progress.

PHS 6040 (Rev. 1/84) GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 30044-13 STB

PERIOD COVERED
October 1, 1988 to September 30, 1989

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:	M. P. Moorman	Engineer	DTRT	NIEHS
	R. A. Sloane	Biologist	DTRT	NIEHS
	G. J. Rosenthal	Microbiologist	DTRT	NIEHS
	M. P. Dieter	Physiologist	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:
.9	.5	.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.)*

A two-year exposure to methylene chloride is in progress as part of a study being conducted in collaboration with several other groups within the NIEHS. This study is designed to investigate cellular and molecular processes responsible for the induction of lung and liver tumors and to measure changes in pharmacokinetic parameters with age and treatment. Two other studies involving collaborations with other researchers in DTRT are presently being designed. Three structurally-related compounds--styrene, alpha-methylstyrene, and divinylbenzene--will be investigated for leukemogenic potential and effects on pulmonary function. Exposures to a pentamidine isethionate aerosol will be used to evaluate general toxicity and effects on the immune system. The ability to accurately control and document the exposure environment continues to be enhanced by refinements in methods of data acquisition and management. An animal identification system which reads implanted microchips has been added to the existing data management system. The use of this system provides a readily accessible history of treatment, environmental conditions, and observations for each animal in a study. The detailed design of a new inhalation facility has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ES 30106-15 STB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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:	M. Taylor	Staff Fellow	STB	NIEHS
	G. Rosenthal	Biologist	STB	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:

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 and relate alterations in immunological functions with general and specific organ toxicity; (2) when applicable, to examine potential mechanism of action; (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 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 are being examined include tetrachlorodibenzo-p-dioxin, polycyclics, pertussis toxin, and tricyclic antidepressants, 2,3,7,8-tetrachlorodibenzo-p-dioxin and compounds that modulate arachidonic acid metabolism. (b) Development of model systems which allow assessment of Kupffer's cells and alveolar macrophage function, including their 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 several therapeutics used in a treatment of AIDS including alpha-interferon and pentamidine.