NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

ANNUAL REPORT OF INTRAMURAL RESEARCH

October 1, 1987 through September 30, 1988

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NICHD Annual Report
October 1, 1987 to September 30, 1988

Cell Biology and Metabolism Branch

INTRODUCTION

The Cell Biology and Metabolism Branch over the past year has continued its work, both in the laboratory and the clinic, in the areas of iron metabolism, gene regulation, receptor biology and immunology. The work of the laboratory, which has been divided into four projects will be summarized below. The work in the laboratory has gone quite well, and the success of the research is demonstrated by the large number of publications in outstanding journals. In addition to the projects involving human iron metabolism, post-transcriptional mechanisms of human gene regulation and receptors involved in initiating and regulating the immune response, this past year we have initiated a study on the cell biology of the membrane proteins encoded by the human immunodeficiency virus (HIV), the etiologic agent responsible for AIDS. During this past year there have been no significant changes in the organization, structure, or facilities of the branch. The branch is comprised of five research groups as follows:

Group Leader	Group
Joe B. Harford	Biology of the Transferrin Receptor
Richard D. Klausner	Ferritin and the Clinical Basis of Iron Metabolism
Lawrence E. Samelson	The T Cell Antigen Receptor and Immune Activation
Richard D. Klausner	Structure, Function and Cell Biology of the T Cell Antigen Receptor
Warren J. Leonard	Biology of the Interleukin-2 Receptor

Molecular basis of human iron metabolism

We have continued the long term interest of this laboratory in understanding the basis of iron metabolism. The importance of iron metabolism in human biology becomes apparent in many areas of research. Because iron is absolutely essential and perhaps rate limiting in the growth of cells, the ability of cells to regulate and assure iron uptake is strictly correlated with the proliferative rate of cells. This is true during development, in normal differentiation, proliferation, inflammation, the immune response, wound healing and neoplasia. In addition to its correlation with cellular growth, iron is absolutely required for maintenance of baseline metabolic activities. Thus, obtaining sufficient iron is critical for cell health of both the individual cell and the entire organism. This importance is reflected in the wide range of clinical consequences of iron deficiency, a common clinical disorder. In contrast to iron deficiency, where not enough iron leads to pathologic consequences, too much iron is extremely toxic and will lead to cell death. The inability to regulate the uptake of iron underlies one of the most common genetic diseases of man, hereditary hemochromatosis. This disease, which affects one in 400 people in our population, is due to a failure to regulate iron uptake and results in a tremendous amount of morbidity and, if untreated, mortality. The Cell Biology and Metabolism Branch is at the forefront of studies aimed at elucidating the molecular basis of iron metabolism. Our studies continue to focus on two genes. We are currently focusing on how these genes are regulated by iron. We are emphasizing this aspect of iron metabolism because it is the ability of iron to be

regulated in a homeostatic way that underlies cellular mechanisms that attempt to prevent both iron deficiency and iron toxicity. We now know that there are two critical elements that underlie the iron regulatory system: the transferrin receptor, along with its ligand, transferrin, and ferritin. Work from this laboratory has brought us very close to a molecular understanding of how these genes are regulated in response to iron. Ferritin is regulated at the translational level such that when there is little iron there is very little translation, and when there is a large amount of iron there is a larger amount of translation. The transferrin receptor is predominantly regulated at the level of the half-life of the mRNA encoding the protein. In contrast to ferritin, when there is little iron, mRNA levels for the transferrin receptor increase due to a longer half-life and when there is more iron the half life of the mRNA shortens, resulting in a drop in receptor number and less iron uptake into the cell.

Work in this laboratory over the past several years has allowed us to define two molecular elements underlying the regulation of these two genes. One is an RNA element, a sequence found within the messenger RNA molecules encoding both ferritin and the transferrin receptor. This element, which we refer to as an iron-responsive element or IRE, is a small stem-loop structure which endows iron sensitivity to the regulation of the fate of the RNA. A single IRE present in the 5' untranslated region (5' UTR) of ferritin mRNA molecules is entirely responsible for the ability of iron to regulate the translation of the ferritin message. In fact, this element has been isolated and can be inserted into the 5' untranslated region of any gene and thereby confer iron-dependent regulation of translation upon that gene. The regions of the mRNA of the transferrin receptor responsible for its regulation in response to iron are contained within the 3' untranslated region. This region has been localized and analyzed and has been shown to contain 5 RNA motifs with sequences resembling the ferritin IRE. That the sequence motifs in this region of the transferrin receptor are in fact capable of being functional IRE's has been demonstrated by cloning these sequences into the 5' UTR of reporter genes and demonstrating that they can confer iron-dependent translational control (identical to that seen in ferritin) to these exogenous genes.

The second molecular element involved in the regulation and expression of these iron related genes is a cytosolic protein (proteins) which specifically binds with high to the IRE RNA regulatory element. This protein has been identified by the use of a combination band shift and RNAse protection assay. As predicted from sequence analysis, the same cytosolic binding protein interacts with the IRE's found in the ferritin 5' UTR and with IRE's identified in the regulatory region of the 3' UTR of the transferrin receptor. These observations have allowed us to formulate a unifying model to explain the apparent disparate regulation of these two genes. According to this model, when there is little iron around, the IRE binding protein becomes activated and binds to the IRE. If the IRE is in the 5' UTR of the gene, the binding of that protein will sterically block the initiation of translation. For the transferrin receptor mRNA the situation is a bit more complex. In order to explain the regulation of message half-life by iron via the IRE and its interaction with the binding protein, the following can be proposed: Once again, the absence of iron activates the IRE binding protein to bind to the RNA regulatory element. We propose that near the IRE in the 3' UTR of the transferrin receptor gene is a site which is a recognition sequence for attack by a endoribonuclease. Attack by this endonuclease results in a short half-life of the receptor mRNA. However, when the neighboring IRE is occupied by the IRE binding protein, access to this site is blocked, the message is not degraded, and message levels rise. In this way the increased binding of the IRE binding protein and the absence of iron can explain both the decreased rate of biosynthesis in ferritin and the increased rate of biosynthesis in the tranferrin receptor. Support for this model has been provided by the finding that when cells are treated with an iron chelator in order to starve them of accessible iron, the specific activity of the IRE binding protein in the cytosol is

considerably increased. In addition to this model for how the IRE binding protein could regulate iron-responsive genes at the post-transcription level, we are beginning to unravel the actual biochemical mechanisms by which the IRE binding protein may both respond in its activity to iron levels and interact with the IRE.

In addition to the tranferrin receptor, transferrin and ferritin, other critical genes are undoubtedly involved in human cellular and total body iron metabolism. The identification of these genes, however, is a major problem. Because the essentials of iron metabolism is likely to be similar in all organisms, be they primitive prokaryotes, yeast, or higher eukaryotic and mammalian cells and organisms, we have embarked on a new approach to solving this problem. Accordingly, we are examining iron metabolism in the yeast Saccharomyces cerevisea. We have shown that this organism is absolutely dependent on the uptake of environmental iron for health, growth and proliferation. We are using both biochemical, protein chemical and, most importantly, genetic approaches in order to identify genes and gene products of this yeast that are involved in iron uptake. In particular we are focusing on an iron reductase gene present in the membrane of yeast cells that is probably responsible for the initial events in the transport of iron across cellular membranes. This is one of the major gaps in our understanding of human iron metabolism. We believe that obtaining the yeast gene encoding this iron reductase will allow us to identify the corresponding higher eukaryotic gene.

One of the goals of all of these studies is the application of the insights gained to diseases of iron metabolism. We continue to have a very active clinic in which we examine patients with hereditary hemochromatosis and establish continuous cell lines from their peripheral blood lymphocytes. This provides us with not only cells from these patients with which to study physiologic abberations, but also a source of genetic material to look at the molecular basis of possible defects underlying hereditary hemochromatosis. In addition, these studies are giving us unique insights into specific mechanisms of gene regulation in human cells. They are also providing us with useful applications of recombinant DNA technology. Accordingly, the discovery of the IRE has led to our patenting this new type of genetic element for possible use as part of an iron-regulated expression vector system.

RECEPTORS OF THE IMMUNE SYSTEM

The T Cell Antigen Receptor

Largely due to the work of this laboratory, the T cell antigen receptor is now understood as one of the most complex integral membrane receptor molecules. This complexity applies both to subunit structure and biochemical function. We now know that the receptor is composed of at least seven different proteins that are assembled in complexes consisting of seven, nine or more chains. We have named the two most recently defined chains of this receptor complex, the zeta and eta chains. Both the murine and the human zeta chain genes have been cloned by us in the past year and this has led to a complete elucidation of the structure of the protein. The zeta chain comes in two different forms, as a homodimer and as a heterodimer. In the latter situation it is linked to a somewhat larger chain which we have called eta. The frequency of finding eta is only about one-tenth the frequency of finding zeta and thus the hetrodimer is most likely only found in the minority of surface receptors. One of the goals of our studies is to correlate the structure of the receptor with its function. We define receptor function by examining both the very proximal biochemical events that ensue upon receptor stimulation or the resulting phenotypic changes in cells (generally referred to as cell activation). In order to make these structure/function correlations we are taking two approaches: 1) the isolation of mutants or variants of antigen specific T cell hybridomas; and 2) the reintroduction of genetically altered T cell receptor subunits into deficient T cell lines. Over the past year much progress has been

made with the first approach. In particular functional consequences of failing to synthesize either the zeta or the eta chain have been examined. The absence of the eta chain has no effect on the surface expression of the receptor complex. However, it does seem to be correlated with severe functional deficiency. We have previously shown that the T cell antigen receptor functionally couples to at least two cellular biochemical pathways. In one, phosphorylated phosphatidylinositides are broken down in response to receptor activation, leading to the release of water soluble inositol phosphates and diacylglycerol. The result of this is the activation of protein kinase C and the mobilization of intracellular calcium. The other pathway involves the activation of a non-receptor tyrosine kinase or tyrosine kinases which result in the tyrosine phosphorylation of a number of cellular substrates (see below). In the absence of eta, receptor mediated tyrosine kinase activation is maintained while the ability to couple the phosphatidylinositide pathway is lost. The eta chain is made in such small quantities that it is likely that only a subpopulation of T cell receptors contains this chain. Despite this, this population is critically correlated with one pathway that leads to full T cell activation. The functional importance of this minor chain of the T cell receptor makes it imperitive that we clearly define exactly what this chain is. Recent data has finally allowed us to determine that it is structurally related to the zeta chain. We are now attempting to determine genetically the nature of this relationship.

As stated above, multiple kinases are activated in response to the stimulation of the T cell antigen receptor. We believe that understanding the nature of these kinases, their pattern of activation, their pattern of regulation, their interaction, and their relevant cellular substrates would be absolutely critical to our ability to understand and possibly manipulate the immune response. We have defined a set of cellular substrates that are tyrosine phosphorylated in response to receptor activation. Recent data suggests that there may be two different sets of substrates phosphorylated in response to receptor occupancy and that these two sets may be the targets of two different tyrosine kinases. One set includes the rapid phosphorylation (within seconds) of a cytosolic protein called pp62. After pp62 is phosphorylated the zeta chain of the T cell receptor is phosphorylated. Another set of substrates has been defined which appear to be extremely susceptible to dephosphorylation by a tyrosine phosphatase within the cell. In order to see the phosphorylation of this set of substrates, one needs to examine the cell in the presence of the tyrosine phosphatase inhibitor. The pharmacologic characteristics of the phosphorylation of these two sets of substrates are quite distinct. We are currently attempting to purify the pp62 tyrosine kinase substrate as it may be the tyrosine kinase linked to the T cell receptor. In response to receptor stimulation an additional cytosolic kinase is phosphorylated. This is the cellular homologue of the raf-oncogene. This kinase is a serine/threonine specific kinase and every molecule of c-raf within the T cell is phosphorylated, perhaps on multiple sites, in response to receptor activation. We are currently exploring the regulatory consequences of the phosphorylation of this proto-oncogene.

In addition to studying structure and function of the T cell antigen receptor, we have been studying the cell biology of this receptor. In particular, we are attempting to understand the mechanisms and pathways by which newly synthesized receptor chains are assembled with each other and finally expressed on the cell surface. This has been a truly productive area in the past year. It has led to some outstanding new concepts in cell biology which will have wide ranging implications and applications. These studies have provided the most complete picture to date of the process whereby the cell assembles multicomponent complexes within the membrane system. Using the seven chain T cell antigen receptor complex we have examined the concept of architectural editing whereby the three-dimensional structure of newly synthesized and assembling membrane protein complexes are recognized by the cell as either being correct or incorrect. Following this recognition the fate of these complexes within the cell is determined. We have defined that multiple fates can befall these complexes. When the complete and correct complex is formed within the

endoplasmic reticulum, it is transferred through the Golgi apparatus, and finally expressed on the cell surface. Certain incomplete complexes are transferred out of the ER through the Golgi but are rapidly delivered to and destroyed with the lysosomes. Finally the majority of incorrect complexes formed are retained within the endoplasmic reticulum system, never reaching the Golgi apparatus. Interestingly, within the endoplasmic reticulum these complexes are degraded. Characterization of this ER degradation has revealed an entirely new degradative system, non-lysosomal in nature. This degradative system is exquisitely sensitive to cytosolic pH, suggesting that it may be regulated by hormones, etc. One feature of this degradation is that different proteins show vastly different rates of susceptibility to this degradation. One principle underlying differential susceptibility is the state of assembly for particular proteins. Thus an assembled subunit of the receptor complex has a relatively long half-life within the endoplasmic reticulum while the free subunit is rapidly destroyed. Retention and rapid degradation within the endoplasmic reticulum system explains a wide variety of phenotypic changes that accompany spontaneous human mutations. For example, in a variety of forms of familial hypercholesterolemia the LDL receptor, is present in extremely low amounts within the cell. The explanation for this, we believe, is retention and degradation in the endoplasmic reticulum. A variety of other examples of ER degradation exist and we are currently pursuing both the physiologic and pathophysiologic roles of this new pathway described in this laboratory. The early stages in the assembly of a multicomponent complex within the endoplasmic reticulum are extremely complex. It has not been described in detail for any system. We are beginning to unravel both the kinetics and mechanism of this assembly. In particular, we are interested in a protein that we have described, called TRAP, for T cell receptor associated protein, which non-covalently assembles with the newly synthesized chains in the endoplasmic reticulum. TRAP stays with these chains for about 15 minutes while they assemble and then TRAP dissociates. We do not know the function of this new protein but we believe that one of its functions may be to catalyze correct assembly of these many chains with each other. Purification, characterization and molecular cloning of the gene encoding TRAP will be of great importance in understanding the fundamental cellular process of multi-chain assembly. Such work is ongoing within the laboratory.

The Cell Biology of the Human Immunodeficiency Virus (HIV)

Having described the complicated pathways for newly synthesized membrane proteins in T cells, we turned our attention to an attempt to describe similar pathways for the envelope glycoproteins of the human immunodeficiency virus, HIV. Little work has been done on the cell biology of assembly of this important human pathogen. In collaboration with Malcolm Martin and Ron Willey, NIAID, we have described the pathway taken by the newly synthesized envelope glycoprotein of HIV. The envelope glycoprotein of HIV is encoded by a single gene. It is inserted into the endoplasmic reticulum, transferred relatively slowly out of the ER into the Golgi and at some point late in the Golgi or immediately thereafter, it is cleaved by an acid dependent protease to the two glycoproteins that make up the membrane proteins of the infectious virus. Interestingly, only 5-10% of the precursor glycoproteins is ever cleaved. The uncleaved glycoprotein is efficiently transferred to lysosomes where it is degraded while the cleaved glycoproteins are efficiently spared from lysosomal degradation and instead are released from the cell, presumably as viral particles. This work has provided the basis for attempts to design new drugs to inhibit the inefficient processing of the glycoprotein. This processing is absolutely essential for the production of infectious particles. We have shown that this process is exquisitely sensitive to membrane permeable amines and we are now examining whether such bases would be valuable in interfering with HIV infection. This work represents a clear example of the applicability of studies aimed at understanding the basic cell biology of membrane proteins to human disease.

The Human Interleukin-2 Receptor and T Cell Activation Genes

T cell activation is accompanied by a genetic program whereby a number of genes are turned on in a predictable and defined pattern. This pattern of genetic program expression is stereotyped both in terms of kinetics, order of genes, and the groups of genes involved. One of the most important sets of genes that are turned on involve the interleukin-2 system. Interleukin-2, or T cell growth factor, provides autocrine, paracrine and endocrine stimulation for the proliferation of T cells. In order for these T cells to respond to this growth factor they must express a receptor that is specific for it. The IL-2 receptor group has continued to examine two aspects of its biology. The first of these is directed towards determining the genetic elements that are involved in the carefully regulated expression of the interleukin-2 receptor alpha subunit gene. Using a variety of techniques for the study of 5' genomic flanking elements as transcriptional elements as well as techniques aimed at looking at specific DNA protein interactions, this group has come a long way in elucidating the sequences involved in the expression of this gene. In addition to the regulated expression of this gene during the activation of T cells, the gene for the IL-2 receptor alpha chain is also highly expressed in an apparently unregulated fashion in cells infected with and transformed by the human T cell leukemia virus, HTLV-I. Again, this group has been defining those elements that appear to be responsive to the expression of gene products encoded by this virus. One area of some interest that has been focused on over the past year is the potential role of a particular DNA binding protein, first described as an enhancer element for the kappa immunoglobulin light chain gene. A consensus sequence that defines this element binds a particular transcription factor called NF-kappa-B. It appears that the same motif is present and capable of binding an NF-kappa-B molecule (or molecule closely related to it) in the regulatory region of the IL-2 receptor gene. The role of this transcriptional factor in the expression of the IL-2 receptor alpha chain gene is being explored. This is of particular interest because of the role of these sequences and this factor in the activation of the human immunodeficiency virus, HIV.

Another aspect of the biology of the interleukin-2 receptor explored by this group is the role of the beta subunit of the receptor (p70) first described by this group two years ago. The ability of the beta subunit to exist on the cell surface in the absence of the alpha subunit and to serve as a receptor in those cells has been demonstrated. This is true of a variety of peripheral blood mononuclear cells such as large granular lymphocytes and the precursors of LAK cells and NK cells. That the beta subunit can be a receptor, or part of a receptor, in the absence of the alpha subunit is demonstrated by both its ability to bind IL-2 and, in fact, mediate biologic responses to that binding. The beta chain of the IL-2 receptor is not restricted to T cells but can be induced to exist on the surface of both peripheral blood B cells and monocytes. The potential roles in immunobiology of this receptor is only beginning to be explored. This group has made considerable progress in purifying the beta subunit of the IL-2 receptor which is an essential step towards the molecular and molecular genetic characterization of this critical protein of the immune system.

Finally, this group has been engaged in characterizing other genes that are rapidly turned on in response to T cell activation. One of these, which has been referred to as Act-II, is rapidly turned on in both T cells and other mononuclear cells in response to stimulation. The gene and cDNA encoding this protein have been characterized. The cDNA predicts a small secreted protein, suggesting that this may be a new lymphokine. In addition, this protein has recently been expressed in a variety of systems including a bacculovirus expression system in cultured insect cells. This system has allowed the secretion of a large amount of protein to enable more biochemical analysis as well as functional studies of this novel T cell activation gene product.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01600-04 CBMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Biochemical Basis of T Cell Activation

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

PI: L. E. Samelson Medical Officer (Research) CBMB, NICHD

Others: Please see attached sheet

COOPERATING UNITS *(if any)*

Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, MD (J. Ashwell)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.45	5.45	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.)*

Activation of the multicomponent antigen receptor on T cells (TCR) results in rapid activation of polyphosphoinositide metabolism and stimulation of a protein tyrosine kinase. We have characterized cells bearing abnormal antigen receptors lacking one or more receptor subunits and can relate defects in phosphoinositol release to absence of the TCR eta chain.

Analysis of the tyrosine kinase pathway in T cells has revealed that the subunit of the antigen receptor phosphorylated on tyrosine residues after activation is the TCR zeta chain. In addition to this zeta chain phosphorylation, activation of the TCR results in rapid tyrosine phosphorylation of a 62 kD cytosolic protein which is currently being isolated. In addition to these studies high level expression of constructs containing the v-src kinase have been prepared and tyrosine phosphatases have been characterized. These multiple approaches have been undertaken in order to fully understand signal transduction and cellular activation in T cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Others:	R. D. Klausner	Head	CBMB, NICHD
	M. Baniyash	Visiting Fellow	CBMB, NICHD
	P. Garcia-Morales	Visiting Fellow	CBMB, NICHD
	J. S. Bonifacino	Visiting Associate	CBMB, NICHD
	J. Siegel	Senior Staff Fellow	CBMB, NICHD
	J. J. O'Shea	Expert	CBMB, NICHD
	Y. Minami	Visiting Fellow	CBMB, NICHD
	E. Hsi	Adjunct Scientist	CBMB, NICHD
	E. T. Luong	Chemist	CBMB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01601-04 CBMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Molecular Aspects of the Regulation of the Human Transferrin Receptor

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

PI:	J. B. Harford	Senior Investigator	CBMB, NICHD
Others:	J. L. Casey	IRTA Fellow	CBMB, NICHD
	D. M. Koeller	Medical Staff Fellow	CBMB, NICHD
	R. D. Klausner	Head	CBMB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The cells of higher eukaryotes acquire iron via the serum protein transferrin (Tf) and a high-affinity cell surface transferrin receptor (TfR). The expression of the TfR is highly regulated (greater than 20-fold) by iron availability with higher levels of expression occurring when iron is scarce. The regulation of the expression of the TfR is achieved by modulation of the level of the mRNA encoding the receptor. At least two genetic loci were found to participate in this regulation of TfR mRNA levels. A modest (2- to 3-fold) transcriptional regulation was found to be mediated by human genomic DNA 5' of the transcription start site. We have molecularly cloned and partially characterized the promoter of the human TfR gene. However, the TfR cDNA even when driven by a heterologous promoter remains highly regulated by iron. The TfR mRNA is 5 kb in length of which approximately half is the 3' untranslated region (UTR). We have found the 3' UTR to be both necessary and sufficient as the major locus of iron responsiveness. We are attempting to understand the mechanism of regulation of TfR expression and to identify and characterize the elements of the 3' UTR that are responsible for this regulation. Among the sequence elements implicated to date are RNA stem-loop structures that resemble the iron-responsive element found in the mRNA of ferritin, another iron-regulated protein of cellular iron metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01602-04 CBMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Intracellular Iron Metabolism

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

PI:	R. D. Klausner	Head	CBMB, NICHD
Others:	M. W. Hentze	Visiting Associate	CBMB, NICHD
	T. A. Rouault	IRTA Fellow	CBMB, NICHD
	S. W. Caughman	Adjunct Scientist	CBMB, NICHD
	A. Dancis	Medical Staff Fellow	CBMB, NICHD
	J. G. Barriocanal	Visiting Fellow	CBMB, NICHD
	J. B. Harford	Senior Investigator	CBMB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.55	4.55	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.)*

The molecular biology of intracellular iron metabolism has been studied by examining the regulation and expression of the gene for human ferritin. Ferritin functions to store, detoxify and regulate intracellular iron. It performs all of these functions via its ability to accumulate large amounts of iron within a 24 subunit shell. The critical determinant of the effect of ferritin upon the cell is its concentration. This is determined by both the level of expression of the 2 subunits not mentioned encoding ferritin and, by iron, through the level of biosynthesis of the protein. We have isolated the gene for human ferritin H chain and have analyzed the molecular basis for the regulation of ferritin biosynthesis by iron. We have demonstrated that a 26 nucleotide region of RNA contained within the 5' untranslated region of ferritin mRNA is only responsible for iron dependent regulation of translation. We have analyzed this RNA element and have referred to it as an iron-responsive element (IRE). We have identified this element as the only element involved in translational control in response to iron. We have identified a cytoplasmic factor or factors which interact specifically with this element and are likely responsible for the iron-dependent translational regulation. In addition, we have begun to examine the molecular basis of iron metabolism in the yeast Saccharomyces cerevisiae in order to illuminate the genes in this primitive eukaryote involved in iron metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

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PROJECT NUMBER

Z01 HD 01604-03 CBMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Interleukin-2 Receptor - Structure, Function, and Regulation

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

PI:	W. J. Leonard	Medical Officer (Research)	CBMB, NICHD
Others:	S. L. Cross	Guest Researcher	CBMB, NICHD
	J. R. Gnarra	IRTA Fellow	CBMB, NICHD
	N. F. Halden	Biologist	CBMB, NICHD
	M. Napolitano	Visiting Fellow	CBMB, NICHD
	M. Sharon	Senior Staff Fellow	CBMB, NICHD
	C. H. Spencer	Biotechnology Fellow	CBMB, NICHD

COOPERATING UNITS (if any) Baylor College of Medicine, Houston, TX (N. T. Chang); Hoffmann La Roche, Inc., Nutley, NJ (R. Chizzonite); FDA, Bethesda, MD (J. P. Siegel); Whitehead Institute for Biomedical Research, Cambridge, MA (M. Lenardo); Brigham and Women's Hospital, Boston, MA (J. Pober); Lab of Molecular Virology, NY (K.-T. Jeang); American Red Cross, Rockville, MD (W. Burgess)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The human interleukin-2 (IL-2) receptor (IL2R) is being studied in order to understand specific critical components of the T cell immune response in normal and neoplastic cells. The approaches used are based on (1) biochemical analysis of high, intermediate, and low affinity IL2Rs; (2) characterization of transcription regulatory sequences in the IL2R-alpha gene; (3) characterization of DNA binding proteins for regulatory regions. We were the first to identify the existence of a 65 to 77 kD glycoprotein (p70, IL2R-beta) which is a component of the high affinity human IL2R, distinct from IL2R-alpha (p55, Tac antigen), and which can bind IL-2. IL2R-beta mediates the generation of LAK cells and IL-2 induced augmentation of NK activity. Further it is present on resting CD4 and CD8 positive T cells, and can be induced on B cells and monocytes. We have partially mapped the region of the IL2R-alpha gene necessary for transcriptional activity using IL2R-alpha-CAT constructs in transfection experiments. We previously found evidence for: (1) a requirement for a larger promoter region in Jurkat cells than in HTLV-I tranformed T cells; (2) the ability to convert the Jurkat pattern to the HTLV-I pattern by cotransfection with tat-I; (3) a region that functions as a negative regulatory region in HTLV-I transformed T cell lines. We now have characterized regions of DNA that bind proteins in vitro. One of these is 3' to the transcription initiation site; the others are 5' to it. One of the 5' sites is PMA inducible in Jurkat and can bind the nuclear factor, NF-kB, which binds to the kappa immunoglobulin gene enhancer. The regulation of expression of the IL2R-alpha gene appears to depend on both positive and negative regulatory elements. We have also identified a new gene, Act-2, which is induced in T cells within 15 min of exposure to PHA, reaching maximal levels of mRNA expression in 4 h and declining significantly by 16 h. This gene encodes a secreted product and has been expressed using a baculovirus vector. Efforts to study its biologic function and molecular regulation are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01605-02 CBMB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

T Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology

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

PI: R. D. Klausner Head CBMB, NICHD

Others: Please see attached sheet

COOPERATING UNITS *(if any)*

National Cancer Institute, NIH, Bethesda, MD (J. Ashwell), American Red Cross, Rockville, MD (W. Burgess), National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD (R. Willey, M. Martin)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	4.75	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The structure, assembly, and regulation of expression of the T cell antigen receptor are the goals of this group. The T cell antigen receptor is a seven chain multicomponent receptor complex responsible for the initiation and specificity of the immune response. The most recently described chains of the T cell receptor are the two zeta chains which exist as a homodimer. The genes for the both murine and human zeta have been cloned, sequenced, and expressed. This has allowed us to complete the picture of the primary structure of all the components of this receptor complex. The gene has been isolated, characterized in terms of its 5'flanking region, its transcription initiation sites, and intron exon structure. The human gene has been localized to chromosome 1. The isolation of variants and mutants of T cells that lack a variety of the chains of the T cell receptor have allowed us to model the allowable subunit interactions when only some of the chains of the receptor are available. This has enabled us to come up with a nearest-neighbor model for the receptor. We have demonstrated that the zeta chain is most likely under the control of negative transacting regulatory elements. Studies on the assembly of the seven chain receptor complex has led to our proposal of the idea of architectural editing of newly synthesized membrane proteins. According to this model, quaternary structure of membrane complexes are recognized by the cell as either being correct or incorrect. Once this recognition occurs, the fate of those complexes within the cell is then determined. In this way only "correct" complexes are expressed on cell surface. In determining possible fates of incorrectly assembled membrane proteins, we have discovered a new pathway within the cell which we have termed the ER degradative pathway.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Others:	A. M. Weissman	Senior Staff Fellow	CBMB, NICHD
	J. S. Bonifacino	Visiting Associate	CBMB, NICHD
	M. Baniyash	Visiting Fellow	CBMB, NICHD
	D. Orloff	Medical Staff Fellow	CBMB, NICHD
	J. Lippincott-Schwartz	PRAT Fellow	CBMB, NICHD
	L. C. Yuan	Biologist	CBMB, NICHD
	C. Chen	Adjunct Scientist	CBMB, NICHD
	D. Antusch	Adjunct Scientist	CBMB, NICHD

DEVELOPMENTAL ENDOCRINOLOGY BRANCH (DEB)

ZO1 HD 00610-08 Puberty and its Disorders: Physiology, Pathophysiology and Therapy
Gordon B. Cutler, Jr., M.D.

ZO1 HD 00613-08 Clinical and Basic Studies of Male Reproduction
Richard J. Sherins, M.D.

Z01 HD 00615-08 Steroid Antagonists
George P. Chrousos, M.D.

Z01 HD 00616-08 Structure, Function, and Physiology of Glycoprotein Hormones
Bruce C. Nisula, M.D.

Z01 HD 00618-07 Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis
George B. Chrousos, M.D.

Z01 HD 00619-07 Hypothalamic-Pituitary-Gonadal Interaction
D. Lynn Loriaux, M.D.

Z01 HD 00621-06 Mechanism of Pubertal Growth
Fernando Cassorla, M.D.

Z01 HO 00622-06 Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormone
George R. Merriam, M.D.

Z01 HD 00623-05 Adrenal Physiology and Pathophysiology
Gordon B. Cutler, Jr., M.D.

ZO1 HD 00625-01 Neuroendocrine Regulation of Reproductive Function
George R. Merriam, M.D.

NICHD Annual Report
October 1, 1987 to September 30, 1988
Developmental Endocrinology Branch

The mission of the Developmental Endocrinology Branch is to enhance our understanding of the role played by the endocrine system in growth, development, and reproduction.

The broad themes of our research derive from questions that emerge from the medical management of men, women, and children with disorders of the endocrine system. We avoid allocating resources to questions that can be addressed equally well by basic scientists in the setting of a university or research institute to insure that we employ optimally the unique resources of the Clinical Center Research Hospital.

Currently, problems in reproduction, growth, development and the endocrine responses to stress are under study. This summary will not describe all studies - they are listed in the individual reports - but will attempt to highlight emerging concepts, show how these findings alter our current understanding, and how they stimulate new or different lines of investigation.

Studies in Reproduction:

Our studies of reproduction in women have taken two lines: First, we wish to understand the endocrinology of the normal reproductive process, including pregnancy and , second, we are attempting to identify critical points in the process that will allow us to enhance fertility or suppress it in a safe, convenient, and reversible way.

Studies directed at understanding the biochemical and physiologic mechanisms of the reproductive cycle in women have yielded some valuable results in the past year.
The "prime mover" of the reproductive cycle is the episodic secretion of GnRH by the hypothalamus. The episodic bursts of secretion occur at a frequency of 90 - 120 minutes until late in the luteal phase when the frequency slows dramatically. It has been tempting to attribute disorders of the reproductive cycle to aberrations in the frequency of GnRH secretion. We have tested this hypothesis by inducing reproductive cycles in women who have GnRH deficiency with GnRH given by intravenous or subcutaneous injection at frequencies ranging between 60 and 180 minutes. All pulse frequencies induced cycles, but the incidence of ovulation was greater when GnRH was given at the 90 to 100 minute frequency. When ovulation occured, all cycles were the same, regardless of pulse frequency. These studies suggest that the tolerance to variations in pulse frequency are great, and that "frequency alterations" are not likely to be a prominent cause of infertility.

There has been considerable attention given to the "Premenstrual Syndrome" by the lay press. Because of our experience with the normal reproductive cycle, we were in a good postition to examine the endocrine profiles of women having the premenstrual syndrome as defined by significant and reproducible changes in "affective" symptoms in the the 5 days preceding menses. Hormones were measured every other day in the first half of the cycle and everyday in the second half. Plasma levels of estradiol, progesterone, cortisol, testosterone, and prolactin, among others, were not different between affected and non-affected women. These findings suggest that premenstraul alterations in mood cannot be attributed to alterations in hormone profiles, and raised

questions as to the rational underlying the current widespread use of progestins to treat the premenstrual syndrome. We have examined the efficacy of one such regimen in a prospective placebo controlled trial. Progesterone suppositories were given for the 7 days preceding the expected time of menses, an women with and without premenstrual symptoms. Preliminary review of the data has revealed no difference in "affective" scores between the progesterone and placebo treated groups. This finding complements our inability to identify an endocrine basis for the premenstrual syndrome.

The availability of the progesterone antagonist, RU 486, has provided two opportunities to advance our understanding of the reproductive cycle: First, our current hypothesis of how the ovary signals the pituitary gland when a dominant follicle is ready for ovulation is that a "progestin" signal is sent by the ovary and read by the pituitary gland. This hypothesis can be tested using the progesterone antagonist RU 486 to interrupt the signal. If the hypothesis is correct, ovulation should be delayed. Women with hypothalamic amenorrhea, ie, GnRH deficiency, were given pulsatile GnRH therapy to induce normal reproductive cycles. RU 486 or a placebo was then given 2 days before the expected time of ovulation and the effect on the timing of ovulation was measured. RU 486 delayed the ovulatory surge of gonadotropins. This supports, but does not prove, the concept that progesterone plays an important role in the transmission of the "readiness" signal from the dominant follicle. It also provides a new rational strategy for modulating the "ovulatory" event. Second, the idea of continuous antagonism of progesterone effect on endometrial function as a "contraceptive strategy" can be tested. Early studies are encouraging. Marked maturational delay of the endometrium was caused by RU 486 in very small amounts, 1 μg/kg/day, or about 1/5000 that of the currently recommended dose for gestational interruption. These findings support the idea that low grade antagonism of progesterone effect may offer a new, effective, and safe approach to contraception.

Improved understanding of the process of implantation and its transition into early pregnancy has been a natural derivative of our interests in reproduction. The examination of this process has concentrated on the study of human chorionic gonadotropin.

Recent studies have yielded significant advances in our knowledge of the structural, functional, and metabolic properties of human chronic gonadotropin (hCG) and other hCG-related molecules prevalent in pregnancy. While pregnancy urine contains appreciable amounts of hCG, hCG β-subunit, and hCG α-subunit, the hCG-related molecule in greatest abundance is a fragment of the hCG β-subunit known as β-core. At certain stages of pregnancy β-core accounts for more than 99% of the immunoreactive hCG in urine. Little is known, however, about the structure of this important molecule. In the past year, β-core has been purified from pregnancy urine and its peptide composition and carbohydrate structure characterized.

A major obstacle to clinical research on the β-core fragment of hCG has been the extensive cross-reactivity of hCG, hCG β-subunit, and LH in the available assays. To circumvent this problem, we have succeeded in developing a sensitive and specific radioimmunoassay for β-core. The concentration of β-core in the urine of pregnant women ranged between 10 and 4000 ug/L. The urine of non-pregnant women contained less than 5ug/L. Urinary β-core concentration was elevated in 5 men with cancer. In one patient, urinary β-core was elevated despite the absence of detectable serum hCG. These findings suggest that β-core may become a valuable cancer marker.

The study of human growth has been a second focus of activity. The optimal approach to the diagnosis of growth hormone deficiency has been under examination. The

measurement of plasma growth hormone concentration every 20 minutes for a 24 hour period is currently in vogue as a diagnostic test. The mean concentration of growth hormone and its secretory pattern can be established from these measurements. Other investigators, using this approach, suggest that the diagnosis of growth hormone deficiency can be made on the basis of these two parameters. We have examined this hypothesis and our data does not support the conclusion. We have shown that the normal range of these parameters is so great that any "mean" level of growth hormone or any pattern of growth hormone secretion is encompassed within the range of normal. These studies suggest that a new approach to the diagnosis of growth hormone deficiency is needed and also suggest that this approach is not likely to be based on tests currently employed for this purpose.

The treatment of growth hormone deficiency with growth hormone releasing hormone has been examined. Earlier studies showed that about 85% of children with growth hormone deficiency respond to GRH, suggesting that most children could be treated with this agent if it could be shown to be effective in stimulating growth. We now have shown that most children can be treated successfully, in the short term, with 10 μg/kg GRH administered once daily as a subcutaneous injection. These findings illustrate that at least one alternative to growth hormone therapy exists for the treatment of growth hormone deficiency.

The pubertal "growth spurt" is known to depend upon sex steroids and upon an adequate plasma concentration of growth hormone. The precise way in which sex steroids modulate the accelerated growth of puberty is unknown. We have shown that estrogens alone and in a very small dose can stimulate maximal growth of the epiphysis. Androgens can also stimulate epiphyseal growth, but it is unknown whether or not androgen stimulated growth is due to the androgens themselves or to the estrogens derived from the androgen precursors. Using the rat as a model, we have shown that the direct administration of androgen into the epiphyseal growth plate can stimulate growth, but only at very high dose. These findings support the idea that androgens must be converted to estrogens to induce epiphyseal growth.

Accelerated growth is one of the serious untoward consequences of precocious puberty. To alleviate this problem, the improved diagnosis and treatment of sexual precocity has been given priority in the work of the branch.

An insight into the pathophysiology of familial male sexual precocity was gained in the past year. We have examined the effect of sera from these patients on testosterone secretion from adult male cynomolgus macaque testes in vivo. In these monkeys, endogenous gonadotropin secretion has been inhibited with a potent luteinizing hormone-releasing hormone (LHRH) antagonist. Sera from patients with familial precocious puberty stimulated greater testosterone secretion than sera from normal prepubertal children matched for gonadotropin level. Although the difference is not yet statistically significant, these data suggest a potential molecular explanation for the disorder.

The treatment for gonadotropin independent sexual precocity has been very unsatisfactory. We have explored the use of antiandrogen for the purpose. The antiandrogen spironolactone decreased the androgenic manifestations of precocious puberty, such as acne and spontaneous erections, but did not control accelerated growth and bone maturation. When the aromatase inhibitor testolactone was added, the accelerated growth was controlled. This finding again highlights the importance of estrogen in regulating linear growth. The results in the first 9 patients to receive the combination appear favorable; growth rate and the rate of bone maturation were

normalized. Studies with each agent individually have shown that the combination of spironolactone and testolactone is superior to either agent alone. The favorable results of this short-term study have encouraged us to begin a long-term pilot study to attempt to normalize the growth rate, bone maturation, and the adult height of these boys.

The approach has also been used to treat patients with the McCune Albright syndrome. In this case, we have used only the aromatize inhibitor testolactone. This approach to treatment has been more successful than expected; inhibition of ovarian aromatase by testolactone has interrupted the process of ovarian cyst formation. Thus, the drug not only blocks estrogen formation by ovarian cysts, but actually prevents cyst formation and shrinks ovarian volume. Since the first publication of these results in the New England Journal of Medicine, the number of subjects under treatment has increased to 20. This should be a large enough group to begin to address the issue of how the treated children compare to normal children in growth rate, bone maturation and, ultimately, adult height.

We have pioneered the use of LHRH analogs for the treatment of gonadotropin dependent sexual precocity. The oldest of our LHRH analog-treated children have reached the normal age of pubertal onset and treatment has been withdrawn. The data from the first year in which the LHRH analog has been discontinued have been analyzed. Gonadotropin secretion returned to normal between 3 and 12 months after stopping treatment. Pubertal progression resumed at the normal rate of approximately one Tanner stage per year. Growth rate after treatment has been appropriate for bone age. Thus, the available data suggest a prompt return normal hypothalamic-pituitary-gonadal function after discontinuation of long-term LHRH analog treatment of central precocious puberty.

The youngest children entered into this study have now been treated for more than 6 years. Puberty, growth rate, and bone maturation have remained suppressed during the entire period. The predicted adult height for this group (20 girls, 7 boys) has increased form 4'10" to 5'5". Thus, the current data from this large, long-term project remain favorable.

The adrenal gland undergoes a series of maturational changes and plays an important role in growth and development. Studies in the past year have focused on the improved diagnosis and treatment of Cushing's syndrome. Application of the techniques of ACTH radioimmunoassay coupled with inferior petrosal sinus sampling and CRF administration has improved diagnostic accuracy for Cushing's syndrome to nearly 100%. Treatment has been improved in parallel. The new technique of magnetic resonance imaging promises to enhance our success even further.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00610-08 DEB

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

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Puberty and its Disorders: Physiology, Pathophysiology and Therapy

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

PI: Gordon B. Cutler, Jr. Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*: (see attached)

LAB/BRANCH: Developmental Endocrinology Branch

SECTION: Section on Developmental Endocrinology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7.5	6.6	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 objective of this project is to advance understanding of the mechanisms that underlie normal and abnormal puberty, and to apply this knowledge to improve existing therapy for disorders of puberty. Principal areas of clinical investigation include the mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the mechanisms of prepubertal and pubertal growth, the role of pubertal sex steroids in the acquisition of normal adult bone density, the treatment of central precocious puberty with an analog of luteinizing hormone-releasing hormone, the treatment of the McCune-Albright syndrome with an aromatase inhibitor, and the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor. A major focus of laboratory investigation involves cloning and structural analysis of the human gonadotropin-releasing hormone gene and studies of the regulation of this gene in transfected cells.

Others:	D. L. Loriaux	Head, SSH	DEB, NICHD
	B. Albertson	Adjunct Scientist	DEB, NICHD
	K. M. Barnes	Chemist (Tech)	DEB, NICHD
	M. Burgueno	Adjunct Scientist	DEB, NICHD
	F. Cassorla	Head, ULGP	DEB, NICHD
	G. Chrousos	Head, UHRF	DEB, NICHD
	P. Feuillan	Adjunct Scientist	DEB, NICHD
	Z. Huang	Visiting Fellow	DEB, NICHD
	L. Laue	Med. Staff Fellow	DEB, NICHD
	S. Malozowski	Adjunct Scientist	DEB, NICHD
	P. Manasco	Med. Staff Fellow	DEB, NICHD
	S. Radovick	Adjunct Scientist	DEB, NICHD
	A. Rahman	Student Volunteer	DEB, NICHD
	S. G. Ren	Adjunct Scientist	DEB, NICHD
	S. Rose	Adjunct Scientist	DEB, NICHD
	J. Levine Ross	Adjunct Scientist	DEB, NICHD
	C. Shen	Student Volunteer	DEB, NICHD
	M. Uriarte	Med. Staff Fellow	DEB, NICHD
	P. Ziaya	Adjunct Scientist	DEB, NICHD

Cooperating Units

LDP, National Institute of Mental Health (E. Susman, E. Nottelmann, G. Inoff, L. Dorn, J. Blue); Clinical Center, NIH (M. Royster, A. McNemar, K. Hench, A. Dwyer, T. Shawker), MCNEB, National Institute of Diabetes, Digestive, and Kidney Diseases (F. Wondisford); Department of Pediatrics, University of Michigan (C. Foster); Department of Obstetrics and Gynecology, SUNY at Stony Brook (D. Kenigsberg); Department of Pediatrics, University of Minnesota (O. Pescovitz); Department of Internal Medicine, McMaster University Medical Center (J. Booth); Department Internal Medicine, University of Dalhousie (R. Rittmaster); Department of Population Dynamics, Johns Hopkins University School of Hygiene and Public Health (L. Ewing).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00613-08 DEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Clinical and Basic Studies of Male Reproduction

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

PI: Richard J. Sherins Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Reproductive Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	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 study were to ascertain biological, physiological and clinical mechanisms of male reproductive disorders and to provide rational strategies of treatment for men with reproductive disorders and to provide rational strategies of treatment for men with reproductive disease.

This project represented a continuum of research begun in 1970 and included studies of 1) the hormonal regulation of spermatogenesis in gonadotropin deficient men, 2) biology of sperm function, 3) adverse effects if cancer therapy on gonadal function, 4) evaluation of treatment of men with reproductive disorders and 5) the role of sex steroids in regulation of gonadotropin secretion.

Major findings from studies performed this year have shown 1) pulsatile GnRH in gonadotropin deficient men enhances testicular growth above that achieved with gonadotropins but sperm production is not facilitated, 2) subjects with Kallmann's syndrome show evidences for subtle neurological deficits which may serve as markers for the genetic disorder, 3) semen from infertile mean show subpopulations of sperm on the basis of linear velocity characteristics, which may be important as a marker of infertility, 4) biodegradable testosterone microspheres appear to provide long-term androgen replacement in hypogonadal men, which offers a potentially practical therapy for men who desire infrequent parenteral injections.

Owing to the retirement of Dr. Sherins from the USPHS after 20 years of active duty, this study terminates on October 1, 1988. Dr. Sherins' laboratory as a unit closes and his personnel, study, patients, and projects are now relocated.

Others:

D.L. Loriaux	Chief	DEB, NICHD
G.B. Cutler	Senior Investigator	DEB, NICHD
B.C. Nisula	Senior Investigator	DEB, NICHD
F. Cassorla	Senior Investigator	DEB, NICHD
L. Liu	Adjunct Scientist	DEB, NICHD
A. Burris	Adjunct Scientist	DEB, NICHD
S. Rose	Adjunct Scientist	DEB, NICHD
D. Vogel	Adjunct Scientist	DEB, NICHD
D. Vantman	Adjunct Scientist	DEB, NICHD
G. Koukoulis	Adjunct Scientist	DEB, NICHD
J. Tezon	Adjunct Scientist	DEB, NICHD
L. Dennison	Adjunct Technician	DEB, NICHD
G. Merriam	Expert	DEB, NICHD

Cooperating Units:

T. Kinsella	ROB, NCI
M. Lippman	DCT, NCI
R. Fischell	APL, Johns Hopkins University
S. Carter	University of Maryland
A. Yergy	LTPB, NICHD
J. Booth	McMaster University, Ontario, Canada
R. Clark	Emory University
J. Davidson	Stanford University
J. Blaquier	Steroid Lab. of Experimental Medicine, Buenos Aires, Argentina

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00615-08 DEB

PERIOD COVERED

October 1, 1987 to September 30,1988

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

Steroid Antagonists

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

PI: George P. Chrousos Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, National Institute of Mental Health, NIH (P.W. Gold); Surgery Branch, National Cancer Institute, NIH (M. Lotze); Clinical Pathology Department, Clinical Center, NIH (T. Fleisher)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Hypothalamic Releasing Factor

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	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.)*

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. Antagonists for the glucocorticoids or the progestins with potential clinical usefulness have been discovered only recently. The objective of this project is to develop and study the molecular mechanisms of action and the human applications of the antagonists for both of these classes of steroids.

Initially, we proved that glucocorticoid antagonists can be developed by modifications of the 11-position of the steroidal C ring of glucocorticoids. Then we tested a prototype glucocorticoid-progestin antagonist (RU 486) developed recently by Roussel-UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects in small experimental animals.

Given to nonhuman primates or man RU 486 causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of a glucocorticoid (dexamethasone). This suggests that antiglucocorticoids could be used for challenging the hypothalamic-pituitary-adrenal axis, when clinical testing is required in patients with disorders of this axis. Antiglucocorticoid therapy of patients with severe Cushing's syndrome due to ectopic ACTH secretion or adrenocortical tumors causes remission of the clinical manifestations of hypercortisolism. We have treated 8 patients and are currently enlarging the therapy series.

Given to women in single monthly doses during the luteal phase of the cycle RU 486 causes vaginal bleeding. The subsequent cycle is of normal duration. This suggests that single doses of RU 486 could be used for contraception.

Other professional personnel

Other:	R. Bernardini	Adjunct Scientist	DEB, NICHD
	D. Brandon	Chemist	DEB, NICHD
	L. Golden	Medical Staff Fellow	DEB, NICHD
	S. Kawai	Adjunct Scientist	DEB, NICHD
	L. Laue	Medical Staff Fellow	DEB, NICHD
	C. Liapi	Adjunct Scientist	DEB, NICHD
	L. Loriaux	Head, SSH	DEB, NICHD
	L. Nieman	Expert	DEB, NICHD
	D. Rabin	Adjunct Scientist, NRSA	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00616-08 DEB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure, Function, and Physiology of Glycoprotein Hormones

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

PI:	B.C. Nisula	Head	DEB, NICHD
Others:	D. Blithe	Sr. Staff Fellow	DEB, NICHD
	S. Rose	Adjunct Scientist	DEB, NICHD
	R. Wehmann	Expert	DEB, NICHD
	R. Jeevanram	Visiting Fellow	DEB, NICHD
	P. Manasco	Medical Staff Fellow	DEB, NICHD
	C. Lyons	Bio Lab Tech	DEB, NICHD

COOPERATING UNITS *(if any)*
(none)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Medical Endocrinology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

The overall objectives of this project are to understand the endocrinology of the human glycoprotein hormones, thyroid-stimulating hormone (TSH), choriogonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and thereby to develop diagnostic and therapeutic clinical applications. Recent research advances in the current period include the following: Purification and structural characterization of a fragment of the beta-subunit of hCG, called beta-core, which is the most abundant hCG-related molecule in pregnancy urine; development of a radioimmunoassay for beta-core in which LH, hCG, and beta-hCG exhibit negligible cross-reactivity, thus making feasible studies of the physiology and cancer biology of beta-core; demonstration in vivo in men of full intrinsic steroidogenic activity in highly purified desialylated hCG; and elucidation of a potential clinical role for immunoassays which detect specific carbohydrate structures in the carboxyl-terminal region of hCG-beta. Future directions of the project will include determination of the physiological pattern of beta-core production in normal pregnancy, investigations of the biochemistry and physiology of beta-core production and metabolism, characterization of the natural evolution of the oligosaccharide structures of hCG and its subunits throughout pregnancy, and assessment of the roles of subtle defects of pituitary-thyroid axis function or of TSH structure as causes of short stature and delayed growth in childhood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00618-07 DEB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis

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

PI: George P. Chrousos Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*
(see attached)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Unit on Hypothalamic Releasing Factors

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.1	6.1	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.)*

In this project we seek to advance the understanding of the physiology and pathophysiology of the hypothalamic-pituitary-adrenal axis. The role of stress-related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress-related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas:

1) Clinical applications of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome, and pseudo-Cushing's syndrome (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary-adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, depression and anorexia nervosa.

2) Regulation of the hypothalamic-pituitary-adrenal axis in vivo and in vitro: The regulation of the axis by opioids, vasopressin, oxytocin, and glucocorticoids has been studied in vivo. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has been examined in vitro in a newly established hypothalamic organ culture system. Athletes have a hyperfunctional pituitary-adrenal axis in the resting state. Hypothalamic-pituitary-adrenal axis reactivity and personality traits have been correlated in developing adolescents.

3) Role and actions of glucocorticoids: The effects of glucocorticoids upon the cardiovascular system during surgical stress are merely permissive. Glucocorticoid resistance is associated with normal size glucocorticoid receptor protein that has decreased affinity for glucocorticoids and normal size mRNA.

Other professional Personnel

Others:	R. Bernardini	Adjunct Scientist	DEB, NICHD
	D. D. Brandon	Chemist	DEB, NICHD
	A. Calogero	Adjunct Scientist	DEB, NICHD
	P. Feuillan	Adjunct Scientist	DEB, NICHD
	W. Gallucci	Adjunct Technician	DEB, NICHD
	T. Gomez	Clinical Associate	DEB, NICHD
	M. Grino	Adjunct Scientist	DEB, NICHD
	T. Kamilaris	Adjunct Scientist	DEB, NICHD
	L. Laue	Medical Staff Fellow	DEB, NICHD
	C. Liapi	Adjunct Technician	DEB, NICHD
	S. Listwak	Adjunct Technician	DEB, NICHD
	D. L. Loriaux	Head, SSH	DEB, NICHD
	A. Margioris	Adjunct Scientist	DEB, NICHD
	E. McClure	Adjunct Scientist	DEB, NICHD
	L. Nieman	Expert	DEB, NICHD
	D. Rabin	Adjunct Scientist	DEB, NICHD
	T. Wheler	Clinical Associate	DEB, NICHD

Collaborating Investigators

Biological Psychiatry Branch, National Institute of Mental Health, NIH (P.W. Gold); Surgical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, NIH (E. Oldfield); Laboratory of Developmental Psychology, National Institute of Mental Health, NIH; Laboratory of Clinical Physiology, National Institute on Aging, NIH (E. Nottleman); Human Performance Laboratory, Dept of Military Medicine, USUHS (Patricia Deuster).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00619-07 DEB

PERIOD COVERED
October 1, 1988 to September 30, 1988

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

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

PI: D. Lynn Loriaux Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*
Roussel-UCLAF, Department of Radiology, Paris, France (E.E. Baulieu)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Steroid Hormones

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.63	1.63	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 this year have focused on the pathophysiology of leydig cell activation in patients with familial precocious puberty, the production rate of cortisol using a new technique employing stably labeled isotopes of cortisol, the development of therapeutic agents for neutron capture mediated receptor specific cell destruction, and the development of specific tests for the evaluation of corpus luteum function. Progress has been made in each area. A "long acting Leydig cell stimulator" has been demonstrated in the plasma of children with familial sexual precocity. The "true" production rate of cortisol has been shown to be less than previously believed, "Carborane" tagged derivatives of CRF and GRF have been developed that retain biologic activity and, for CRF, have been shown to be internalized and have cytolytic activity in an epithermal neutron beam. The response of the corpus luteum to hCG has been characterized in normal women, setting the stage for the application of this knowledge to the evaluation of unexplained infertility.

Other Professional Personnel

Others:	L. Nieman	Expert	DEB, NICHD
	B. Albertson	Adjunct Scientist	DEB, NICHD
	P. Manasco	Medical Staff Fellow	DEB, NICHD
	P. Platia	Medical Staff Fellow	DEB, NICHD
	H. Tracer	Medical Staff Fellow	DEB, NICHD
	J. Zawadski	IPA	DEB, NICHD
	M. Batista	Fogarty Fellow	DEB, NICHD
	T. Loughlin	Fogarty Fellow	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-06 DEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Mechanisms of Linear Growth

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

PI: Fernando Cassorla Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

Clinical Center, NIH (M. Skerda, G. Heavner, L. Long): Catholic University of Nijmegen, The Netherlands (I.M. Valk); Hahnemann Medical School, Philadephia, Pennsylvania (J.L. Ross)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Growth Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	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 objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include improving the accuracy of the methods employed to diagnose growth hormone deficiency. We are also studying the effects of growth hormone and sex steroid administration on linear growth in patients with Turner's syndrome and delayed puberty. In addition, we are studying the mechanism of catch up growth in a primate model. We are also attempting to define the optimal dose of hydrocortisone for growth in patients with adrenal insufficiency and of thyroid hormone in monkeys with hypothyroidism. We are also studying the effects of administering somatomedin-C, a growth hormone-dependent peptide, to hypophysectomized cynomolgus monkeys to determine its growth-promoting activity. In addition, we are examining the effect of inducing pubertal delay in children with extreme short stature, in order to prolong prepubertal growth prior to the pubertal spurt and possibly enhance ultimate height by delaying epiphyseal fusion. We are also investigating the effects of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature through a randomized, double-blind, placebo-controlled clinical trial. In addition, we are investigating the growth hormone secretory dynamics in patients with hypophosphatemic rickets. Finally, we are studying the effects of growth hormone-releasing factor on linear growth in growth hormone-deficient children by using different treatment regimens in order to optimize growth.

Others:	G.B. Cutler	Head, SDE	DEB, NICHD
	G.R. Merriam	Head, UCN	DEB, NICHD
	B. Linder	Medical Staff Fellow	DEB, NICHD
	A. Cristiano	IRTA	DEB, NICHD
	G. Marin	Visiting Fellow	DEB, NICHD
	S. Rose	Adjunct Scientist	DEB, NICHD
	S. Malozowski	Adjunct Scientist	DEB, NICHD
	S.G. Ren	Adjunct Scientist	DEB, NICHD
	G. Municchi	Adjunct Scientist	DEB, NICHD
	D.L. Loriaux	Head, DEB	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00622-06 DEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormone

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

PI: George R. Merriam Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Steroid Hormones

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.55	2.52	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.)*

Growth hormone-releasing hormone (GHRH) and somatostatin (SRIF) are the two hypothalamic peptides which together control growth hormone (GH) synthesis and release. This project aims: a) To explore the efficacy of GHRH and its analogues in treating GH deficiency and GH excess (acromegaly): b) to study the neuroendocrine regulation of GH secretion; and c) to define alterations of GH regulation in different physiologic states, and to determine their cause. Therapy of GH deficiency: We have established that GH deficiency is usually due to a deficiency of hypothalamic GHRH. While this disease is usually treated with GH, we have found that GHRH is also highly effective in normalizing growth velocity in these patients. Since it can be effective even with a single daily dose, GHRH may also be as practical as GH for treatment. We are now testing whether drugs which lower SRIF, which blocks GH secretion, can enhance the therapeutic effect of GHRH. Evaluation and therapy of acromegaly: We are testing a new approach to the treatment of acromegaly, a form of pituitary tumor with overproduction of GH leading to severe illness, which is not well treated by current means. GHRH is linked to an organoboron conjugate which emits radiation under exposure to neutrons. We are testing whether this compound will localize in tumor cells, and allow them to be killed selectively.

Other Investigators: (NIH)

Fernando Cassorla, M.D.	DEB, NICHD
Hao-Chia Chen, Ph.D.	ERRB, NICHD
Constance Chik, M.D., Ph.D.	DEB,NICHD
Audrey Cristiano, M.D.	DEB, NICHD
William Gahl, M.D., Ph.D.	HGB, NICHD
Philip Gold, M.D.	BPB, NIMH
En-Hui Hao, M.D.	DEB, NICHD
S. Mitchell Harman, M.D., Ph.D.	GRC, NIA
Ijaz Khan, M.D.	BPB, NIMH
Mitchell Kling, M.D.	BPB, NIMH
Therese Loughlin, M.D.	DEB, NICHD
D. Lynn Loriaux, M.D., Ph.D.	DEB, NICHD
Nina Ma, Ph.D.	DEB, NICHD
Saul Malozowski, M.D.	DEB, NICHD
Edward Oldfield, M.D.	SNB, NINCDS
Susan Rose, M.D.	DEB, NICHD

Others: (non NIH)

Rosario D'Agata, M.D.	University of Catania, Italy
Marie C. Gelato, M.D., Ph.D.	State University of New York, Stony Brook
Frederick Hawthorne, Ph.D.	University of California, Los Angeles
Santiago Muzzo, M.D.	INTA, Chile
Ora Pescovitz, M.D.	Dept. of Pediatrics, Univ. of Indiana
Roger Rittmaster, M.D.	Dept. of Medicine, Dalhousie University
Yi-Fan Shi, M.D.	Chinese Academy of Medical Sciences, Beijing

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00623-05 DEB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Adrenal Physiology and Pathophysiology

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

PI: Gordon B. Cutler, Jr. Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.1	0.1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

We seek to advance understanding of the mechanisms that cause adrenal androgen secretion by the fetal adrenal zone prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen or glucocorticoid secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing's syndrome. We also seek to clarify the pathophysiology of primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency and to improve the treatment of these conditions.

Other professional personnel

Others:	D.L. Loriaux	Chief	SSH, DEB, NICHD
	B. Albertson	Adjunct Scientist	(Georgetown University)
	K.M. Barnes	Chemist (Tech)	DEB, NICHD
	F. Cassorla	Head, ULGP	DEB, NICHD
	C. Chik	Adjunct Scientist	(MRC of Canada)
	G. Chrousos	Head, UHRF	DEB, NICHD
	P. Feuillan	Adjunct Scientist	(Amer. Diabetes Found.)
	L. Laue	Med. Staff Fellow	DEB, NICHD
	T. Loughlin	Visiting Fellow	DEB, NICHD
	L. Nieman	Expert	Roussel-UCLAF
	J. Levine Ross	Adjunct Scientist	(Hahnemann Univ.)
	H. Tracer	Medical Staff Fellow	DEB, NICHD

Collaborating Investigators:

Chief, Radiology, Clinical Center, NIH (J. Doppman); Chief, SNE, BPB, National Institute of Mental Health (P. Gold); Acting Chief, SNB, NINCDS, NIH (E. Oldfield); Staff Radiologist, Radiology, CC, NIH (A.J. Dwyer); Department of Internal Medicine, McMaster University Medical Center (J. Booth); Department of Internal Medicine, University of Dalhousie (R. Rittmaster); Department of Pediatrics, University of Minnesota (O. Pescovitz).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00625-01 DEB

PERIOD COVERED
October 1, 1987 to September 30, 1988

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

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

PI: George R. Merriam Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Sex Steroids

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.5	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 spece provided.)*

This project aims to clarify some of the central mechanisms controlling reproduction. The secretion of hypothalamic gonadotropin-releasing hormone (GnRH) is episodic, and both the frequency and amplitude of the pulses are important modulators of the pituitary response. Abnormalities of pulse frequency or amplitude may underlie disorders such as hypothalamic amenorrhea. We have developed protocols to characterize the patterns of these pulses in normal subjects and in patients, and statistical methods to analyze these patterns to distinguish pulses from noise. Using these methods we have shown that the midcycle gonadotropin surge is largely the result of change in pulse amplitude rather than pulse frequency, and that pulsatile gonadotropin secretion is largely abolished in hypogonadotropic hypogonadism and lactational amenorrhea. Several of these methods independently confirm a linkage between pulses of luteinizing hormone (LH) and prolactin (PRL), hitherto thought to be under independent control. We have used normative data to optimize GnRH therapy of hypothalamic amenorrhea.

Senior Staff

D. Lynn Loriaux, M.D., Ph.D.	Chief, DEB, NICHD
Richard J. Sherins, M.D.	Head, SRE, DEB, NICHD

Other Professional Staff

Osborne F.X. Almeida, Ph.D.	Visiting Fellow, DEB, NICHD
Marcello Batista, M.D.	Visiting Fellow, DEB, NICHD
Henry C. Bohler, M.D.	NRSA Fellow, DEB, NICHD
Constance Chik, M.D., Ph.D.	MRC of Canada Fellow, DEB, NICHD
Charles C. Coddington, M.D.	Guest Worker (US Navy), DEB, NICHD
Robert Collins, M.D.	Guest Worker (US Air Force), DEB, NICHD
Renée Eger	Summer Student (CC), DEB, NICHD
Roy Hertz, M.D.	Adjunct Scientist, DEB, NICHD
Gerard S. Letterie, M.D.	Guest Worker (US Army), DEB, NICHD
Eric Libre, B.A.	Summer student, DEB, NICHD
Linda Liu, M.D.	Adjunct Scientist, DEB, NICHD
Lynnette K. Nieman, M.D.	Expert, DEB, NICHD
Ning Ma, Ph.D.	Adjunct Scientist, DEB, NICHD
Jeselle Mathews, M.D.	Summer student (CC), DEB, NICHD
Lawrence M. Nelson, M.D.	NRSA Fellow, DEB, NICHD

Collaborators

John Booth, M.D.	McMaster University, Canada
William F. Crowley, Jr., M.D.	Massachusetts General Hospital, Boston
Gunter Emons, M.D.	Medizinische Hochshule, Lübeck, West Germany
Marco Filicori, M.D.	University of Bologna, Italy
Thomas Fleischer, M.D.	CC, NIH
Anne Kiblanski, M.D.	Massachusetts General Hospital, Boston
Gerald Lincoln, Ph.D.	MRC Reproductive Biology Unit, Edinburgh
Neil J. MacLusky, Ph.D.	Yale University
Mortimer Mishkin, Ph.D.	NIMH
Pia Platia, M.D.	Clinical Center, NIH
David Rubinow, M.D.	NIMH
Michael O. Thorner, M.B., B. Ch.	University of Virginia
Johannes Veldhuis, M.D.	University of Virginia
Kenneth W. Wachter, Ph.D.	University of California, Berkeley
Thomas Zoeller, Ph.D.	NINCDS, NIH

ENDOCRINOLOGY AND REPRODUCTION RESEARCH BRANCH (ERRB)

Z01 HD 00022-15 Renin-Angiotensin System and Aldosterone Regulation
Greti Aguilera, M.D.

Z01 HD 00035-16 The Structure and Function of Biologically Active Molecules
Hao-Chia Chen, Ph.D.

Z01 HD 00146-13 Structure and Function of Chorionic Gonadotropins
Hao-Chia Chen, Ph.D.

Z01 HD 00147-13 Mechanism of Action of Peptide Hormones in Steroidogenic Cells
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00149-13 Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00150-13 Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00151-13 Regulation of Gonadal and Placental Function
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00184-10 Regulation of Pituitary Hormone Secretion
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00187-09 Hormonal Regulation of Cellular Metabolism
Kuo-Ping Huang, Ph.D.

Z01 HD 00190-06 Adrenocortical Zonation: Regulation of Steroidogenesis and Cholesterol Metabolism
Charles A. Strott, M.D.

Z01 HD 00191-04 Mechanisms of Neuroendocrine Regulation
Greti Aguilera, M.D.

Z01 HD 00192-03 Purification, Immunology, and Functional Activity of Adrenocortical Proteins
Charles A. Strott, M.D.

Z01 HD 00193-03 Angiotensin II Receptors and Activation Mechanisms
Kevin J. Catt, M.D., Ph.D.

Endocrinology and Reproduction Research Branch

The research programs of the Endocrinology and Reproduction Research Branch are directed at the elucidation of cellular mechanisms involved in hormone secretion and action. These programs include studies on the characterization of peptide hormones and their cellular receptors; the structure-function relationships of peptide and glycoprotein hormones; the regulation of hormone biosynthesis and secretion; and the mechanisms of peptide hormone action in endocrine target cells. Of particular interest are the analysis of pituitary-gonadal and pituitary-adrenal regulation, the control of ovarian activity during the reproductive cycle and pregnancy, and the receptor-mediated control of pituitary, gonadal, and adrenal function. During the current year, research has been performed on the receptors and signalling processes that are responsible for the control of differentiation and secretion in endocrine target cells. The role of hormones in cellular regulation has also been examined in selected forms of normal and disordered human endocrine function, and in appropriate animal model systems for the analysis of hormone secretion and the stimulatory and inhibitory control of target-cell function. The staff of the ERRB share common interests in the mechanisms of action of peptide and glycoprotein hormones, the role of neuropeptides in hypothalamic-pituitary regulation and stress, the control of gonadal and adrenal function by pituitary hormones, the renin-angiotensin system and aldosterone secretion, and the mechanisms and roles of protein phosphorylation in metabolic regulation. The major research programs of the Branch are supervised by the respective senior investigators under the following organizational units within the ERRB:

(a) The Section on Hormonal Regulation (Dr. Kevin Catt) performs research on the control of endocrine target cells by peptide hormones, in particular the characterization, regulation, and activation mechanisms of membrane receptors for gonadotropin-releasing hormone (GnRH), corticotropin-releasing factor (CRF), angiotensin II (AII), and gonadotropins. The receptor-mediated actions of hypothalamic releasing peptides and other regulators of pituitary hormone secretion are studied in cultured anterior pituitary cells. The actions of angiotensin II are investigated in rat and bovine adrenal glomerulosa cells, and those of gonadotropins are analyzed in ovarian granulosa and luteal cells.

The hypothalamic control of reproductive function is expressed through the actions of GnRH, which regulates gonadotropin secretion by binding to calcium-mobilizing receptors in the plasma membrane of pituitary gonadotrophs. GnRH receptors appear to be confined to the pituitary and placenta in primates, but are present in gonads, brain, and other sites in the rat. The consequences of GnRH receptor activation in gonadotrophs have been shown to involve the integrated actions of several intracellular messenger systems, including polyphosphoinositide breakdown and mobilization of intracellular calcium, as well as influx of extracellular calcium through plasma-membrane calcium channels. In isolated gonadotrophs, GnRH stimulates the hydrolysis of phosphatidylinositol bisphosphate to diacylglycerol (DAG) and inositol trisphosphate ($InsP_3$). A role for activation of protein kinase C in gonadotrophs has been indicated by studies on LH release during the translocation and regulation of protein kinase C by endogenous DAG by activators (phorbol esters, synthetic diglycerides) and inhibitors (retinal). The generation of $InsP_3$ and promotion of calcium mobilization and entry provides a mechanism for the early elevation of $[Ca^{2+}]i$ during GnRH action. GnRH also stimulates the production of higher inositol phosphates including $InsP_4$ and $InsP_5$,

and causes marked elevation of Ins-4-P rather than Ins-1-P as the major product of polyphosphoinositide metabolism. Arachidonic acid (AA) and its lipoxygenated metabolites are also generated during GnRH action, via activation of diacylglycerol lipase as well as phospholipase A_2. The actions of AA on LH release are related to its effects on calcium mobilization and activation of an AA-dependent protein kinase in pituitary cytosol. The role of calcium entry in GnRH action is largely related to the time course of the LH response, which is at first independent of extracellular calcium but is subsequently dependent on calcium influx during the sustained phase of LH release in GnRH-stimulated gonadotrophs.

The properties of angiotensin II (AII) receptors and their intracellular signalling pathways were studied in the adrenal zona glomerulosa and in Xenopus oocytes injected with adrenal mRNA. The mechanisms leading to stimulation of steroidogenesis were analyzed in isolated glomerulosa cells from the rat and bovine adrenal cortex. Purification of AII receptors from the bovine adrenal gland was pursued by detergent solubilization of photoaffinity-labeled membrane sites and fractionation by ion exchange, lectin-affinity, and immunoaffinity chromatography. Elevation of cytoplasmic calcium by AII depends upon mobilization of intracellular calcium stores by the products of ligand-stimulated phosphoinositide turnover, and also on calcium entry through voltage-sensitive channels. Intracellular receptors for inositol-1,4,5-trisphosphate, previously identified in adrenal, pituitary and hepatic microsomes, were further analyzed in hepatic subcellular fractions and were found to co-enrich with the plasma membrane. The association of high-affinity $InsP_3$ receptors and $InsP_3$-responsive calcium release with the membrane fraction suggests that $InsP_3$ releases calcium from a storage site associated with the plasma membrane of the rat liver. It is likely that a specialized vesicular system to which $InsP_3$ can bind and release calcium is located close to the plasma membrane and is thus adjacent to the site at which $InsP_3$ is formed during stimulation by calcium-mobilizing hormones.

The Ins-1,4,5-P_3 formed from PIP_2 breakdown during AII action was rapidly eliminated via two major metabolic routes. In addition to breakdown to inositol via Ins-1,4-P_2 and Ins-4-P by the previously identified 4-monophosphate pathway, the calcium-mobilizing 1,4,5-trisphosphate is rapidly converted to Ins-1,3,4,5-P_4 which is then degraded to the inactive 1,3,4-trisphosphate isomer. The latter is metabolized by degradation to Ins-3,4-P_2 and Ins-1,3-P_2, and also undergoes a further cycle of phosphorylation to form a new tetrakisphosphate isomer that has been identified as Ins-1,3,4,6-P_4. These studies revealed the importance of the 4-monophosphate pathway in inositol polyphosphate catabolism, as well as new phosphorylation pathways and formation of inositol metabolites with potential roles in intracellular signalling and AII action.

Further studies on the multiple pathways of inositol phosphate production in bovine glomerulosa cells provided additional details of the formation and metabolism of inositol derivatives in the presence and absence of lithium. During stimulation with angiotensin II, Ins-1,4,5-P_3 increased to a peak of 15-fold above basal within 10 sec, followed by a second phase of continuous increase over the next 30 min. The Ins-1,4,5-P_3 formed during agonist stimulation was rapidly eliminated by two distinct metabolic pathways. The more direct metabolic route was via degradation by sequential dephosphorylations to form inositol 1,4-bisphosphate and inositol 4-phosphate, and ultimately inositol. Lithium ions inhibited both the formation and dephosphorylation of inositol 4-monophosphate, which is a specific product of inositol polyphosphate metabolism. In addition, a cyclical metabolic sequence was initiated by the 3-phosphorylation of Ins-

1,4,5-P_3 to form inositol 1,3,4,5-tetrakisphosphate. The Ins-1,4,5,-P_3-kinase responsible for this reaction was stimulated by increased Ca^{2+} concentrations in the micromolar range. Inositol 1,3,4,5-tetrakisphosphate was then dephosphorylated to inositol 1,3,4-trisphosphate, which in turn was either further degraded to inositol 3,4-bisphosphate or rephosphorylated to inositol 1,3,4,6-tetrakisphosphate. Lithium ions also inhibited the production of inositol 3,4-bisphosphate, explaining the large accumulation of inositol 1,3,4-trisphosphate in cells stimulated in the presence of lithium. Prolonged exposure to angiotensin II in the presence of Li^+ caused a progressive decline in inositol polyphosphate formation without depletion of the lipid precursor, phosphatidylinositol 4,5-bisphosphate, suggesting that an accumulating product of polyphosphoinositide hydrolysis (possibly diacylglycerol) has an inhibitory effect on the phospholipase C-catalyzed breakdown process. These newly defined pathways may provide additional regulatory steps in the mechanism of cell activation by angiotensin II and other Ca^{2+} mobilizing hormones.

Previous findings on the role of guanine nucleotide regulatory (G) proteins in AII binding and action indicated that the adrenal AII receptor is coupled to Gi as well as to the functionally identified but as yet undiscovered protein (Gp) responsible for coupling of calcium-dependent hormone-receptor systems to phospholipase C. Direct evidence for the role of G nucleotides in the stimulation of polyphosphoinositide hydrolysis by AII was obtained in permeabilized, inositol-labeled adrenal glomerulosa cells. In such cells, nanomolar concentrations of AII stimulated $InsP_3$ formation within 15 sec., and similar but less rapid responses were elicited by guanine nucleotides and fluoride. In adrenal membrane preparations, GTPγS-stimulated polyphosphoinositide hydrolysis was enhanced by Ca^{2+}, with half-maximal activity at 300 nM free Ca^{2+}. Ins-1,4,5-P_3 formation was also increased by NaF, further indicating the involvement of a guanine nucleotide regulatory protein. In addition to Ins-1,4,5-P_3 and its metabolites formed during degradation via the 4-monophosphate pathway, AII and GTPγS stimulated the formation of the phosphorylated metabolite inositol 1,3,4,5-tetrakisphosphate and inositol 1,3,4-trisphosphate in permeabilized cells. The absence of a significant rise in inositol 1-monophosphate indicated that phosphatidylinositol hydrolysis was not stimulated by AII or GTPγS. Pretreatment of glomerulosa cells with pertussis toxin for 12 h before permeabilization did not inhibit AII- or GTPγS-stimulated inositol polyphosphate formation. However, treatment with cholera toxin, forskolin, or 8-Br-cAMP for 12 h enhanced both basal and ligand-stimulated Ins-1,4,5-P_3 production. These observations suggest that agonist binding to the AII receptor activates a polyphosphoinositide-specific phospholipase C in the adrenal glomerulosa cell, and that a distinctive guanine regulatory protein is involved in this mechanism.

Characterization and purification of the AII receptor has been pursued by two approaches based on detergent solubilization of receptors from the bovine adrenal cortex. The complete isolation of the AII receptor by conventional purification techniques has been hampered by its extreme instability, which prevents the use of ligand-affinity procedures employed to purify several other peptide hormone receptors. For this reason, purification of photoaffinity-labeled AII receptors was performed by ion-exchange, lectin-affinity, and immunoaffinity homatography, the latter employing solid-phase antibody specific for the N-terminal region of AII that is accessible in the hormone-receptor complex. Since this method did not provide sufficient quantities of receptors for sequencing, the rapidly-inactivating free AII receptor has also been purified from solubilized bovine adrenal membranes by rapid lectin and ligand-affinity

chromatography. This method has been more successful in providing large quantities of the putative receptor protein, and promises to yield sufficient material to permit microsequencing of the receptor.

An additional approach to characterization of the AII receptor, as well as to the analysis of its activation mechanisms, has been to express the AII receptor from mRNA extracted from the adrenal glomerulosa zone and other AII target tissues. The expression of several neurotransmitter and drug receptors from injected exogenous mRNA in *Xenopus laevis* oocytes has been demonstrated by electrophysiological measurements on ion channel activation. The expression of specific receptors for peptide hormones in such a translation system would facilitate studies on the structure and regulation of cell-surface receptors as well as their coupling to membrane transduction mechanisms. The expression of receptors for calcium-mobilizing hormones in *Xenopus* oocytes was sought by analysis of phospholipid turnover in hormone-stimulated oocytes.

For this purpose, *Xenopus* oocytes were injected with mRNA extracted from bovine adrenal and pituitary glands and incubated with *myo*-[^{3}H]inositol to label plasma-membrane phosphatidylinositol phosphates. The expression of functionally active receptors for AII and thyrotropin-releasing hormone (TRH) was demonstrated by the stimulation of [^{3}H]inositol phosphate production by AII and TRH in the mRNA-injected, [^{3}H]inositol-prelabeled oocytes. The ability of AII and TRH to act by way of newly synthesized receptors from mammalian endocrine tissues to stimulate phosphatidylinositol polyphosphate hydrolysis in *Xenopus* oocytes suggests a generalized and conserved mechanism of receptor coupling to the transduction mechanism responsible for activation of phospholipase C in the plasma membrane.

(b) The Section on Endocrine Physiology (Dr. Greti Aguilera) investigates physiological and pathological aspects of circulatory homeostasis and neuroendocrine regulation, including mechanisms of adaptation to stress. This program also includes studies on the role of the renin-angiotensin system in the regulation of mineralo-corticoid secretion and blood pressure, and the effects of AII in other systems including the pituitary and gonads. AII has been shown to mediate the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomulosa zone to AII. Previous studies in the rat have demonstrated that adrenal responsiveness to AII depends not only on the trophic effects of the peptide, but also upon the modulatory effects of other regulators including dopamine, atrial natriuretic factor (ANF) and somatostatin (SRIF).

Studies on the effects of the dopaminergic antagonist metoclopramide (MCP) in sodium-loaded hypophysectomized rats showed that the sensitizing effect of MCP on the adrenal response to AII is blunted in the absence of the pituitary gland. In addition, abundant receptors for AII, which undergo regulatory changes during altered sodium and during administration of AII and MCP, are present in the intermediate lobe of the pituitary. These findings suggest the involvement of the pars intermedia of the pituitary gland in adaptation to changes in sodium intake, and provide an additional mechanism whereby dopaminergic regulation of an intermediate lobe peptide could modulate the physiological changes in responsiveness of the adrenal glomerulosa zone.

Studies on the ontogeny of the AII receptor revealed dramatic changes in receptor concentration and tissue distribution during development. In addition to a marked decrease in AII receptor concentration in the adrenal capsule and smooth muscle with age, the fetal and neonatal rat and mouse were found to possess abundant AII

receptors widely distributed in muscular and mesenchymal tissue throughout the body. Other components of the renin-angiotensin system were found in the fetus, suggesting a unique role of AII during development. Analysis of the role of the receptor-bound AII in adrenal glomerulosa cells revealed marked internalization of the agonist ligand following binding to receptors, but not of the bound antagonist ligand. In addition, there was significant accumulation of the internalized agonist in the nucleus, suggesting that AII has direct actions at the genomic level in addition to its recognized effects on plasma membrane transduction mechanisms.

In the pituitary gland, investigations were focused on the properties and regulation of the corticotropin releasing factor receptors and the mechanism of interaction between CRF and other regulators of ACTH secretion. In studies on the properties of the CRF receptor, gel electrophoresis analysis of detergent-solubilized CRF receptors crosslinked with ^{125}I-Tyr-oCRF indicated that the receptor is a single protein with a molecular weight of 67 kDa. The characteristics of the CRF receptor are similar in several different target tissues, including the anterior and intermediate lobes of the pituitary and the cortex, amygdala and olfactory bulb of the brain. Previous studies have shown that the down-regulation of pituitary CRF receptors that accompanies the increase in plasma ACTH following adrenalectomy is dependent on hypothalamic factors such as CRF and VP. In the rat, studies during stress showed transient increases in plasma ACTH during prolonged immobilization. The subsequent decrease in plasma ACTH in the continuous presence of stress is accompanied by CRF receptor down-regulation and desensitization of the pituitary. However, pituitary responsiveness *in vivo* as well as the potentiating effect of VP on CRF action *in vitro* are maintained, emphasizing the importance of the interaction between regulators during the physiological control of ACTH secretion. CRF receptors in the intermediate pituitary and brain are unchanged during chronic immobilization stress.

In studies on the mechanism of action of ACTH regulators, the synergistic effect of VP on CRF action was previously found to involve potentiation of CRF-stimulated cAMP production, suggesting that activation of protein kinase C is part of the mechanism of action of VP. Studies in isolated pituitary cells showed that inhibition of endogenous protein kinase C abolishes the effects of VP in the corticotroph. In addition, VP was shown to stimulate inositol phosphate formation in pituitary cells and to induce translocation of protein kinase C from cytosol to the membrane compartment.

(c) The Section on Molecular Endocrinology (Dr. Maria Dufau) investigates the molecular basis of peptide hormone action, with particular emphasis on the characterization of gonadotropin receptors, activation of steroid biosynthesis in gonads and adrenal, and analysis of the biological activity of circulating gonadotropins. A major aspect of this program is concerned with the characterization of gonadal gonadotropin and prolactin receptors, and of the physical and functional relationships of the LH receptor site and adenylate cyclase.

The LH/hCG receptor from rat ovary and testis was purified by sequential affinity column chromatography and isolated as a single protein species on SDS-PAGE under reducing conditions. The purified testicular receptor was shown to be phosphorylated *in vitro* by the catalytic subunit of cAMP-dependent protein kinase. Occupancy of the receptors by hCG significantly increased the rate of phosphorylation by the catalytic subunit of cAMP-dependent protein kinase, while maximal stoichiometry of phosphorylation was not affected by hCG. However, preincubation of receptors with hCG for 30-60 min reduced the subsequent rate of receptor phosphorylation.

Phosphorylation by protein kinase A did not affect the binding characteristics of the testicular LH/hCG receptor. The phosphorylated testicular and ovarian LH/hCG receptors bound effectively to hCG-Sepharose and wheat germ lectin, and when eluted were resolved as single bands of Mr 90,000 and 85,000 in SDS-PAGE. These studies indicate that occupancy by hCG leads to conformational changes which initially facilitate but subsequently reduce receptor phosphorylation, and that tight binding of hCG renders the phosphorylation sites less accessible.

In further studies on the differences in Mr of LH/hCG receptors from testis (90 kDa) and ovary (80-85 kDa) trypsin digestion of phosphorylated ovarian and testicular LH/hCG receptors showed six radioactive peaks with almost identical retention times. This finding suggests that the two receptors have homologous amino acid sequences and phosphorylation sites for protein kinase A, and that post-translational modifications are responsible for the differences in size of the testicular and ovarian receptors. Neuraminidase treatment of purified receptors caused reductions in Mr to 82,000 ± 3,400 (testis) and 77,000 ± 3,700 (ovary), and further treatment with O-glycanase had little effect on molecular size. However, endoglycosidase F caused a reduction in apparent Mr of the LH/hCG receptor, and deglycosylation with N-glycosidase and Endoglycosidase F produced a single labeled polypeptide of Mr=59,000 ± 3,000 for both receptors. These results indicate that LH/hCG receptors are sialoglycoproteins with predominately N-linked glycosylation, and suggest that changes in the glycosylation pattern could account for the size difference between testicular and ovarian receptors. The various enzyme treatments also suggested that the LH/hCG receptor contains sialylated N-linked carbohydrate chains of the biantennary and/or hybrid type.

Studies on the transduction mechanism of prolactin action were initiated in the Nb_2 lymphoma cell line, which is dependent upon lactogen for proliferation. The ability of cAMP to modify PRL-stimulated Nb_2 lymphoma cell mitogenesis, and differences between the effects of pertussis toxin and cholera toxin (i.e. biphasic effect, degree of inhibition) and also the differential effect of PMA on Nb_2 cell replication, suggests the involvement of one or more G proteins in PRL action or its modulation, including a cAMP-independent mechanism.

Previous studies in human and experimental animals have suggested that sex steroid hormones modulate the pituitary secretion of biologically active gonadotropin (i.e. decrease in the B:I ratio of LH after castration in rats is attenuated by androgen replacement; in human menopause/or gonadal failure increased B:I ratios are reversed by E_2 administration; young men have higher B:I ratio than cycling women). To examine the role of E_2 in modulating biologically active LH secretion, bioactive LH release was examined in men subjected to steady state E_2 infusion, and the feedback actions of endogenous E_2 on spontaneous and exogenous GnRH-stimulated pulsatile bioactive LH release were analyzed. Steady state intravenous infusion of estradiol at a dosage that mimics its endogenous production rate preferentially suppressed mean circulating bioactive LH concentrations, with a consequent significant decline in the plasma bio/immuno LH ratio. Conversely, antiestrogen treatment enhanced spontaneous bioactive LH pulse frequency, increased bioactive LH pulse amplitude, and augmented plasma intrapulse and interpulse bio/immuno LH ratios. Low-dose pulsed injections of exogenous GnRH also increased plasma bio/immuno LH ratios. However, tamoxifen attenuated the ability of exogenous GnRH to further enhance the bio/immuno LH ratio, suggesting that endogenous LH release was already maximally enriched in LH bioactivity during antiestrogen administration. The ability of estradiol to modulate specific properties of the LH pulse signal as well as its frequency may have significant

implications in relation to pituitary function, and may also reflect direct actions of estradiol on the gonadotrope with effects on cellular processing and/or terminal glycosylation of LH molecules.

In earlier studies on GnRH-induced LH release *in vivo* a single large bolus of GnRH and/or continuous GnRH infusions did not cause any change in the plasma bio/immuno LH ratio. However, it was recently observed that two consecutive submaximal pulses of exogenous GnRH resulted in an immediate and preferential release of bioactive LH, with a consequent increase in the plasma bio/immuno LH ratio. These findings might be explained by functional compartmentalization of releasable LH pools, such that pharmacological GnRH stimulation increases secretion from all pituitary LH pools to yield an integrated bio/immuno value, rather than selective release from a highly bioactive pool. Also, during continuous GnRH infusion there may be lack of definition of a small early pool (presumably of high bioactivity) and/or a mixture with a late pool of reduced bioactivity. Thus, it is inferred that spontaneous LH pulses, putatively generated in response to endogenous GnRH stimulation of gonadotropes, exhibit a relative enrichment in biological activity, and this degree of enrichment can be modulated by estrogen action. Such observations suggest a critical role for estradiol in regulating the functional attributes of the pituitary-gonadal axis in man.

Studies on the dynamics of bioactive LH release in healthy older men (ages 60-75) revealed significant attenuation of the pituitary's capacity to release biologically active gonadotropic hormone. The diminution of bioactive LH release could be unmasked by maneuvers designed to enhance endogenous secretion of LH enriched in biological activity. Such evocative procedures consisted of mimicking endogenous GnRH action by consecutive intravenous pulses of low-dose (10 μg) exogenous GnRH, or by presumptively augmenting endogenous GnRH secretion with antiestrogen treatment. Some healthy older men exhibited evidence of neuroendocrine dysfunction, reflected by irregular bursts of bioactive LH release followed by transiently low plasma bio:immuno (B:I) LH ratios. However, mean basal plasma bioactive LH concentrations, B:I ratios, and spontaneous LH pulse properties (peak frequency, amplitude, duration, and enhanced B:I ratios within LH peaks) were not altered in older men. On the other hand, augmentation of bioactive LH secretion and enhancement of plasma B:I ratios by pulsed injections of exogenous GnRH were either significantly reduced or absent in older men. In addition, although tamoxifen increased bioactive LH pulse frequency in both age groups and facilitated exogenous GnRH action in some subjects, older men increased their 12-h mean bioactive LH concentrations, B:I ratios, and bioactive LH peak amplitudes to a significantly lesser degree than young men. In summary, young and older healthy men exhibit similar mean basal plasma bioactive LH concentrations and spontaneous LH pulse properties. However, pituitary bioactive LH reserve is markedly attenuated in older men challenged with either exogenous GnRH or antiestrogen. Accordingly, we conclude that healthy aging men manifest an impaired secretory reserve for biologically active LH release.

In the fetal rat Leydig cell, estradiol causes up-regulation of its receptor and induction of the late steroidogenic lesion at 17α-hydroxylase/17-20 desmolase that is observed in the adult Leydig cell. The ability of adult Leydig cells to respond to sustained gonadotropin stimulation with increased androgen production was previously found to be is limited by an estrogen-dependent refractory state, with decreased activity of microsomal P-450$_{17\alpha}$ and decreased testosterone production. Such inhibitory regulation of the testis by endogenous estrogen was not observed in fetal life, due to a very low level of aromatization capacity, with lack of up-regulation and/or induction of

testicular estrogen receptors by estradiol. More recent studies have revealed that higher doses or frequent treatment of fetal cultures with LH increase aromatase activity and consequent E_2-receptor-mediated action for the induction of gonadotropin-mediated desensitization in fetal cells. Resolution of fetal Leydig cells by centrifugal elutriation demonstrated that in addition to a predominant cell type with fetal characteristics, a small population of adult-like Leydig cells is present in the fetal testis, and that a functional adult-like population emerges from the fetal Leydig cells during gonadotropin treatment.

In related studies, the extent to which modulatory actions related to changes in P-$450_{17\alpha}$ mRNA levels could account for steroidogenic stimulation and desensitization was evaluated. For this purpose, a partial length rat P-$450_{17\alpha}$ cDNA clone was characterized and identified. The 1 Kb cDNA insert, displaying high similarity with the previously isolated P-$450_{17\alpha}$ cDNA sequences from human, bovine and porcine species, and containing the conserved tridecapeptide and heme regions and termination codon, was employed to examine the regulation of mRNA levels in adult animals treated with hCG and in cultured fetal Leydig cells. During low-dose hCG treatment, an early increase in mRNA levels was followed by a return to control values at later times, while higher desensitization doses caused a marked reduction in the mRNA at 24 h and minor recovery at 48 h. After estradiol treatment, fetal rat Leydig cells maintained in the presence of LH showed 70% decreases in P-$450_{17\alpha}$ mRNA levels and testosterone production. These studies suggest that gonadotropin stimulation and desensitization of P-$450_{17\alpha}$ dependent enzymes in the adult rat testis as well as estradiol-induced desensitization in fetal Leydig cells are related to levels P-$450_{17\alpha}$ mRNA. These studies have also demonstrated that a short loop feedback control by products of the androgen pathway leads to marked reduction of P-$450_{17\alpha}$ mRNA in the Leydig cell. This finding rules out mechanisms based on the generation of an inhibitor protein acting at the translational level, or inactivation of P-$450_{17\alpha}$ by reactive oxygen-free radicals derived from breakdown of the interaction of pseudosubstrate (testosterone) with P-450 of oxygen (P450-oxygen-complex), proposed in earlier reports by others demonstrating the reduction of enzyme mRNA. It is extremely interesting that a hormone known to be trophic to its target cell has such a dual effect, causing increased mRNA levels at low doses but a subsequent major reductions at higher doses and hence steroidogenic desensitization. The above studies were made possible by the isolation of the cDNA for rat P-$450_{17\alpha}$, since a bovine cDNA probe was found to be unsuitable for use in the rat. It has been recognized that P-$450_{17\alpha}$ is present in the human and bovine adrenal and not in the rat adrenal, but only now with the isolation of rat cDNA and the use of an homologous probe was it possible to conclusively demonstrate the absence of adrenal message in this species.

Other studies using fetal Leydig cell cultures have demonstrated that Leydig cells are a site of β-endorphin synthesis *in vitro* and that testicular β-endorphin is under direct control of gonadotropins. Acute stimulation of Leydig cells by hCG can markedly enhance β-endorphin secretion, and these changes are not mediated by testosterone. In contrast, testosterone or its metabolites may exert a negative autocrine modulation of β-endorphin, since inhibition of steroid biosynthesis markedly increased basal and hCG-stimulated β-endorphin output (by 100-200%). In addition, β-endorphin did not affect testosterone production, and opiate binding was not detected on Leydig cells. Since we have demonstrated functional β-endorphin receptors and opioid inhibition of FSH-stimulated androgen binding protein production in Sertoli cells, the β-endorphin

produced in the Leydig cell may have paracrine effects that contribute to the quiescent state of the testis from early life to sexual maturation and could also be involved in the modulation of seminiferous tubule function during adult life.

(d) The Section on Adrenal Cell Biology (Dr. C. Strott) investigates the physiology and regulation of adrenal steriodogenesis, by characterization of cellular steroid binding proteins and soluble factors which mediate steroidogenic responses to ACTH, and analysis of cellular mechanisms of cholesterol utilization in steroid biosynthesis. The Section is also interested in the development of adrenocortical zonation and the regulation of adrenal steroidogenesis, and is currently concentrating on two areas of research: 1) adrenocortical calmodulin, calcium- and calmodulin-binding proteins, protein kinase systems, and the post-translational modification of proteins; 2) purification, immunology, and functional activity of soluble and membranous adrenocortical proteins including steroid-binding proteins.

The steroidogenic action of ACTH can be separated into acute and chronic phases. The acute ACTH response (sec-min) occurs primarily at the level of the mitochondria in regulating the rate-limiting step in steroidogenesis, the conversion of cholesterol to pregnenolone. The chronic action of ACTH (hours) occurs at the level of the genome and involves synthesis of various steroidogenic enzymes and co-factors. Both the acute and chronic actions of ACTH are dependent on the cytoplasmic synthesis of protein in that both responses are blocked by cycloheximide. In addition, both the acute and chronic actions of ACTH can be mimicked by cAMP. Based on mutation studies performed with an ACTH-responsive murine adrenocortical tumor cell line (Y_1), as well as ACTH receptor studies involving various adrenocortical cell-types, it is now accepted that in the adrenal cortex ACTH stimulates membrane-bound adenylate cyclase activity which leads to an increase in intracellular cAMP and the activation of cAMP-dependent protein kinase followed by steroid synthesis. The role of other protein kinases in this process such as Ca^{2+}-regulated kinases, if such a role exists, is not well understood. No adrenocortical regulatory phosphoprotein has yet been identified, and there is no evidence that the P-450 cholesterol side-chain cleavage enzyme is regulated by phosphorylation-dephosphorylation. The guinea pig is used as an animal model to examine ACTH steroidogenic action for the reason that its adrenal gland is composed of an ACTH-responsive outer zone and an ACTH-unresponsive inner zone. In this model, adenylate cyclase activation and cAMP formation in response to ACTH are similar for the two zones, suggesting that in the inner zone a defect has developed beyond the formation of cAMP. When cAMP-dependent protein kinase activity was measured it was found to be significantly less in the inner zone than in the outer zone. The meaning of this finding, however, is unclear since the activities of Ca^{2+}-regulated protein kinases were also significantly lower in the inner zone than in the outer zone. It has been suggested that calmodulin, a protein that mediates certain intracellular actions of Ca^{2+}, may play an important role in ACTH-stimulated steroidogenesis. For this reason, the calmodulin system has been examined in this model and evidence was found for calmodulin kinase III activity and an endogenous substrate, similar to elongation factor-2 (M_r 100,000). This latter system is known to be hormonally regulated in the rat corpus luteum, and may also be a significant regulatory system in the adrenal gland.

The initial reaction of steroidogenesis is the cleavage of the cholesterol side chain by a specific cytochrome P-450 enzyme located on the inside face of the inner mitochondrial membrane. The resultant steroid, pregnenolone, is then metabolized by enzymes having extramitochondrial locations. Thus, pregnenolone must move out of the

mitochondria, crossing the organelle's inner and outer membranes. Despite the significance of pregnenolone efflux from mitochondria, the process remains poorly characterized. This process is currently being investigated in the guinea pig adrenal cortex, in which a specific pregnenolone-binding protein (PBP) has been identified. Although PBP behaves as a M_r 58,000 globular protein on gel permeation chromatography, it migrates as a M_r 34,000 protein on SDS-PAGE. A polyclonal antibody has been generated against the purified 34 kDa protein. At all stages of purification, including the starting material, Western blot analysis of isoelectric focusing gels reveals a similar pattern of apparent microheterogeneity with pIs of 6.8, 6.6, 6.4, and 6.2. The major dilemma at the moment is to distinguish between microheterogeneity of a single protein and a co-purifying contaminant. Additional purification steps are under investigation, and an effort to generate an N-terminal sequence is also under way. Studies with the polyclonal antibody demonstrate that the PBP is present only in the soluble fraction of the adrenal cortex. Immunocytochemistry indicates that the PBP is most abundant in the outer, ACTH-responsive adrenocortical zone. The latter finding is interesting because the pregnenolone-binding activity is far greater in the inner, ACTH-unresponsive zone of the adrenal cortex. It thus appears that there could be active and inactive forms of the binding protein, and this phenomenon is currently under investigation. It is possible that the active/inactive forms and the microheterogeneity of PBP are related. Success with the N-terminal sequencing will resolve the problem of purity unless the PBP is composed of two non-identical subunits (it is probable that the PBP exists as dimer in its native form). Once the polyclonal antibody specificity has been ascertained, it will be used to isolate the PBP mRNA. The ultimate goal is to develop a cDNA clone for the PBP and to determine the complete amino acid PBP sequence.

(e) The Section on Molecular Structure and Protein Chemistry (Dr. H.-C. Chen) conducts research on the analysis, synthesis, and structure-function relationships of biologically active peptides and proteins. This includes the identification and synethesis of unusual structures and sequences in amino acids and peptides, and the development of new techniques for peptide sequencing and synthesis. Of particular interest are the structural design, chemical synthesis, and modification of molecules important to reproductive and developmental biology. A major component of this project focuses on the role of carbohydrate structures in the subunit association and receptor interactions of human chorionic gonadotropin (hCG), employing chemically modified derivatives of hCG for receptor binding and target cell activation studies. The HF deglycosylated derivative of asialo hCG was found to bind with 2-5 fold higher affinity in corpus luteum homogenates and 5-10 fold higher in testis homogenates than hCG. When added to granulosa luteal cells or testicular minces it caused no cAMP production at 0.1 microgram/ml and inhibited the production of cAMP by hCG. In recent studies, pure hCG-alpha subunit was specifically deglycosylated at Asn^{78} by an enzymatic procedure. The heterodimer formed by combination of this subunit with intact hCG-beta displayed similar potency to HF-deglycosylated hCG in the rat uterine weight assay and in cAMP and testosterone production in rat Leydig cells.

hCG-beta chains isolated from urines of 5 choriocarcinoma patients were compared with those from molar and normal pregnant women by SDS polyacrylamide gel electrophoresis and by Western blotting using anti-hCG-beta-COOH peptide antiserum. The sizes found in the choriocarcinoma patients were either larger or smaller than those from molar or normally pregnant women and should permit distinguishing these

cases from the normal range. A triantennary oligosaccharide structure in chorio-carcinoma hCG implies different mechanisms of post-translational processing in the malignant trophoblast.

Other parts of this project focus on partial or total synthesis of biologically important peptides which 1) incorporate unique structural features as functionally important probes or for defined linkages to proteins, 2) represent portions of the sequence of proteins and are useful for immunological investigations, 3) display agonism or antagonism through substitutions of amino acid residues or changes in secondary structures. As an example of this approach, specific toxicity has been programmed into ovine corticotropin-(oCRF) and human growth hormone (hGHRH) releasing hormones by way of derivatization with an active ester of ^{10}B-enriched 1-(2-carboxymethyl)-1,2-dicarba-closododecarborane after the standard Merrifield solid phase peptide synthesis. In order to prevent perturbation of biological activity, carboranyl-acetylation was directed to the alpha-amino terminus of oCRF or the introduced epsilon-amino group of Lys at residue 41 for GHRH. The derivatives are fully active for their respective hormone-releasing activities, and the labelled oCRF was able to suppress the ACTH release system upon irradiation with slow neutron gas without affecting the releases of GH, LH and prolactin in a pituitary cell culture system. Neutron radiation experiments with the labelled hGHRH are now in progress.

In other studies on biologically active peptides, substitutions of Ala at Gly^{13} and Gly^{18} within the magainin 2 amide sequence was performed in order to enhance the potential of an amphiphilic alpha-helical conformation as revealed by the circular dichroism. This manoeuver resulted in one to two orders of magnitude increase of antimicrobial activity over magainin 2 in a wide variety of bacteria. The importance of a free amino-terminus for the full activity of magainin was also established. In addition, the synthesis of a 27-residue peptide deduced from the mouse protooncogene *c-fos* with Tyr as the amino-terminus was accomplished. The peptide was conjugated to bovine thyroglobulin by diazotized benzidine through the phenolic function of Tyr to produce a complex suitable for immunization to raise antisera to the *c-fos* gene product.

(f) The Section on Metabolic Regulation (Dr. K.-P. Huang) studies the role of protein kinases and phosphorylation-dephosphorylation of proteins in the regulation of cellular functions. Also, the regulation and hormonal control of glycogen metabolism, and the activities of glycogen synthase and phosphorylase kinase. The receptor-mediated turnover of membrane phospholipids plays an important role in the regulation of many cellular functions. Inositol 1,4,5-trisphosphate is believed to trigger the release of calcium from an intracellular nonmitochendrial pool, whereas diacylglycerol activates protein kinase C to modulate numerous cellular responses. This signal-transduction pathway has been implicated in the regulation of cell growth, differentiation, gene expression, hormone and neurotransmitter release, cell-surface receptor function, and cellular metabolism.

Three major classes of protein kinase C isozyme were found to be highly enriched in mammalian brains. Using isozyme-specific antibodies to determine the cellular distributions of these enzymes by light microscopic immunocytochemistry, strong staining was revealed in neuronal somata and their dendrites and weak or no reaction in axons and astroglial structures. In the cerebellum, type I PKC antibodies stained the Purkinje cell bodies and dendrites, type II PKC antibodies stained the granule cells, and the type III PKC antibody stained both Purkinje and granule cells. In the cerebral cortex, all antibodies stained neurons resembling pyramidal cells and their apical

dendrites in layers II to VI, while layer I was nearly devoid of staining. However, the various isozyme-specific antibodies revealed distinct laminar distribution patterns of the positively stained neurons, and the type III PKC-positive neurons exhibited a higher density than those of type I or II PKC-positive ones, especially in layer II of the cingulate (retrosplenial) and piriform cortices. In the hippocampal formation, both pyramidal cells of the hippocampus and granule cells of the dentate gyrus were stained by all PKC antibodies. Subcellularly, type III PKC appeared mostly in the cytoplasm of these neurons whereas type I and II PKC seemed to associate with the nucleus as well. In the olfactory bulb, both type II and III PKC antibodies stained the periglomerular and granular cells, and the latter also stained the mitral cells. The distinct cellular and subcellular distribution of PKC isozymes suggest that each isozyme plays a unique role in various specific neural functions.

Using PKC isozyme-specific antibodies for immunoblot analysis, heterogeneous distribution of PKC isozymes was demonstrated in various regions of monkey and rat brains, and type I PKC was most abundant in cerebellum, hippocampus, amygdala, and cerebral cortex. By immunocytochemical analysis, type I PKC-specific antibody showed strong reactivity in various types of neuron in hippocampal formation, amygdala, cerebellum, and neocortex. In hippocampal formation, granule cells of dentate gyrus and pyramidal cells of hippocampus were heavily stained. The relative levels of PKC isozymes in several areas of monkey cerebral cortex involved in the visual information processing were determined by immunoblot analysis. While type II and III PKCs appeared to be evenly distributed thoroughout the areas, type I PKC formed a gradient of increasing concentration rostral along the cerebral cortex of occipital to temporal and then to the entorhinal areas. Neurobehavioral studies have demonstrated that the perirhinal and entorhinal cortices of the temporal lobe participate more than the striate and prestriate cortices of the occipital lobe in the storage of visual representation and that both hippocampus and amygdala are important in memory formation. Since type I PKC is present at high levels in hippocampus, amygdala, and temporal lobe cortex, we predict that the type I protein kinase C may be important for mnemonic function.

Types I, II, and III protein kinase C have been shown to be products of the γ, β, and α genes of this enzyme family, respectively. Incubation of the highly purified rat brain protein kinase C isozymes with trypsin (kinase/trypsin=100) under identical conditions resulted in a preferential degradation of the type I and II enzymes, whereas the type III enzyme was relatively resistant to tryptic proteolysis. Degradation of the type III enzyme by trypsin could be facilitated by the addition of Ca^{2+}, phosphatidylserine, and dioleoylglycerol; none of these components alone was effective. Limited proteolysis of the three protein kinase C isozymes generated distinctive fragments from each isozyme, indicating that each molecule has specific trypsin-sensitive sites. Tryptic digestion of the type III protein kinase C was used as a model to determine the effects of various modulators on protein kinase C degradation. While Ca^{2+} and phosphatidylserine together were sufficient to convert the type III protein kinase C from a trypsin-insensitive to a sensitive form, addition of dioleoylglycerol greatly reduced the Ca^{2+} requirement for such conversion. Among the various phospholipids tested for trypsinization, phosphatidic acid and phosphatidylserine were most effective, whereas phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol were the least effective. With the exception of phosphatidic acid, the order of effectiveness of these phospholipids for trypsinization of the kinase paralleled that for the stimulation of protein kinase C activity. The relevance of these proteolytic reactions to physiological responses was assessed with phorbol ester on rat basophilic leukemia cells 2H3, which

contained both type II and III protein kinase C and showed synergistic interactions between Ca^{2+} ionophores and the tumor-promoting phorbol esters for histamine secretion. Immunoblot analysis with the isozyme-specific antibodies revealed that phorbol ester induced a faster degradation of the type II than that of the type III isozyme in these cells. These results demonstrate that the various protein kinase C isozymes have different susceptibility to proteolysis *in vitro* when tested with trypsin, as well as toward endogenous proteases in intact cells.

Rat basophilic leukemia cells (2H3) can be stimulated to secrete histamine by aggregation of the I_gE receptors either directly with antireceptor antibodies or indirectly with antigen when cells are primed with the appropriate antigen-specific I_gE. The secretory response of the parental 2H3 cells appears to require an increase in the intracellular level of Ca^{2+} and activation of protein kinase C based on evidence including the synergism between Ca^{2+} ionophore and tumor-promoting phorbol ester, and the inhibition of secretion by inhibitors of PKC. In 2H3 cell variants having reduced secretory response to antigen, the level of PKC isozymes were determined by immunoblot analysis and by chromatographic separation on hydroxyapatite. Among ten 2H3 cell variants tested, all contained normal levels of the type III PKC as compared to parental 2H3. Several mutants having reduced responses to antigen for histamine secretion were found to contain reduced level of the type II PKC. Analysis of the PKC substrates in 2H3 and type II PKC-deficient mutant cells by *in vitro* phosphorylation with a proteolytically activated PKC revealed that the two cell lines had similar substrates for PKC. Hence, the different physiological responses observed in the mutant cells may result from the deficiency of type II PKC. These findings suggest that the type II PKC is involved in the ligand-mediated secretory response in 2H3 cells.

Effects of phorbol 12-myristate 13-acetate (PMA) on the fate of protein kinase C in two mouse thymoma cell lines, which are either responsive (EL4) or unresponsive (lEL4) to PMA-induced interleukin-2 (IL-2) production, were investigated with polyclonal antibodies raised against rat brain enzyme. These antibodies immunoprecipitated completely the protein kinase C from both cell lines and detected mainly an 82-KDa protein by immunoblot analysis of the crude homogenates as well as the partially purified kinase preparations. PMA elicited a time- and dose-dependent redistribition of protein kinase C from cytosol to the particulate fraction and proteolytic degradation of the kinase from both cell lines. The dose of PMA required for half-maximum protein kinase C translocation and degradation was at least 5 times lower for EL4 than for IEL4. In the presence of 16 nM PMA the rates of protein kinase C translocation and degradation were faster in EL4 than in IEL4, and the half-lives of protein kinase C in EL4 and IEL4 were less than 5 min and greater than 2 h, respectively. Analysis of the tryptic fragments of the immunoprecipitated enzyme, previously phosphorylated in the presence of [γ-^{32}P]ATP, revealed minor structural differences between the protein kinase C from these two cell lines. In neither cell line did the PMA-induced degradation of protein kinase C result in accumulation of the Ca^{2+}/phospholipid-independent kinase (catalytic unit) as analyzed by immunoblotting and gel filtration chromatography. Thus, activation of protein kinase C through its proteolytic conversion to the effector-independent catalytic unit plays little role in IL-2 production. The role of protein kinase C translocation and degradation in the PMA-induced responses in EL4 cells in unknown. However, IL-2 production in EL4 cells were reduced when PMA-induced degradation of protein kinase C was retarded by exogenously added protease inhibitors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00022-15 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Renin-Angiotensin System and Aldosterone Regulation

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

PI:	G. Aguilera	Head, SEP	ERRB, NICHD
Others:	K. J. Catt	Head, SHR	ERRB, NICHD
	M. A. Millan	Sr. Staff Fellow	ERRB, NICHD
	S. Nakano	Visiting Fellow	ERRB, NICHD
	S. Zemel	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this project is to analyze physiological and pathological aspects of the renin-angiotensin system, including the effects of AII in circulatory homeostasis, pituitary and gonadal function. AII mediates the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomulosa zone to AII. Previous studies in the rat have demonstrated that the adrenal responsiveness to AII depends on the trophic effects of the peptide and the modulatory effect of the other regulators such as dopamine, atrial natriuretic factor (ANF) and somatostatin (SRIF).

Studies using the dopaminergic antagonist metoclopramide (MCP), in sodium loaded hypophysectomized rats showed that the sensitizing effect of MCP on the adrenal effects of AII is blunted in the absence of the pituitary. In addition, abundant receptors for AII, which undergo regulatory changes during altered sodium intake, AII and MCP administration, are present in the intermediate lobe of the pituitary suggesting that an intermediate lobe factor is involved in the control of the adrenal responsiveness to AII.

Studies on the ontogeny of the AII receptor revealed dramatic changes in receptor concentration and distribution during development. In addition to a marked decrease in AII receptor concentration in the adrenal capsule and smooth muscle with age, in fetal and neonatal rat and mouse there are abundant AII receptors widely distributed in muscular and mesenchymal tissue throughout the body. Other components of the renin-AII system were found in the fetus suggesting a unique role of AII during development.

Analysis of the role of the receptor bound AII in adrenal glomerulosa cells indicated marked internalization of the ligand following binding of the agonist but not the antagonist. In addition, there was significant accumulation of the internalized agonist in the nucleus suggesting direct actions of AII at the genomic level in addition to the recognized membrane transduction mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00035-16 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The Structure and Function of Biologically Active Molecules

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

PI:	H. C. Chen	Head, SMSPC	ERRB, NICHD
Others:	J. L. Morell	Research Chemist	ERRB, NICHD
	J. H. Brown	Research Chemist	ERRB, NICHD
	F. A. Ghazanfari	Staff Fellow	ERRB, NICHD
	G. Aguilera	Head, SEP	ERRB, NICHD

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NICHD (G. Merriam, L. Liu)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Structure & Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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.)*

This project focuses on partial or total synthesis of biologically important peptides which 1) incorporate unique structural features as functionally important probes or for defined linkages to proteins, 2) represent portions of the sequence of proteins and are useful for immunological investigations, 3) display agonism or antagonism through substitutions of amino acid residues or changes in secondary structures.

A. Specific toxicity has been programmed into ovine corticotropin-(oCRF) and human growth hormone (hGHRH) releasing hormones by way of derivatization with an active ester of 10B enriched 1-(2-carboxymethyl)-1,2-dicarba-closododecarborane after the standard Merrifield solid phase peptide synthesis. In order to prevent perturbation of biological activity, carboranyl-acetylation was directed to the alpha-amino terminus of oCRF or the introduced epsilon-amino group of Lys at residue 41 for GHRH. The derivatives are fully active for their respective hormone releasing activities, and the labelled oCRF was able to suppress the ACTH release system upon irradiation with slow neutron gas without affecting the releases of GH, LH and prolactin in a pituitary cell culture system. Neutron radiation experiments with the labelled hGHRH are now in progress.

B. Substitutions of Ala at Gly13, Gly18 within the magainin 2 amide sequence in order to enhance the potential of an amphiphilic alpha-helical conformation as revealed by the circular dichroism resulted in one to two order of magnitude increase of antimicrobial activity over magainin 2 in a wide variety of bacteria. The importance of a free amino-terminus for the full activity was also established.

C. Synthesis of a 27 residue peptide deduced from mouse proto-oncogene c-fos with Tyr as the amino-terminus was accomplished. The peptide was conjugated to bovine thyroglobulin by diazotized benzidine through phenolic function of Tyr.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00146-13 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The Structure and Function of Chorionic Gonadotropins

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

PI:	H. C. Chen	Head	ERRB, NICHD
Others:	J. H. Brown	Research Chemist	ERRB, NICHD
	T. C. Chang	Visiting Fellow	ERRB, NICHD
	C. A. Owens	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

New York State Department of Health (N. J. Ellish)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Structure & Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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.)*

This project focuses on studying the role of carbohydrate structures in the subunit association and receptor interactions of hCG.

A. HF deglycosylated asialo hCG was found to bind with 2-5 fold higher affinity in corpus luteum homogenates and 5-10 fold higher in testis homogenates than hCG. When added to granulosa luteal cells or testicular minces it caused no cAMP production at 0.1 microgram/ml and inhibited the production of cAMP by hCG.

B. Pure hCGalpha subunit was specifically deglycosylated at Asn78 enzymatically. This subunit when combined with intact hCGbeta displayed potency in the rat uterine weight assay and in cAMP and testosterone production in rat Leydig cells similar to HF deglycosylated hCG.

C. hCGbeta chains isolated from urines of 5 choriocarcinoma patients were compared with those from molar and normal pregnant women by SDS polyacrylamide gel electrophoresis and Western blotting using anti-hCGbeta-COOH peptide antiserum. The sizes found in the choriocarcinoma patients were either larger or smaller than those from molar or normally pregnant women and should permit distinguishing these cases. A triantennary oligosaccharide structure in chorio-carcinoma hCG implies different mechanisms of processing in the malignant trophoblast.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00147-13 ERRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Action of Peptide Hormones in Steroidogenic Cells

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

PI:	M.L. Dufau	Head	ERRB, NICHD
Others:	M. Namiki	Visiting Fellow	ERRB, NICHD
	Marie Nishihara	Visiting Fellow	ERRB, NICHD
	Christine A. Winters	Chemist	ERRB, NICHD
	Juan Calvo	Visiting Scientist	ERRB, NICHD
	Andrea Fabbri	Guest Researcher	ERRB, NICHD
	Ellen Buczko	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

In the fetal rat Leydig cell, E2 causes an up-regulation of its receptor and an induction of the regulatory mechanism (late steroidogenic lesion, 17 hydroxylase/17-20 desmolase) that is similar to that observed in the adult rat Leydig cell. The absence of this regulation in fetal life is due to a very low aromatization capacity. Our recent studies has revealed that high doses or frequent administration of LH is able to elevate aromatase activity and consequent E2-receptor-mediated action for the induction desensitization in fetal cells. Resolution of fetal Leydig cells by centrifugal elutriation have demonstrated in addition or predominant cell type with fetal characteristics, a small population of adult-like Leydig cells and the emergence of a functional adult-like population from the fetal Leydig cell induced by gonadotropin treatment. In further studies we assessed whether hormonal modulatory actions related to changes in P-45017alpha mRNA levels could account for steroidogenic stimulation and desensitization. We have characterized, cloned and identified a partial length rat P-45017alpha cDNA clone. The (1 Kb) rat cDNA insert, displaying high similarity with the P-45017alpha cDNA structures from human, bovine and porcine species, containing the conserved regions and termination codon was employed to evaluate the hormonal regulation of mRNA levels in adult and cultured fetal Leydig cells. Low hCG dose showed an early increase in mRNA levels returning to control values at later times, while a higher desensitizing dose caused a marked reduction in the mRNA (24 h) and a small recovery at 48 h. Fetal rat Leydig cells treated with E2 showed a 70% decrease in P-450 mRNA levels and testosterone production followed closely the changes in mRNA. These studies suggest that desensitization of P-45017alpha dependent enzymes in the adult desensitization and fetal Leydig cells are related to levels mRNA. In other studies has demonstrated that Leydig cells are a site of beta-endorphin synthesis *in vitro* and that is under control of gonadotropins. Testosterone or its metabolites may exert a negative autocrine modulation of beta-endorphin, as inhibition of steroid biosynthesis markedly increased basal and hCG-stimulated beta-endorphin output (by 100-200%). Since we have demonstrated functional beta-endorphin receptors in Sertoli cells, the beta-endorphin produced in the Leydig cell may have paracrine effects that contribute to the quiescent state of the testis from early life to sexual maturation and it could also be involved in the modulation of tubule function during adult life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00149-13 ERRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

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

PI:	M.L. Dufau	Head, SME	ERRB, NICHD
Others:	K.J. Catt	Head, SHR	ERRB, NICHD

COOPERATING UNITS *(if any)*
Dept. Medicine, Charlottesville, VA, Dept. of Pediatrics, Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

1) Previous studies in human and experimental animals have suggested that sex steroid hormones may modulate the pituitary secretion of biologically active gonadotropin. We examine the role of E2 in biologically active LH secretion, in men subjected to steady state E2 infusion and feedback actions of endogenous E2 on spontaneous and exogenous GnRH stimulated pulsatile bioactive LH release. E2 suppressed mean circulating bioactive LH concentrations, with a consequent significant decline in the plasma bio/immuno LH ratio. Conversely, antiestrogen treatment enhanced spontaneous bioactive LH pulse frequency, pulse amplitude, and intrapulse and interpulse bio/immuno LH ratios. Low-dose pulsed injections of exogenous GnRH also increased plasma bio/immuno LH ratios. However, tamoxifen attenuated the ability of exogenous GnRH to further enhance the bio/immuno LH ratio, which suggests that endogenous LH release was already maximally enriched in LH bioactivity during antiestrogen administration. The ability of E2 to modulate specific properties of the LH pulse signal as well as its frequency may have significant implications in relation to target tissue function, and also reflect direct actions of estradiol on gonadotrope function, through influence the cellular processing and/or one or more aspects of terminal glycosylation of LH molecules. 2) In our early studies using a single large bolus of GnRH and/or continuous GnRH infusions were not able to disclose a stimulatory effect of exogenous GnRH on the plasma bio/immuno LH ratio. In contrast, a schedule of two consecutive **submaximal** pulses (10 micrograms one hour apart) of exogenous GnRH did result in an immediate and preferential release of bioactive LH with a consequent increase in the plasma bio/immuno LH ratio. These findings might be explained by functional compartmentalization of releasable LH pools. 3) The dynamics of bioactive LH release in healthy older men (ages 60-75) have revealed significant attenuation of the pituitary's capacity to release biologically active gonadotropic hormone. The diminution of bioactive LH release could be unmasked by low-dose (10 micrograms) exogenous GnRH, or by antiestrogen treatment. Young and older healthy men exhibit similar mean basal plasma bioactive LH concentrations and spontaneous LH pulse properties. However, pituitary bioactive LH reserve is markedly attenuated in older men challenged with either exogenous GnRH or antiestrogen, indicating that healthy aging men manifest an impaired secretory reserve for biologically active LH release.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00150-13 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase

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

PI:	M.L. Dufau	Head	ERRB, NICHD
Others:	T. Minegishi	Visiting Fellow	ERRB, NICHD
	D. Pineda	NRSA Fellow	ERRB, NICHD
	C. Delgado	Guest Researcher	ERRB, NICHD
	J. Larsen	Guest Researcher	ERRB, NICHD
	E. Buczko	Guest Researcher	ERRB, NICHD
	R. Barkey	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.5	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.)*

1) The LH/hCG receptor from rat ovary and testis has been purified by sequential affinity column chromatography and isolated as a single protein species on SDS-PAGE under reducing conditions. The purified testicular receptor was shown to be phosphorylated *in vitro* by the catalytic subunit of cAMP-dependent protein kinase. Occupancy of the receptors by hCG significantly increased the rate of phosphorylation by the catalytic subunit of cAMP-dependent protein kinase, while maximal stoichiometry of phosphorylation was not affected by hCG. However, prolonged preincubation of hCG with the receptor reduced the rate of receptor phosphorylation. Identical phosphopeptide maps were obtained by reverse phase FPLC following trypsinization of both phosphorylated receptors. Six peaks contained phosphoserine and the major component was also phosphorylated on threonine. The phosphorylated receptor, like the native receptor, bound wheat germ lectin and hCG-Sepharose, and migrated as a single band of Mr=90,000 (testis) and Mr = 85,000 (ovary) respectively on SDS-PAGE. Neuraminidase treatment of purified receptors caused reductions in Mr to 82,000 ± 3,400 (testis) and 77,000 ± 3,700 (ovary), and further treatment with O-glycanase had little effects on molecular size. However, deglycosylation with N-glycosidase and Endoglycosidase F produced a single labeled polypeptide of Mr=59,000 ± 3,000 for both receptors. These results indicate that LH/hCG receptors are sialoglycoproteins with predominately N-linked glycosylation, and suggest that changes in the glycosylation pattern could account for the size difference between testicular and ovarian receptors. The various enzyme treatment also suggested that the LH/hCG receptor contains sialylated N-linked carbohydrate chains of the biantennary and/or hybrid type. Our studies indicate that receptor occupancy by hCG leads to a conformational change which facilitates its phosphorylation during initial binding and reduces the rate of phosphorylation after more prolonged exposure to gonadotropin. 2) The Nb2 lymphoma cell line which is dependent upon. lactogen for proliferation was used to initiate studies on the transduction mechanism of prolactins action. cAMP modified PRL-stimulated Nb2 lymphoma cell mitogenesis. The differences between the effects of pertussis toxin and cholera toxin (i.e. biphasic effect, degree of inhibition) and also the differential effect of PMA on Nb2 cell replication suggests the involvement of one or more G protein in PRL action or its modulation, including a cAMP-independent mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00151-13 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Gonadal and Placental Function

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

PI:	K. J. Catt	Head	ERRB, NICHD
Others:	M. Knecht	Sr. Staff Fellow	ERRB, NICHD
	P. Feng	Visiting Fellow	ERRB, NICHD
	M. Zilberstein	Research Associate	ERRB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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)*

Studies on the molecular basis of hormone action during granulosa cell differentiation included evaluation of the functions and mechanisms of action of growth factors and plasminogen activator. TGF-beta was previously found to exert bifunctional actions on the maturation of granulosa cells, and to modulate FSH-induced stimulation of cAMP formation, steroidogenesis, and LH receptor expression in a concentration-dependent manner. TGF-beta amplified gonadotropin responses in the presence of small amounts of FSH, but had less effect or even inhibited the action of higher FSH levels in the presence of insulin. A novel action of TGF-beta on ovum maturation was observed in oocytes of immature gonadotropin-secreted rats. The growth factor accelerated the maturation of both follicle-enclosed oocytes and cumulus-oocyte complexes, with significant increases in the rate of germinal vesicle breakdown. This effect of TGF-beta was manifested with unusual rapidity, being detectable after one hour, and required the presence of the surrounding cumulus cells. Other growth factors including IGF-I, IGF-II, and EGF also stimulated germinal vesicle breakdown. These actions of growth factors, in conjunction with the effects of gonadotropic hormones, may regulate the meiotic maturation of oocytes during follicle development. Studies on the actions of gonadotropins on granulosa cell function revealed that FSH regulates the biosynthesis of a cell-associated tissue plasminogen activator. The enzyme was produced during the initial hours of granulosa cell maturation and was localized in the extracellular matrix laid down by the cells, suggesting that its presence in the basement membrane could be an important factor in the acquisition of the epithelial phenotype by granulosa cells during differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00184-10 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Pituitary Hormone Secretion

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

PI:	K. J. Catt	Head	ERRB, NICHD
Others:	G. Aguilera	Head, SEP	ERRB, NICHD
	S.-I. Izumi	Visiting Fellow	ERRB, NICHD
	S. Stojilkovic	Guest Researcher	ERRB, NICHD
	M. Virmani	Guest Researcher	ERRB, NICHD
	J. Chang	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The hypothalamic control of reproductive function is expressed through the actions of GnRH on gonadotropin secretion after binding to high-affinity receptors in the plasma membrane of pituitary gonadotrophs. The mechanism of cellular activation by GnRH involves the integrated actions of several messenger systems, including phosphoinositide breakdown and mobilization of intracellular and extracellular calcium. GnRH stimulates the hydrolysis of phosphatidylinositol bisphosphate to diacylglycerol and inositol trisphosphate (InsP3). The role of diacylglycerol formation and activation of protein kinase C in gonadotropin secretion was indicated by the impaired action of GnRH in pituitary cells depleted by kinase C by prolonged treatment with phorbol esters. An extracellular Ca2+-independent component of GnRH action was defined by studies on the effects of Ca2+ channel agonist and antagonist analogs on GnRH- and K+-induced LH secretion from pituitary cells in normal and calcium-depleted incubation medium. The initiation of the secretory response to GnRH was found to be largely independent of calcium entry, whereas the prolongation of gonadotropin secretion was maintained by calcium influx, in part through voltage-sensitive calcium channels. The role of arachidonic acid metabolites in GnRH action is probably related to the calcium-independent component of GnRH-induced LH secretion. Since GnRH is secreted episodically and for short periods, much of its physiological action on pulsatile gonadotropin release could be independent of calcium influx from the extracellular fluid. Further evidence for this mechanism was obtained by analysis of cytosolic calcium concentration ([Ca2+]i) during GnRH stimulation of enriched gonadotrophs, in which rapid peak increases of [Ca2+]i and LH release were followed by sustained elevations of both [Ca2+]i and hormone secretion. Whereas the rapid peak of [Ca2+]i was largely attributable to mobilization of Ca2+ from intracellular stores by InsP3, and showed little dependence on extracellular Ca2+, the sustained increase in [Ca2+]i and LH release were highly dependent on intracellular Ca2+ and are partly mediated by influx through dihydropyridine- and voltage-sensitive calcium channels. The regulation of calcium release in pituitary microsomes was shown to be mediated by high-affinity InsP3 binding sites that were characterized in pituitary membranes and serve as receptors through which InsP3 triggers Ca2+ mobilization in the pituitary gland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00187-09 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Hormonal Regulation of Cellular Metabolism

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

PI:	K.-P. Huang	Head	ERRB, NICHD
Others:	F. Huang	Expert	ERRB, NICHD
	H. Nakabayashi	Visiting Associate	ERRB, NICHD
	Y. Yoshida	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Section on Growth Factors, NICHD, NIH, (G. Guroff); Laboratory of Immunology, NIAID, NIH (W.E. Paul); Lab. of Chemical Pharmacology, NHLBI, NIH (M.A. Beaven); Lab. of Cell Biology, MH, NIH (W.S. Young); Lab. of Developmental & Molecular Immunity, NICHD, NIH (E. Hanna)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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.)*

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C, a Ca2+/phospholipid-dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those stimulated by many hormones and growth factors. Three isozymic forms of protein kinase C have been identified from rat and monkey brains. Polyclonal and monoclonal antibodies against these enzymes were prepared for their immunochemical characterization. These enzymes were found to have distinct tissue, cellular, and subcellular distributions and were differentially expressed during development. The type I protein kinase C, which is expressed only in the central nervous system, was synthesized most actively during synaptogenesis. The content of this enzyme was highest in hippocampus, amygdala, cerebellum, and cerebral cortex. In the cortical regions of the monkey brain visual information processing pathway, the type I protein kinase C was found to be high in regions important for memory formation, suggesting its possible role in mnemonic function. The role of each protein kinase C isozyme in cellular regulation was investigated by selecting mutant cell lines deficient in an isozyme. Several type II protein kinase C-deficient basophilic leukemia cell lines have been identified. These cell lines will be transfected with the type II protein kinase C gene to determine whether physiological responses are modified in the presence and absence of the isoenzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00190-06 ERRB

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

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Adrenocortical Zonation: Regulation of Steroidogenesis & Cholesterol Metabolism

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

PI:	C. A. Strott	Head	ERRB, NICHD
Others:	M. Kubo	Visiting Fellow	ERRB, NICHD
	T. Demura	Guest Researcher	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH: Endocrinology and Reproduction Research Branch

SECTION: Section on Adrenal Cell Biology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The steroidogenic action of ACTH can be separated into acute and chronic aspects. The acute ACTH response (sec-min) occurs primarily at the level of the mitochondria in regulating the rate-limiting step in steroidogenesis, the conversion of cholesterol to pregnenolone. The chronic action of ACTH (hours) occurs at the level of the genome and involves synthesis of various steroidogenic enzymes and co-factors. Both the acute and chronic actions of ACTH are dependent on the cytoplasmic synthesis of protein in that both responses are blocked by cycloheximide. In addition, both the acute and chronic actions of ACTH can be mimicked by cAMP. Based on mutation studies performed with an ACTH-responsive murine adrenocortical tumor cell line (Y1), as well as ACTH receptor studies involving various adrenocortical cell-types, it is now accepted that in the adrenal cortex ACTH stimulates membrane-bound adenylate cyclase activity which leads to an increase in intracellular cAMP and the activation of cAMP-dependent protein kinase followed by steroid synthesis. The role of other protein kinases in this process such as Ca2+-regulated kinases, if such a role exists, is not well understood. No adrenocortical regulatory phosphoprotein has yet been identified; and there is no evidence that the cholesterol side-chain cleavage P450 is regulated by phosphorylation-dephosphorylation. The guinea pig is used as an animal model to examine ACTH steroidogenic action for the simple reason that it is composed of an ACTH-responsive outer zone and an ACTH-unresponsive inner zone. In this model, adenylate cyclase activation and cAMP formation in response to ACTH are similar for the two zones suggesting that in the inner zone a defect has developed beyond the formation of cAMP. When cAMP-dependent protein kinase activity was measured it was found to be significantly less in the inner zone than in the outer zone. The meaning of this finding, however, is unclear particularly since the activities of Ca2+-regulated protein kinases were also significantly lower in the inner zone than in the outer zone. It has been suggested that calmodulin, a protein that mediates certain intracellular actions of Ca2+, may play an important role in ACTH-stimulated steroidogenesis. Thus, the calmodulin 'system' has been examined in this model. There is evidence for calmodulin kinase III activity and an endogenous substrate, elongation factor-2 (Mr 100,000). It is known that this latter system is hormonally regulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00191-04 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Mechanisms of Neuroendocrine Regulation

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

PI:	G. Aguilera	Head, SEP	ERRB, NICHD
Others:	K. J. Catt	Head, SHR	ERRB, NICHD
	P. Carvallo	Visiting Fellow	ERRB, NICHD
	M. A. Millan	Sr. Staff Fellow	ERRB, NICHD
	M. Flores	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

NIA, NIH (J. P. Harwood)
Dept. of Psychiatry, UCSD (R. L. Hauger)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

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

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

Investigation has focused on the properties and regulation of the corticotropin releasing factor receptors and the mechanism of interaction between CRF and other regulators of ACTH secretion.

A. CRF receptor properties: Gel electrophoresis analysis of detergent-solubilized CRF receptors crosslinked with 125I-Tyr-oCRF indicated that the receptor is a single protein with a molecular weight of 67 kDa. The characteristics of the CRF receptor are similar in the different target tissues, including anterior and intermediate lobes of the pituitary and the cortex, amygdala and olfactory bulb of the brain.

B. CRF receptor regulation: Previous studies have shown that pituitary CRF receptor down-regulation that accompanies the increase in plasma ACTH following adrenalectomy is dependent on hypothalamic factors, such as CRF and VP. Studies during stress showed transient increases in plasma ACTH following chronic immobilization. The subsequent decrease in plasma ACTH in the continuous presence of stress is accompanied by CRF receptor down-regulation and desensitization of the pituitary. However, pituitary responsiveness *in vivo* as well as the potentiating effect of VP on CRF action *in vitro* are maintained emphasizing the importance of the interaction between regulators during physiological control of ACTH secretion. CRF receptors in the intermediate pituitary and brain are unchanged during chronic immobilization stress.

C. Mechanism of action of ACTH regulators: Previous studies demonstrated that the synergistic effect of VP on CRF action involves potentiation of CRF-stimulated cAMP production suggesting that protein kinase C activation is part of the mechanism of action of VP. Studies in isolated pituitary cells showed that inhibition of endogenous protein kinase C abolishes the effects of VP in the corticotroph. In addition VP was shown to stimulate inositol phosphate formation and to induce translocation of protein kinase C from cytosol to the membrane compartment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00192-03 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Purification, Immunology and Functional Activity of Adrenocortical Steroid-binding Proteins

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

PI:	C. A. Strott	Head	ERRB, NICHD
Others:	Y. C. Lee	Snr. Staff Fellow	ERRB, NICHD
	W. J. Driscoll	IRTA	ERRB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Adrenal Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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 initial reaction of steroidogenesis is the cleavage of the cholesterol side chain by a specific cytochrome P-450 located on the inside face of the inner mitochondrial membrane. The resultant steroid, pregnenolone, is then metabolized by enzymes having extramitochondrial locations. Thus, pregnenolone must move out of the mitochondria, crossing the organelle's inner and outer membranes. Despite the significance of pregnenolone efflux from mitochondria, the process remains poorly characterized. This process is currently being investigated with the guinea pig adrenal cortex model. In this model there exists a specific pregnenolone-binding protein (PBP). Although PBP behaves as a Mr 58,000 globular protein on gel permeation chromatography, on SDS-PAGE it migrates as a Mr 34,000 protein. A polyclonal antibody has been generated against the 34 kDa protein. At all stages of purification, including the starting material, Western blot analysis of isoelectric focusing gels reveals the same pattern of apparent microheterogeneity with pIs of 6.8, 6.6, 6.4, 6.2. The major dilemma at the moment is to distinguish between microheterogeneity of a single protein and a co-purifying contaminant. Additional purification steps are under investigation, and an effort to generate an N-terminal sequence is also under way. Studies with the polyclonal antibody demonstrate that the PBP is present only in the soluble fraction of the adrenal cortex. Immunocytochemistry indicates that the PBP is most abundant in the outer adrenocortical zone (the guinea pig adrenal cortex can be divided into an ACTH-responsive outer zone and an ACTH-unresponsive inner zone). The latter finding is quite interesting for the pregnenolone-binding activity is far greater in the inner zone. It, thus, appears that there are active and inactive forms of the binding protein. The latter phenomenon is also under investigation. It is possible that the active/inactive forms and the microheterogeneity are related. Success with the N-terminal sequencing will resolve the problem of purity unless the PBP is composed of two non-identical subunits (it is probable that the PBP exists as dimer in its native form). Once the polyclonal antibody specificity has been ascertained, it will be used to isolate the PBP mRNA. The ultimate goal is to develop a cDNA clone for the PBP and determine the complete amino acid PBP sequence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00193-03 ERRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Angiotensin II Receptors and Activation Mechanisms

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

PI:	K. J. Catt	Head, SHR	ERRB, NICHD
Others:	G. Aguilera	Head, SEP	ERRB, NICHD
	A. Baukal	Biomedical Engineer	ERRB, NICHD
	M. Carson	IRTA Fellow	ERRB, NICHD
	K. Sandberg	IRTA Fellow	ERRB, NICHD
	T. Balla	Visiting Fellow	ERRB, NICHD
	L. Hunyadi	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Dept. of Physiology, Semmelweiss University Medical School, Budapest (A. Spat)
Contract for preparation of adrenal and pituitary cells ND1-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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 properties of angiotensin II (AII) receptors and their intracellular signalling pathways were studied in the adrenal glomerulosa cell and other target tissues. Purification of photolabeled AII receptors of the bovine adrenal gland was performed by detergent solubilization and fractionation by ion exchange, lectin-affinity, ligand-affinity, and immunoaffinity chromatography. The steroidogenic action of AII depends upon mobilization of intracellular calcium by the products of polyphosphoinositide turnover, and also on calcium entry through voltage-sensitive channels. A new method for the analysis of hormone receptors coupled to calcium mobilization was developed by the use of [3H]inositol-labeled *Xenopus* oocytes, in which AII receptors expressed from adrenal and pituitary mRNA were shown to be coupled to phosphoinositide hydrolysis. In conjunction with procedures being developed to measure receptor concentration and calcium mobilization in individual oocytes, this method will be applied to the screening of mRNA from cDNA expression libraries to isolate functional cDNA encoding receptors for calcium-mobilizing hormones including AII, vasopressin and GnRH. Direct evidence for coupling of the AII receptor to phospholipase C by a guanine nucleotide regulatory protein was obtained in permeabilized adrenal cells, in which InsP3 production was stimulated by guanine nucleotides and fluoride, as well as by AII. In adrenal glomerulosa cells, the conversion of Ins-1,4,5-P3 to Ins-1,3,4,5-P4 was shown to be catalyzed by a calcium-calmodulin dependent 3-kinase, and the identity of the new InsP4 formed from Ins-1,3,4-P3 was determined chemically to be Ins-1,3,4,6-P4. A further inositol tetrakisphosphate was identified in AII-stimulated cells and bears a precursor-product relationship with InsP5. The effects of lithium on inositol phosphate metabolism were shown to include inhibition of inositol-1,3,4-P3 breakdown to Ins-3,4-P2 as well as of Ins-4-P to inositol, and also marked attenuation of Ins-1,4,5-P3 production during prolonged incubation due to inhibition of phospholipase C-catalyzed breakdown of plasma-membrane polyphosphoinositides. The intracellular receptors which mediate the calcium-mobilizing action of InsP3 were found to co-purify with InsP3-sensitive calcium-releasing vesicles in the hepatic plasma membrane fraction, suggesting the existence of membrane-associated organelles from which InsP3 promotes calcium release into the cytoplasm during peptide hormone action.

HUMAN GENETICS BRANCH (HGB)

ZO1 HD 00131-14 Human Biochemical Genetics
William A. Gahl, M.D., Ph.D.

Z01 HD 00133-11 Study of Glycogen Storage Disease
James B. Sidbury, Jr., M.D.

Z01 HD 00403-07 Magnesium Metabolism in Mothers and Neonates
Joan L. Caddell, M.D.

Z01 HD 00404-06 Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases
Jean DeB. Butler, Ph.D.

Z01 HD 00408-05 Pathophysiology and Treatment of Human Genetic Diseases
Joan C. Marini, M.D., Ph.D.

Z01 HD 00410-03 Metabolism in Children with Glycogen Storage Disease, Type I
James B. Sidbury, M.D.

ZO1 HD 00412-01 Molecular Regulation of Gene Expression
Samuel Adeniyi-Jones, M.D., Ph.D.

Z01 HD 00909-09 Fetal Alcohol Syndrome
Anil B. Mukherjee, M.D., Ph.D.

Z01 HD 00910-09 Biochemistry, Molecular Biology, and Physiology of Phospholipase A_2 Inhibitory Proteins
Anil B. Mukherjee, M.D., Ph.D.

Z01 HD 00912-09 Gene Regulation and Cellular Differentiation
Janice Y. Chou, Ph.D.

HUMAN GENETICS BRANCH

William A. Gahl, M.D., Ph.D., Acting Chief

This Branch conducts research to elucidate the pathophysiology of human genetic and developmental disorders through an understanding of basic biological mechanisms. Clinical research projects include studies on the natural history, treatment, and methods of diagnosis of several heritable disorders of man. Basic research involves the use of in vitro systems to study aspects of cell biology with immediate or potential applications to human genetic diseases.

Human Biochemical Genetics

The Section on Human Biochemical Genetics, under the direction of William Gahl, studies a wide range of inborn errors of metabolism from both clinical and basic research aspects. Some emphasis is placed upon investigations into lysosomal storage.

There are two known storage diseases due to defective transport of small molecules out of lysosomes. The first is cystinosis, a multi-systemic disease of children resulting in kidney failure by age 10 years. Members of the Section care for 35 pre-renal transplant patients, offering the cystine-depleting agents cysteamine and phosphocysteamine as efficacious preventative therapy against impaired growth and renal function in these children. Cysteamine eyedrops are also being successsfully studied to reduce the number of corneal crystals in affected patients. Cysteamine and phosphocysteamine are offered to approximately 20 post-renal transplant patients in whom non-renal complications of longstanding cystinosis are being described. These include exocrine and endocrine pancreatic insufficiency and ophthalmic and renal involvement. One 22 year old patient suffered from a myopathy with muscle cystine crystals and represented the first documented example of clinical muscle impairment in cystinosis. Members of the Section also described the first pregnancy to a cystinosis patient with a normal delivery and a normal infant despite cystine crystals in the maternal portion of the placenta. In a different infant affected by the disease cystinosis, cysteamine therapy from two weeks of age attenuated the severity of the renal Fanconi syndrome, a tubular reabsorption defect, which is part of the clinical presentation of cystinosis. The same syndrome results in urinary loss of the nutrient carnitine, which is prescibed to our patients as replacement therapy. Blood carnitine levels are rapidly restored to normal by this treatment.

The second known lysosomal transport disorder is Salla disease, a Finnish disorder in which free sialic acid is stored in cellular lysosomes. In 1986, members of the Section demonstrated that the basic defect of Salla disease (and the more severe variant, infantile free sialic acid storage disease) is impaired transport of free sialic acid out of the lysosomes. This resulted in the referral of several cell strains from patients with other disorders of sialic acid metabolism, and these defects are being aggressively investigated. In particular, two cell strains store free sialic acid in their cytosol, not their lysosomes.

In a basic research pursuit involving lysosomal transport of small molecules, members of the Section have described a carrier-mediated transport system for monoiodotyrosine (MIT) in rat thyroid cells in culture. MIT, a product of thyroglobulin hydolysis in lysosomes, is a small molecule whose iodine needs to be salvaged by the cell for reincorporation into thyroglobulin and subsequent production of thyroid hormone. This iodine reutilization takes place in the cytosol and the newly discovered

lysosomal transport system for MIT explains how MIT's iodine travels from lysosome to cytosol for salvage. An impairment of this system in humans may result in iodine or thyroxine responsive hypothyroidism.

The Section has also recently become involved in investigating the cause of new lysosomal storage diseases. We have collected several fibroblast strains from patients with an unidentified storage disorder. Lysosomes are being isolated from fibroblasts and their contents analyzed for amino acids by ion exchange chromatography, sugars by pulsed amperometric detection and lipids by thin layer chromatography. Later, liquid chromatography-mass spectrometry and electron probe analysis will be applied to the analysis.

A clinical protocol for the study of Lowe (oculocerebrorenal) syndrome has been established. The renal, ophthalmic, neurological, and joint manifestations of the disease are studied, and optimal treatment modalities are forthcoming. One of the Section's patients has been found to have central demyelination and peripheral neuropathy and other patients are being examined for these complications. Involvement of heterozygote mothers in the X-linked disease is also under investigation.

In a recently completed clinical study, oral betaine therapy was shown not to improve trabecular bone density in patients with pyridoxine-nonresponsive homocystinuria. However, the double-blind, placebo-controlled crossover study did demonstrate that quantitative computerized tomography will detect the reduced bone density in homocystinuria before standard radiographs.

The continued investigation of sulfur metabolism in a man with methionine adenosyltransferase deficiency has been extremely illuminating. Sulfur and methyl balance studies in this individual demonstrated that, in vivo, S-adenosylmethionine regulates the partitioning of homocysteine between degradation to inorganic sulfate and remethylation to methionine. In addition, transamination was shown to provide an active though minor pathway for methionine metabolism in the human.

A 2 year old boy was diagnosed by workers in the Section as having a hepatic copper storage disease which, by clinical, histological, and biochemical criteria resembles Indian Childhood Cirrhosis. The boy's fibroblasts are now being studied in attempts to determine the cell biological causes of copper storage in this disease and to discover how cells handle copper in general.

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

In this separate project, Jean Butler, of the Section on Human Biochemical Genetics, studies the lysosomal storage of cholesterol in mutant cells, in collaboration with other NIH scientists. The exact causes of two lysosomal storage disorders, Niemann-Pick Types C and D, remain unknown. It has now been demonstrated that the fibroblasts from the Niemann-Pick Type C patients store cholesterol in their lysosomes and fail to esterify the cholesterol. Niemann-Pick Type D fibroblasts also fail to esterify cholesterol, but do not manifest lysosomal storage of cholesterol. These discoveries are expected to help elucidate the biochemical defects in these disorders, and to reveal new aspects of cellular cholesterol metabolism.

Glycogen Storage Disease

Adjunct Scientist James Sidbury has continued to care for patients with glycogen storage disease and to study the disorder at the bench. He has demonstrated the variability in the ability of affected children to hydrolyze a standard load of starch. Among several types of starch tested, corn starch remains the starch hydrolyzed in the most consistent fashion, and its long-term use in maintaining blood glucose concentrations in patients with glycogen storage diseases is now being studied. To date, the metabolic regulation that accompanies corn starch therapy has not resulted in a reduction in the size or frequency of the hepatomas which characterize type 1 glycogen storage disease in post pubertal patients.

An extremely important finding of the past year has been the discovery that 80% of post-pubescent patients with type 1 glycogen storage disease have glomerulosclerosis. This natural history information has important prognostic and therapeutic implications, among them the fact that renal disease should serve as a measure of the efficacy of dietary and pharmacological interventions.

Another active pursuit involves determination of the rate of maximum glucose production in patients with hepatic glycogenosis. Stable isotope studies have revealed a rate of 2 gm/Kg body weight, although these results may not apply for very young children.

Magnesium Metabolism in Mothers and Neonates

Adjunct Scientist Joan Caddell studies the audiogenic seizure-shock episode in weanling rats with acute magnesium deficiency as a model system for Sudden Infant Death Syndrome (SIDS). The rats show capillary aggregates of platelets, leukocytes, erythrocytes and lymphocytes in the lung and heart. The seizure-shock episodes could be almost completely aborted by administration of a thromboxane A2 receptor antagonist. Magnesium deficiency is associated with the release of thromboxane A2 which causes platelet aggregation. Work on this model system may broaden our knowledge of the physiological consequences of magnesium deficiency in animals and man.

Pathophysiology and Treatment of Human Bone Disease

This group, headed by Joan Marini of the Section on Molecular Biology, studies osteogenesis imperfecta (OI) and related bone diseases from molecular, biochemical and clinical perspectives.

On the molecular level, the group has detected 5 point mutations in Type I collagen mRNA by developing a system for identifying mismatches in RNA/RNA hybrids using RNase A digestion. The point mutations are in patients with Types II, III, and IV OI. Two Type IV mutations have been well localized using three overlapping probes near the 3' end of the alpha 1(I) cDNA. Sequencing of both alpha 1(I) alleles of one patient is being pursued using a cDNA library.

On the biochemical level, overmodification of Type I collagen chains in Types II and IV OI has been characterized using cyanogen bromide fragmentation of the chains in fibroblasts in chorionic villi. This approach, as well as investigation into proteoglycan and osteonectin production, is also being pursued in osteoblasts cultured in collaboration with Dr. Pamela Robey of the National Institute of Dental Research.

Clinically, chorionic villus sampling is employed for the prenatal diagnosis of Types II and IV OI by examining the collagen for defects. In studies of the endocrine parameters responsible for growth failure in OI, nine of eighteen patients studied displayed a neurosecretory deficiency of growth hormone. Five children with OI and poor growth are being treated with either clonidine or growth hormone itself; four have manifested improved growth. A lower limb bracing program for OI patients continues to provide benefits to selected young children in terms of support and ambulation.

The group also studies the treatment of fibrodysplasia ossificans progressiva, a disorder involving atopic bone formation, with the retinoid Accutane. They report a toxic effect of the drug on bone growth associated with the ability of retinoids to de-differentiate chondrocytes. Surgical treatment of a jaw calcification has also been performed on a patient receiving Accutane and didronal. His clinical progress is being followed, and his tissue is now in culture for examination of its growth factor content, in particular EGF and TGF-b.

Molecular Regulation of Gene Expression

Samuel Adeniyi-Jones, a member of the Section on Molecular Biology, continues to study the role of short repeated sequences (Alu-sequences) in the regulation of gene expression. His group has reported the identification of 63 Kd protein in Xenopus oocytes which binds primary and processed transcripts of injected repeated sequence genes in both the nucleus and cytoplasm. Antibody to the protein inhibits the Alu gene expression, and the protein appears conserved in evolution. Its specific role in Alu gene expression is under continued investigation.

Biochemistry, Molecular Biology, and Physiology of Phospholipase A_2 Inhibitory Proteins

The Section on Developmental Genetics, under Anil Mukherjee, conducts both basic and clinical research on the mechanism(s) of action and genetic regulation of endogenous steroid induced antiinflammatory proteins.

A fundamental question in biology is how an organism protects its epithelial lining from the external environment. Organs such as the tracheobronchial, gastrointestinal and genitourinary tracts come into contact with myriads of foreign antigens and yet, as a rule, do not respond with an inflammatory/immunological response. The mucosal epithelia of these organs secrete antimicrobial and antiinflammatory substances to modulate uncontrolled inflammatory/immunological reactions. Recently, Zasloff and others have described an antimicrobial defense system in vertebrates like Xenopus. However, the presence of an antimicrobial activity does not fully explain why there is an absence of inflammation, since dead microbes can still be antigenic to a host. For the past ten years we have suggested that small molecular weight proteins such as uteroglobin (UG) in the rabbit may be responsible for the modulation of an inflammatory response in the mucosal epithelium in mammals. Several workers have demonstrated, in mammalian species other than the rabbit, that proteins similar to uteroglobin also serve as modulators of the immune system. Therefore, we compared the structures and amino acid sequences of three proteins, human lipocortin, rat seminal vesicular protein (RSV IV) and rabbit uteroglobin. A consensus sequence, a nonapeptide, was discovered; the synthetic nonapeptide proved a potent inhibitor of phospholipase A_2 (PLA_2) enzyme activity in vitro and an extremely potent antiinflammatory agent in vivo. In fact, the nonpeptide was more potent than indomethacin, ibuprofen and even dexamethasone in certain instances. We also discovered that the N-terminal fragments of Xenopus derived antimicrobial peptides

from an amphipathic structure similar to the peptides derived from uteroglobin and/or lipocortin-1 and are potent PLA_2 inhibitors in vitro and antiinflammatory agents in vivo. Thus, in Xenopus, the antimicrobial peptides degrade to produce antiinflammatory peptides. This explains the observation of Zasloff that the Xenopus wet epithelium neither gets infected nor inflamed even if the organism is allowed to live in a contaminated environment after surgery.

Because of the potential scientific, therapeutic, and pharmaceutical importance of these agents a patent application has been filed with the U.S. Patent and Trademark Office for these compounds.

To delineate the physiological role of endogenous antiinflammatory agents large quantities of this protein are required. An inexpensive way to obtain this protein would be to use recombinant DNA technology to express uteroglobin in a bacterial host. Furthermore, expression of the UG gene in E. coli will allow site-directed mutagenesis studies to ascertain the function of this protein. Last year we reported the construction of a plasmid cloning vector system for this purpose. Transformation of E. coli with those plasmids and induction with IPTG yielded UG but to a much lower level than required for preparative purposes. Therefore, this year reconstruction of this cloning vector was undertaken where the "trc" promoter controlling the transcription of the UG structural gene in the previous vector (pLE 101) is now replaced with a 89-bp DNA fragment controlling the "10" promoter of phage T_7 and the translational start signal of the major T_7 capsid protein. The resulting plasmid vector (pLE 103-1) was used to transform bacterial host BL21(DE3). These transformed bacterial cultures when induced with IPTG expressed 20-30 μg of UG per milliliter of bacterial culture. This high level of expression is adequate for preparative purposes. The recombinant UG thus produced appears to be a dimer as secreted naturally by the endometrial cells of the rabbit when stimulated with progesterone. To our knowledge, this is the first demonstration that a protein with a quarternary structure such a UG can be produced in a bacterial host in its natural form. The successful development of this expression system paves the way for future site-directed mutagenesis studies on this protein.

Because this novel plasmid cloning vector has the potential to express complex proteins such as the antibodies and other medically important substances, an invention disclosure has been filed with the NIH Patent Office for possible U.S. and International patents.

Using cocrystallographic techniques in collaboration with Drs. Keith Ward and Virginia Pett at the Naval Research Laboratories attempts are being made to study the possible interaction of the antiflammin peptides with the active site of PLA_2. Preliminary studies using fluorescence of PLA_2 active-site-tryptophan indicates that the peptides indeed interact with the active site tryptophan of PLA_2. Confirmation of this and other observations will delineate the mechanism of action of these antiinflammatory peptides.

Other studies on transformed cell lines from the endometrium and tracheobronchial epithelium are continuing. Two cell lines (RBE-7 and H5DC) have been fully characterized and found to secrete UG in vitro upon stimulation with progesterone. These cell lines provide a model system for investigating regulation of steroid action in vitro without the use of animal models; they provide a means to assess the biological potency of synthetic progestins and progestogens in vitro. There is currently no in vitro system available to determine the biological properties of a steroid hormone at this time. A U.S. patent is pending on these cell lines.

Recently, it has been proposed that patients with cystic fibrosis have impaired arachidonate metabolism. The resultant increased arachidonate levels would increase tissue levels of eicosanoids (some of which are proinflammatory), perhaps leading to the profound inflammation observed in CF tracheobronchial epithelium and elsewhere. A preliminary collaborative study suggests a lack of UG-like immunoreactivity in cystic fibrosis epithelial cells compared with normal controls; this finding might explain the increased inflammation in CF. Using a UG cDNA probe a human lung and prostatic expression library is now being screened for UG-like genes in humans.

Fetal Alcohol Syndrome

The Section on Developmental Genetics is also studying the genetic factors predisposing to the development of fetal toxicity due to ethanol. During the past year members of the Section have studied the rate of survival in thiamine deficient medium of cultured amniotic fluid cells derived from 10 pregnancies resulting in the birth of FAS babies and compared them to 12 normal control cell lines. These ongoing studies will delineate whether or not the high transketolase K_m for thiamine pyrophosphate, observed previously in some of these cell lines, make them more sensitive to thiamine deficiency. In a clinical protocol one additional patient, who will be admitted to the ward this year for further studies, was recruited.

Cellular Differentiation

The Section on Cellular Differentiation, led by Janice Chou, conducts research to understand the regulation of gene expression during normal and abnormal differentiation processes. Several problems in gene regulation are emphasized: expression of the α-fetoprotein (AFP) gene in liver; establishment and maintenance of functional liver cells in vitro, and cloning and expression of the human pregnancy-specific β_1-glycoprotein (PSβG) gene.

Over the past several years, this group has studied expression of the AFP gene in fetal and adult rat livers. They found that the adult rat liver contains three AFP mRNAs of 2.2 (minor), 1.7 and 1.5 kb. These transcripts share a common 3' sequence, but the 1.7- and 1.5-kb AFP mRNAs lack sequences present in the first seven 5' exons of the 2.2-kb AFP mRNA. S1 nuclease analysis maps the 1.7-kb mRNA at the 5' boundary of the eighth exon of the 2.2-kb AFP mRNA and the 1.5-kb mRNA in the middle of the eighth exon. A cDNA clone (ARFP5) encoding the 1.7-kb RNA has been isolated from an adult rat liver cDNA library. The 90-bp 5' sequence of ARFP5 is not present in the 2.2-kb fetal AFP mRNA, although ARFP5 does contain nucleotide sequence present in the 2.2-kb AFP mRNA extending from the beginning of its eighth exon (nucleotide 873) to the 3' end. The 1.7-kb AFP mRNA found in adult liver is indistinguishable from a variant AFP mRNA identified by Chou and coworkers in a fetal liver cell line. However, the 1.7-kb RNA could not be reliably identified in fetal rat liver. The developmental profile of these AFP transcripts shows that fetal rat liver contains mainly the 2.2-kb mRNA which decreases to a very low level around the fifth week after birth. The 1.7- and 1.5-kb AFP mRNAs can be visualized about the 3rd week after birth and the levels of these mRNAs increase to about 0.01% of the AFP mRNA level in 18-day-old fetal liver by the 5th week after birth. These two RNAs are the major AFP mRNAs in adult rat liver. Both the 1.7- and 1.5-kb AFP mRNAs are translationally active; they direct the cell-free synthesis of two polypeptides of 50 and 44K.

This group has examined factors required to promote liver differentiation in vitro using primary fetal rat hepatocytes as a model system. They found that in the absence of effectors, primary fetal hepatocytes dedifferentiated. Cells maintained in the presence of glucocorticoid hormone or cAMP produced high levels of albumin and transferrin or albumin and AFP, respectively. Both glucocorticoid and cAMP induced expression of adult liver-specific genes, suggesting that these fetal hepatocytes have matured. This study demonstrated that both glucocorticoid hormone and cAMP are necessary for optimal differentiation of fetal hepatocytes in vitro.

As an initial step towards a better understanding of the functions of human pregnancy-specific β_1-glycoprotein (PSβG), the Section has isolated and characterized four cDNA clones (PSG16, PSG93, PSG95, and PSG9) encoding human PSβG. PSG16 (1.9-kb), PSG93 (2.1-kb), and PSG95 (2.2-kb) encode three polypeptides of 417, 419, and 426 amino acids with apparent molecular masses of 46.9, 47.2, and 47.8K, respectively; these three PSβG species diverge only at the 3' end of the coding regions (after amino acid 414). PSG9 (1.6-kb) encodes a polypeptide of 326 amino acids with an apparent molecular mass of 36.4K. In placenta, four nonglycosylated polypeptides of 50, 48, 46, and 36K have been identified by in vitro translation of placental poly(A)$^+$ RNA. The apparent molecular masses of the native placental PSβGs are glycoproteins of 72 (major), 64, and 54K. Three PSβG mRNAs of 2.3, 2.2 and 1.7 kb can be detected by the four cDNAs identified. Thus, PSβG is heterogeneous at both mRNA and protein levels.

This group has found that the amino acid sequences of PSβG as deduced from the cDNA sequences of PSG16, PSG93, and PSG95 contain two repeated protein domains (1a and 2a) of 93 amino acids each and a 1b domain. PSβG species encoded by PSG9 contains the 1a and 1b domains. PSβG shows strong homology to human carcinoembryonic antigen (CEA) at both nucleotide and amino acid levels. CEA contains eight domains including a N-terminal domain, three repeated domains (I, II, and III) each containing two subdomains (A and B), and a hydrophobic carboxyl-terminal domain. PSβG contains a CEA-like N-terminal domain, one or two repeated domains (a) similar to the A subdomains of CEA which is followed by a domain (1b) similar to the B subdomains of CEA, but lacks a hydrophobic carboxyl-terminal domain. The positions of the cysteine residues in each domain are also conserved, indicating that PSβG and CEA are two members of the same gene family. The similarity between CEA and PSβG suggests that both proteins may play similar roles in growth control in development and differentiation.

An immunoreactive PSβG-like molecule has also been detected in human nonpregnant serum and fibroblast cultures, thus raising the question of the functional role of PSβG in pregnancy. This Section has demonstrated that although placental fibroblasts produced all three PSβG mRNAs of 2.3, 2.2, and 1.7 kb, the major PSβG molecule synthesized is a 62K variant PSβG, while the major placental species is 72K. Since cellular functions persisting in vitro are functions essential for growth, the difference in the major PSβG species between placenta and fibroblasts may be due to different functions of the various PSβGs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00131-14 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Human Biochemical Genetics

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

PI:	William A. Gahl	Medical Officer	HGB, NICHD
Others:	Isa Bernardini	Chemist	HGB, NICHD
	Martin Renlund	Visiting Scientist	HGB, NICHD
	Megan Adamson	NRSA Fellow	HGB, NICHD
	Hans Andersson	IRTA Fellow	HGB, NICHD
	Raili Seppala	Visiting Associate	HGB, NICHD

COOPERATING UNITS *(if any)*

See Attached

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Human Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	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.)*

1.) Thirty-five children with cystinosis pre-renal transplant contribute data to a national protocol aimed at determining whether high dose cysteamine/phosphocysteamine is preferable to standard dose therapy. Cysteamine eyedrops (0.5%) are being used to dissolve corneal crystals in children over 2 years of age. Late complications of cystinosis are described, including exocrine and endocrine pancreatic insufficiency, myopathy, and ophthalmic and neurological involvement. One infant developed renal Fanconi syndrome despite cysteamine therapy from 14 days of age, and one cystinotic woman gave birth to a normal boy despite cystine crystals in her placenta. Carnitine therapy for patients with Fanconi syndrome continues to be pursued.

2.) Sialic acid transport across the lysosomal membrane was shown to be defective not only in Salla disease but also in infantile free sialic acid storage disease fibroblasts. Free sialic acid was shown to be filtered but not reabsorbed by the human kidney.

3.) Central demyelination and peripheral neuropathy were described in oculocerebrorenal syndrome of Lowe. It was found that heterozygotes can have nervous system involvement. A protocol was established to study the clinical and biochemical aspects of this X-linked disease.

4.) The lysosomal transport system for tyrosine and other neutral amino acids, discovered in rat FRTL-5 thyroid cell lysosomes, was shown to be TSH-responsive. So was a lysosomal transport system for monoiodotyrosine (MIT). The existence of this carrier, which may be identical to the tyrosine carrier, explains how thyroid cells can salvage thyroglobulin's iodine for reutilization.

5.) Sulfur and methyl balance studies on an MAT-deficient patient demonstrated that, in vivo, S-adenosylmethionine regulates the partitioning of homocysteine between degradation to inorganic sulfate and remethylation to methionine. Betaine therapy was shown not to improve bone density in pyridoxine-nonresponsive homocystinuria.

6.) A 2-year old boy with hepatic copper storage and aggregates in his fibroblasts helped demonstrate that Indian Childhood Cirrhosis is a genetic disease.

7.) Fibroblasts from patients with unknown lysosomal storage diseases are being screened to identify the stored material.

Cooperating Units:

F. Tietze, NIDDK
S. Mudd, NIMH
J. Schneider, University of California at San Diego
J. Thoene, University of Michigan
G. Thomas, Johns Hopkins University
W. Rizzo, Medical College of Virginia
M. Kaiser-Kupfer, NEI
H. Levy, Massachusetts General Hospital
J. Schulman, IVF Institute, Fairfax, Virginia
J. Hoofnagle, NIDDK
P. Fox, NIDR
B. Baum, NIDR
V. Hascall, NIDR
M. Dalakas, NINCDS
J. Finkelstein, VA Hospital, Washington, D.C.
B. Fivush, Johns Hopkins Medical Center
C. Porter, George Washington University Medical Center
R. Chesney, University of Tennessee, Memphis
G. Merriam, NICHD
A. Tangerman, Nijmegen, The Netherlands
J. Fink, NINCDS
L. Kohn, NIDDK
E. Grollman, NIDDK
G. Reed, NICHD
J. Balfe, Toronto
S. O'Regan, Montreal
K. Ishak, AFIP
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T. Kuwabara, NEI
J. Hoeg, NHLBI
Z. Goodman, AFIP
J. Olson, Johns Hopkins Hospital
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R. Reiss, Ohio State University at Columbus
P. Ozand, King Faisal Hospital, Saudi Arabia
A. Yergey, NICHD
T. Chen, St. Agnes Hospital, Fresno, CA
L. Charnas, NICHD
G. Harper, Biomedicinska Centrum, Uppsala, Sweden
J. Hopwood, Adelaide Children's Hospital, Australia
K. Horvath, Clinical Center, NIH
C. Oliver, NIDR
V. Chaudhry, Clinical Center, NIH
B. Sonies, Clinical Center, NIH
L. Racussan, Johns Hopkins Hospital
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R. Leapman, Div. of Research Services, NIH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00133-11 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Study of Glycogen Storage Disease

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

P.I.: James B. Sidbury Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*

Pamela Brye (RD, CC)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The study is designed to evaluate the immediate physiological response of patients with glycogen storage disease to different types of raw starches. With time the emphasis has shifted to the potential role of cornstarch therapy in preventing long-term complication.

The attempt to demonstrate a correlation with the response to starch loading in vivo and the splitting of the various starches by the analyses in serum was disappointing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00403-07 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Magnesium Metabolism in Mothers and Neonates

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

P.I.: Joan L. Caddell Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*

Joan Blanchette-Mackie (LCDB, NIADDK); George Reed (PRP, NICHD); Michael A. Kaliner (LCI, NIAID); Don Harris (Squibb Institute for Medical Research)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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.)*

Studies on the audiogenic seizure-shock episode in weanling rats with acute magnesium deficiency have been directed toward the pulmonary and cardiac ultrastructural changes which have revealed aggregates of platelets, leukocytes, erythrocytes and lymphocytes in the capillaries, with occasional reticulocytes.

Increased release of thromboxane A2 (TxA2), a platelet aggregator, is associated with magnesium deficiency. A specific TxA2 receptor antagonist at sufficiently high doses almost completely aborted the seizure-shock episode in rats, and the animals (and their lungs) were normal.

Histamine was ruled out as a contributing factor in the seizure-shock episode. A histamine antagonist did not abort the seizure-shock episode, and although plasma histamine from unstressed Mg deficient rats was elevated over control levels, it did not further increase during seizure-shock. The kidney in furosemide-treated Mg deficient weanling rat have shown high levels of calcium. The kidney was studied in adult rats' treated with furosemide (Lasix). Marked renal tubular calcification was demonstrated biochemically and histologically in moderately deficient rats (10 mg magnesium/100 g). Rats fed the National Research Council's Recommended 40 mg/100 g diet showed sporadic elevations of renal calcium. The calciuric effect of furosemide was blocked at higher levels of dietary magnesium, with normal renal calcium levels.

The parenteral Mg load test provides and evaluation of the Mg stores; we evaluated the relationship between parenteral Mg load retention to the young adult rat's dietary, plasma. and bone magnesium. The relationship between the logarithm of percent retention and plasma or femur magnesium level was approximated by a decreasing straight line. Plasma and femur concentrations of magnesium varied linearly. This is a model for the magnesium retention test for young adult humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00404-06 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

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

PI: Jean DeBrohun Butler Senior Investigator HGB, NICHD

COOPERATING UNITS *(if any)*

P. Pentchev (NINCDS); S. Padilla (EPA)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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

1. Continued studies of mutant mouse which stores cystine in lysosomes as do cystinotic patients; anamolies in cholesterol metabolism uncovered similar to:

   a. Niemann-Pick C cells which show lysosomal storage of cholesterol and lack of intracellular cholesterol esterification.

   b. Niemann-Pick D cells which do not store cholesterol but do show a lack of cholesterol esterification.

2. Studies of cholesterol metabolism and transport in Niemann-Pick C and D fibroblasts.

3. Characterization of cystinotic cell metallothionein present in a 2-fold excess in cystinotic versus normal fibroblasts.

4. Investigation of metabolism of ascorbic acid in cystinotic fibroblasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00408-05 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Pathophysiology and Treatment of Human Genetic Diseases

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

P.I.	Joan C. Marini	Senior Staff Fellow	HGB, NICHD
Others:	Dorothy K. Grange	Medical Staff Fellow	HGB, NICHD
	Gary S. Gottesman	Adjunct Scientist	HGB, NICHD
	Mary Beth Lewis	Stay-in School	HGB, NICHD

COOPERATING UNITS *(if any)*

Pamela G. Robey, (BMB, NID); Naomi L. Gerber, (CC); George Chrousos, (DEB, NICHD)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.5	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.)*

We have continued studies to elucidate the molecular basis of heritable connective tissue disorders, and to apply this information to treatment of the disease. We have developed a system for the detection of point mutations in Type I collagen mRNA by RNase A digestion of mismatches in RNA/RNA hybrids. Anti-sense riboprobe is hybridized to the mRNA of the patient. This system allows for more rapid detection and more accurate localization of mutations than had been possible with the collagen protein system. Several mutations have been localized in patients with Osteogenesis Imperfecta and are now being sequenced.

Continued work on the collagen protein of fibroblasts and osteoblasts of Osteogenesis Imperfecta patients has allowed the delineation of the extent and direction of overmodification. The effect on the osteoblast metabolism of non-collagen matrix proteins has revealed some abnormalities. We have demonstrated that chorionic villi express the same collagen defect as is expressed by the fibroblasts of OI patients; this will allow earlier prenatal diagnosis in selected cases.

In clinical protocols on Osteogenesis Imperfecta, we have demonstrated abnormalities of growth hormone secretion and IGF-I stimulation associated with short stature. A pilot study of growth stimulation was encouraging. We have continued our rehabilitation and bracing protocol for children with moderately severe OI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00410-03 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Metabolism in Children with Glycogen Storage Disease, Type I

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

P.I.: James B. Sidbury Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*

N. Esteban (LTPB, NICHD); A.L. Yergey (LTPB, NICHD)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The study is designed to determine the rate of glucose production by the liver in individuals with hepatic glycogenosis. There does not appear to be a significant difference in glucose production by the liver in several different types of hepatic glycogenosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00412-01 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Molecular Regulation of Gene Expression

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

P.I.:	Samuel Adeniyi-Jones	Visiting Scientist	HGB, NICHD
Others:	Richard Maraia	Medical Staff Fellow	HGB, NICHD
	Susan Adeniyi-Jones	Adjunct Scientist	HGB, NICHD

COOPERATING UNITS *(if any)*

Steve Josephs (LTCB, NCI); Mary Klotman (LTCB, NCI); Alan Wolfe (NICHD); Beverly White (NIADDK)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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

Work has continued on the expression of alu-like genes and their role in the regulation of gene expression. A lot of our previous work has suggested that the expression of alu sequences is significant during development. We have therefore developed several model systems for studying their importance during development. We have employed both the Xenopus laevis and mouse system for this study - using both Xenopus and early mouse embryos and the F9 teratocarcinoma cell - line to study this. In addition, we are studying the role of these sequences in muscle development, a tissue which has shown a considerable level of expression of the alu-binding 63K protein. We have partially purified the protein and the role in the transcription and further expression of alu gene transcription is being pursued vigorously.

The Xenopus oocyte which was traditionally used to study the expression of several genes has proved to be a big bonus for the study of the regulation of HIV genes. Work done in collaboration with Steve Josephs in Dr. Gallo's lab has for the first time given concrete proof of translational regulation by the HIV tat gene. In addition, we have been able within this system to demonstrate the presence of a cellular tat like factor which appears important in the expression of HIV genes in this system. The study of this cellular system affords a completely new strategy for interfering with the expression of the AIDS virus.

The transactivation of polymerase III genes by both the Bovine papilloma E2 gene and the HIV tat gene is also a likely candidate in the regulation of expression of these viruses. Our continued investigation of the novel area of gene expression will shed some light on the interaction. We have also begun work in collaboration with Dr. Beverly White on the study of several enzymes related to the expression of the fragile X phenotype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00909-09 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Fetal Alcohol Syndrome

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

PI:	Anil B. Mukherjee	Head	HGB, NICHD
Others:	Sondra W. Levin	Adjunct Scientist	HGB, NICHD
	MoonJohn Kim	Stay-in-School	HGB, NICHD

COOPERATING UNITS *(if any)*

M. Evans (Wayne State University, Detroit, MI); B. Cowan (University of Mississippi, Jackson, MS); P. Martin (Vanderbilt University, Nashville, TN)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

The search for genetic predisposing factors in developing fetal toxicity of ethanol is continued. Last year we admitted one patient at the Clinical Center with the diagnosis of Fetal Alcohol Syndrome (FAS) under the clinical protocol 83-CH-228. We have continued the recruitment process for FAS patients for this clinical protocol. Recently, a fourth patient with the diagnosis of FAS has been referred from the Kennedy Institute in Baltimore who will be admitted this year for further studies. During the past year we have studied the rate of survival of amniotic fluid cells, derived from pregnancies which yielded FAS babies, in thiamine deficient medium as compared to amniotic fluid cells obtained from normal pregnancies under the same conditions. These studies were conducted in order to delineate whether or not there is any difference in survival between the FAS and normal amniocytes when cultured in thiamine deficient medium. If there is a difference in survival in this medium then the cells will be tested for their transketolase Km for TPP. An assumption was made that cells with a high Km for TPP will be more sensitive to thiamine deficiency states. The preliminary data indicate that amniotic cells derived from FAS pregnancies which were grown in thiamine-deficient medium have a mortality rate of 90% compared to 5% in control cells grown in the same medium for ten days. Since all cells were grown to confluence in thiamine enriched medium there was no difference in the thiamine level in these cells at the beginning of the experiment. Thus, the observed differences seem to be real rather than an artifact of culture conditions. These results may suggest an increased susceptibility to thiamine deficiency among the FAS amniocytes compared to normal controls. Ongoing studies will attempt to ascertain if the FAS amniocytes have a higher Km for TPP for transketolase compared to normal amniocytes in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00910-09 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Biochemistry, Molecular Biology and Physiology of Phospholipase A2 Inhibitory Proteins

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

P.I.:	Anil B. Mukherjee	Head	HGB, NICHD
Others:	Lucio Miele	Visiting Fellow	HGB, NICHD
	Antonio Facchiano	Visiting Fellow	HGB, NICHD
	Lalita Murty	Biologist	HGB, NICHD
	Elenora Cordella-Miele	Adjunct Scientist	HGB, NICHD

COOPERATING UNITS *(if any)* M. Manyak (NCI), B. Cowan (University of Mississippi), H. Zacur (Johns Hopkins), N. Dubin (Johns Hopkins), R. Dhanireddy (Georgetown Univ), P.L. Ogra (SUNY at Buffalo), B. Wallner (Biogen), J. Carlstedt-Duke (Karolinksa Institute, Sweden), E. Schiffman (NCI), V. Pett (Naval Research Lab), K. W. (Naval Research Lab), R. Feldman (DCRT).

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

NICHD/ NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.25	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.)*

We have synthesized oligopeptides corresponding to a region of high amino acid sequence similarity between two phospholipase A2 (PLA2) inhibitory proteins: Uteroglobin(UG) and lipocortin-1(lip-1). These novel peptides (antiflammins) are potent PLA2 inhibitors in vitro and very potent antiinflammatory agents in vivo suggesting possible therapeutic applications. Additionally, these peptides inhibit collagen and thrombin induced platelet aggregation at micromolar concentrations. The mechanism of action of these oligopeptides seems to be by interaction with the active site of the PLA2 enzyme. In order to obtain UG at a preparative scale for further studies we reconstructed the plasmid cloning vector pLE-101 and pLE-102 (described last year) so that we can obtain higher level of expression than was possible by using the above plasmids. This has been achieved and the new plasmid pLE 103-1, when introduced into a bacterial strain BL21 (DE-3) and induced with IPTG produced 20-30 mg of UG per ml of bacterial culture. The recombinant UG seems to be a dimer as normally secreted by the rabbit endometrial and tracheobronchial epithelium. To our knowledge, this is the first report of a dimeric mammalian protein that has been expressed in a bacterial host in its natural dimeric form. The full characterization of this recombinant protein is now under way. The successful development of this expression system paves the way for future site-directed mutagenesis studies to delineate the structural-functional relationships of this protein. We have also discovered that major N-terminal fragments of Xenopus antimicrobial peptides are potent PLA2 inhibitors in vitro and antiinflammatory agents in vivo suggesting that these antimicrobial peptides degrade to produce antiinflammatory agents. The presence of UG-like protein is now confirmed in three human organs eg. tracheobronchus, uterus, and the prostate. The prostatic and tracheobronchial protein have been partially purified and preliminary results suggest that these proteins are also PLA2 inhibitors as rabbit UG. Using cDNA probes for PLA2, UG and lip-1 the regulation of expression of these genes is being investigated in established cell lines from several organs. In a clinical study an inverse relationship between the level of UG-like protein and leukotriene C4 has been found. During viral infections of the upper and lower respiratory tracts this inverse relationship was even more pronounced. Studies, currently in progress, may delineate a cause and effect relationship between UG-like protein and proinflammatory leukotriene C4 in the human tracheobronchial mucosa.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00912-09 HGB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Gene Regulation and Cellular Differentiation

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

PI:	Janice Y. Chou	Head	HGB, NICHD
Others:	Yu-Jui Yvonne Wan	Staff Fellow	HGB, NICHD
	Kimberly Leslie	Biologist	HGB, NICHD
	Shuichiro Watanabe	Visiting Fellow	HGB, NICHD
	Juan L. Jimenez-Molina	Visiting Fellow	HGB, NICHD
	Cathie Plouzek	NRC Biotech Fellow	HGB, NICHD
	Adam Sartwell	Bio Aid	HGB, NICHD
	Chi-Jiunn Pan	Adjunct Technician	HGB, NICHD

COOPERATING UNITS *(if any)*

Drs. I. Sun and F. L. Crane (Purdue Univ., IN); Dr. G. Yeoh (Univ. of Western Australia, Australia); Dr. W. Hoppner (Universitats-Krankenhaus, Federal Republic of Germany)

LAB/BRANCH

Human Genetics Branch

SECTION

Cellular Differentiation Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.0	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.)*

Our studies have concerned regulation of gene expression during normal and abnormal differentiation processes. We have shown that adult rat liver contains low levels of three alpha-fetoprotein (AFP) mRNAs of 2.2, 1.7, and 1.5 kb. The 2.2-kb AFP mRNA is expressed mainly in fetal rat liver. The 1.7- and 1.5-kb transcripts share a common 3' sequence with the 2.2-kb RNA, but lack sequences present in the first seven 5' exons of the 2.2-kb AFP mRNA. A cDNA clone encoding the 1.7-kb AFP mRNA has been isolated from an adult liver cDNA library. This cDNA shares similar sequences to the 2.2-kb AFP mRNA at the 3' region, but contains a 90-bp 5' sequence which is absent from the 2.2-kb mRNA. The 90-bp, 1.7-kb AFP mRNA-specific sequence is located in the seventh intron of the fetal rat AFP gene.

Primary fetal rat hepatocytes were employed to examine factors required to promote liver differentiation in vitro. In the absence of effectors, primary fetal hepatocytes dedifferentiated. Cells maintained in the presence of glucocorticoid hormone or cAMP produced high levels of albumin and transferrin or albumin and AFP, respectively. Both glucocorticoid and cAMP induced expression of adult liver-specific genes, suggesting that these fetal hepatocytes have matured. Our study demonstrated that both glucocorticoid hormone and cAMP are necessary for optimal differentiation of fetal hepatocytes in vitro.

cDNA clones encoding human pregnancy-specific B_1-glycoprotein (PSBG) have been isolated and characterized. The amino acid sequence of PSBG as deduced from the cDNA sequence contains two repeated protein domains of 93 amino acids each. PSBG exhibits a strong homology to human carcinoembryonic antigen (CEA) at the nucleotide and amino acid levels. Both proteins contain structurally similar domains and conserve the positions of the cysteine residues in each domain. However, PSBG does not contain a hydrophobic carboxyl-terminal domain. Our data indicate that PSBG and CEA are two members of the same gene family.

Differentiation of ts adult rat hepatocytes depends upon the presence of glucocorticoid hormone, as characterized by the expression of liver-specific genes such as albumin and tyrosine aminotransferase gene (TAT) in the presence of this hormone. These cells contain high levels of retinoic acid receptor mRNA and the addition of retinoic acid inhibits the glucocorticoid-mediated differentiation processes.

LABORATORY OF COMPARATIVE ETHOLOGY (LCE)

Z01 HD 00054-14 Structural and Behavioral Analysis of Vocal Communication in Squirrel Monkeys
D. Symmes, Ph.D.

Z01 HD 00062-12 Brain Mechanisms of Vocal Production in Primates
J. D. Newman, Ph.D.

Z01 HD 00702-08 Genetics of Primate Vocal Behavior
J. D. Newman, Ph.D.

Z01 HD 01106-05 Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity
Stephen J. Suomi, Ph.D.

ZO1 HD 01107-05 Adaptation of Laboratory Reared Monkeys to Field Environments
Stephen J. Suomi, Ph.D.

Z01 HD 01108-04 Comparative Studies of Play Behavior
Maxeen Biben, Ph.D.

Z01 HD 01110-03 Intuitive Parenting of Infants in Comparative Perspectives
(Inactive)

Z01 HD 01111-03 Factors Affecting Nurturant Behavior Toward Infants
Frank A. Pedersen, Ph.D.

Z01 HD 01112-02 Effects of Home- and Out-of-Home Care on Child Development
Michael E. Lamb, Ph.D.

Z01 HD 01113-02 Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood
Michael E. Lamb, Ph.D.

ZO1 HD 01114-01 Individual Differences in Physical and Affective Functioning in Infancy
Michael E. Lamb, Ph.D.

ZO1 HD 01115-01 Effects of Domestic Violence on Children's Development
Michael E. Lamb, Ph.D.

ZO1 HD 01116-01 Pattern of Childrearing Across Cultures and Ecologies
Michael E. Lamb, Ph.D.

LABORATORY OF COMPARATIVE ETHOLOGY
(continued)

ZO1 HD 01117-01 The Hospitalization Experience: Children's Coping with the Stress of Surgery
Marc H. Bornstein, Ph.D.

ZO1 HD 01118-01 Latent Behavioral Effects of Diverse Forms of Caretaking in the First Year of Life
Marc H. Bornstein, Ph.D.

ZO1 HD 01119-01 Specificity of Mother-Infant Interaction
Marc H. Bornstein, Ph.D.

ZO1 HD 01120-01 Observations of Caretaking in Three Societies
Marc H. Bornstein, Ph.D.

ZO1 HD 01121-01 Maternal Activities in Children's Language and Play
Marc H. Bornstein, Ph.D.

ZO1 HD 01122-01 Assesment of Children's Mental and Social Abilities
Marc H. Bornstein, Ph.D.

NICHD Annual Report
October 1, 1987 to September 30, 1988

Laboratory of Comparative Ethology

The Laboratory of Comparative Ethology (LCE) carries out a program of research directed toward the study of behavioral and biological development in humans and in nonhuman primates. The influences on developmental processes of both genetic and environmental factors--and their multiple interactions--are explored in a comparative approach in order to determine the origins, ontogeny, and evolution of various behavioral phenotypes. Longitudinal designs are employed to address issues of ontogenic continuity vs. change, and a variety of both behavioral and biological measures reflecting multiple levels of analysis are collected concomitantly in investigations of developmental processes. A major emphasis is placed on characterizing and understanding normative patterns of biobehavioral development so that deviant patterns can then be readily recognized and their consequences evaluated with respect to established norms. Experimental results in nonhuman primates are correlated with the results of longitudinal studies of human infants and their families, as well as with results obtained by various neuroscience techniques.

The LCE consists of four sections. The Comparative Behavioral Genetics Section, headed by Dr. Suomi, investigates various processes underlying biological and behavioral development in selected nonhuman primate species by focusing on interactions between genetic and environmental factors that affect the course of an individual's ontogeny. Within the Section, the Unit on Neuroethology, headed by Dr. Newman, uses neuroscience techniques to study brain mechanisms involved in the production of various types of primate vocalizations by squirrel monkeys and to examine subtle acoustical differences in characteristic calls between closely related New World primate species. Parallel analyses of vocal patterns in human infant developmental studies and other investigations of parent-infant relationships are carried out in the Unit on Parent and Infant Studies headed by Dr. Pedersen. The Brain, Behavior, and Communication Section, headed by Dr. Symmes, studies the production and utilization of vocal signals by group-living squirrel monkeys in terms of both the acoustical properties of the signals and their information content for group members. Parallel acoustical analyses are conducted on selected vocal patterns of other primate species, including humans. The Child and Family Research Section, headed by Dr. Bornstein, examines perceptual, cognitive, and dispositional development in human infants and children, with special emphasis on studying the relationships among early attentional processes, social stimulation from caretakers, and subsequent cognitive capabilities. Finally, the Section on Social and Emotional Development, headed by Dr. Lamb, studies the effects of different types of caretaking arrangements on infant and toddler social and emotional development and cognitive competence. Special attention is given to longitudinal approaches that involve cross-cultural comparisons and those examining nonnormative samples of both parents and infants.

During FY88 the LCE largely completed a major phase of expansion of staff, space, and research programs. The recently created Section on Social and Emotional Development moved into its new research facilities in the Marriott Scouts Services Building immediately adjacent to the main NIH campus in Bethesda, while remodelling plans for the Child and Family Research Section in the LCE's Building 31 research/office suite were completed. The Comparative Behavioral Genetics Section finished its move into the new facilities in Building 112 at the NIHAC, and the remaining subjects in its rhesus monkey colony from Wisconsin were finally shipped to the NIHAC. During the past year the laboratory's neonatal nursery became functional, and a new 1/4 acre

outdoor test enclosure with easy access for the monkeys living in Building 112 was constructed and successfully pilot-tested. In addition, construction of Building 110A, a joint NICHD-NIMH facility for housing of New World primates at the NIHAC was begun with occupancy scheduled for the fall of 1988. Finally, a Program of Requirements for a new set of multiacre outdoor enclosures with year-round indoor shelters was completed and forwarded through the relevant NIH administrative channels. These new facilities have greatly expanded the LCE's research capabilities and opportunities. As a consequence several major research projects were initiated in FY88 and other active projects were continued or expanded. The overall program of research produced a number of significant and wide-ranging findings, some of which are summarized below.

This past year research in the Comparative Behavioral Genetics Section (CBGS) continued to pursue broad-based investigation of individual differences in biobehavioral response to environmental challenge among rhesus monkeys, with special emphasis on characterizing the genetic and environmental factors that influence the development of such differences. First, standardized neonatal measures predictive of individual differences emerging later in development were refined and their use extended. Of particular interest was the finding, replicated on 3 independent groups of subjects, that relative amounts of quiet sleep, agitated sleep, and awake/alert states during the first month of life predicted individual differences in adrenocortical, behavioral, and immunological responses to stress throughout infancy and early childhood. Rhesus monkeys who as neonates spent significantly less time in both quiet sleep and awake/alert states and more time in agitated sleep subsequently displayed the highest levels of ACTH, the greatest incidence of disturbance behavior, and the lowest lymphocyte/neutriphil ratios when exposed to novel stimuli or challenging situations during their first and second years of life. In fact, these neonatal state measures proved to be as accurate predictors of individual differences in subsequent response to challenge as the rest of the entire standardized neonatal test battery. These findings are of considerable practical importance, in addition to their theoretical interest, in that useful predictions regarding individual differences in response to environmental challenge later in life can apparently be obtained relatively easily and nonobtrusively from both nursery and mother reared infants without physically handling the infants or disrupting the mother-infant bond. It thus may be possible to obtain comparable neonatal activity state data nonobtrusively from rhesus monkey infants reared by their mothers both within complex social groups in captivity and in natural troops living in the wild.

The standardized neonatal test battery was also modified for use with chimpanzee infants in a collaborative study with the Yerkes Regional Primate Research Center in Atlanta, with clear-cut differences in reflex and orientation scores emerging between full-term and premature subjects. Detailed analysis of the orientation scores also revealed that all subjects were much more responsive to both visual and auditory stimuli that are social in nature (human face, human voice) than nonsocial stimuli (e.g., colored balls and rattles). In addition, close examination of visual orienting behavior suggested that chimpanzee neonates have an optimal focal distance about twice that of human infants, a finding that may well account to the apparent failure of chimpanzee infants to maintain prolonged eye-to-eye contact when held by caretakers, in marked contrast to human infants in the arms of competent caretakers.

A second series of studies completed in FY88 examined the long-term consequences of differential early rearing in a number of domains. In these studies rhesus monkey infants were either reared by their biological mothers in dyad cages throughout the first 6 months of life or hand-reared in the neonatal nursery for the first 30 days of

life and then placed into small groups of like-reared peers. At 6 months of age both mother reared and nursery-peer reared monkeys were merged into larger social groups where they remained together until puberty. Thus, except for the first 6 months of life, both mother and nursery-peer reared monkeys grew up under the same environmental conditions. Neuroendocrine differences between these two groups were apparent during the first 6 weeks of life, with mother reared subjects having higher basal levels of growth hormone and lower basal levels of plasma cortisol than nursery-peer reared subjects. Equivalent patterns of body weight increases in the two rearing conditions provided no evidence for an increased risk for failure-to-thrive syndrome in nursery-peer reared infants, as had been suggested by other investigators.

On the other hand, differences between mother and nursery-peer reared monkeys in response to brief social separation at 6 months of age were apparent for a variety of behavioral and physiological measures, although such differences largely disappeared when these monkeys were subsequently combined into large social groups. Nursery-peer reared subjects displayed greater behavioral disruption than did mother reared monkeys in response to the separation manipulations. Nursery-peer reared subjects also displayed greater hypothalamic-pituitary-adrenal responsiveness and higher levels of CSF MHPG than their mother reared counterparts, but there were no significant rearing condition differences in separation levels of other CSF monoamine metabolites or in a variety of measures of immune system responsiveness. Moreover, rearing condition differences in response to similar separations carried out at 18, 30, 48, and 60 months of age were much more limited in scope and degree. Statistically significant rearing condition differences obtained at these later ages were largely limited to measures of self-directed behavior and CSF levels of MHPG and the serotonin metabolite 5-HIAA.

A third series of studies focused on continuity in biobehavioral response to challenge across developmental epochs. Data collected in FY88 in ongoing longitudinal studies of rhesus monkey biobehavioral ontogeny continued to demonstrate strong developmental continuities in virtually every aspect of response to environmental challenge and to link such continuities increasingly to genetic factors. Individuals who exhibited extreme behavioral reactions to challenge early in life tended to do so again in adolescence and early adulthood, although the nature of the reactions changed substantially across developmental epochs. Thus, infant who vocalized most frequently at one month were the ones most likely to exhibit behavioral withdrawal during brief separations at 6 months of age -- and to display the most stereotypy during comparable brief separations in adolescence. Infants who displayed the highest levels of plasma cortisol during early separations were most likely to have the highest levels as juveniles and adolescents, even though the absolute levels of cortisol during brief separation declined with increasing age. Juveniles who had extreme CSF levels of MHPG, HVA, and 5-HIAA when assessed at 6 months of age continued to display extreme levels at 18 months despite significant declines in absolute levels of HVA and 5-HIAA.

Pedigree analyses of the patterns of developmentally stable individual differences continued to provide compelling evidence that these individual differences are highly heritable. Comparisons involving both paternal half-sibs living together and apart (and, in both cases, with no direct experiences with their common biological fathers), as well as cross-generational comparisons, consistently demonstrated reduced variance between same-aged half-sibs compared with unrelated age-mates, between different-aged half-sibs compared with unrelated controls, and between fathers and offspring compared with unrelated members of successive generations, on most the various behavioral and physiological measures for which long-term developmental continuities have been

demonstrated. More sophisticated pedigree comparisons involving blood group factors with established loci are currently underway in collaboration with Dr. Stone's research group.

All of the above-described studies demonstrating long-term stability of individual differences in biobehavioral response to environmental challenge involved rhesus monkeys of known parentage born into a captive colony and followed longitudinally under well-controlled laboratory conditions. In an effort to assess the generality of these various findings and to address questions regarding the adaptive significance of stable individual differences in response to environmental challenge we recently began collaborative studies with the Caribbean Primate Research Center (CPRC) utilizing their populations of wild-born rhesus monkeys living in natural troops. Observations of these rhesus monkey troops during FY88 documented the frequent occurence of "natural" sequences of repeated short-term mother-infant separations during the annual 2-3 month breeding season. Throughout the breeding season adult females frequently leave their family groups and enter into consort relationships with individual males that will keep them occupied and away from their offspring for 1-3 days at a time. During these consort periods the infants who have been "left behind" typically display separation reactions that closely resemble those reported in the laboratory separation studies. In particular, there appears to be a wide range of individual differnces in the severity of the infants' responses to these "natural" separations. These observations clearly indicate that repeated short-term separations from mothers are normative events for wild-living rhesus monkey infants and juveniles, with at least as wide a range of behavioral responses to these "natural" separations as has been reported in the extensive laboratory literature.

A second collaborative study currently underway at the Cayo Santiago Field Station involves careful prospective tracking of the process of adolescent male natal troop emigration through longitudinal study of a cohort of 19 juvenile males from one representative troop at the field station. In addition to behavioral observations conducted throughout the year, annual measurements of physical growth and maturation, hormonal profiles, and psychophysiological reactivity are being obtained from these juvenile and adolescent males during their annual capture by CPRC veterinary staff for tetanus shots and TB testing. At this point in the prospective study approximately half of the young males have already emigrated from their natal troop, so it is possible to compare emigrant with nonemigrant males on a number of variables. A striking finding to date is that personality factors, and underlying psychophysiological characteristics, provide the best means of differentiating emigrant from nonemigrant males. Emigrant males are less fearful and more exploratory prior to emigration than are their nonemigrant agemates, and these differences are reflected in lower and more variable heartrates in standardized reactivity tests for the emigrant males (the relevant adrenocortical data are still under analysis). In contrast, neither social dominance status of the mother nor relative physical and hormonal maturational status differentiates emigrant from nonemigrant adolescent males. This finding is of special significance in that it suggests that individual differences in a behavioral tendency of clear biological (adaptive) relevance are more closely related to individual personality/constitutional factors than those of physical maturation or family social status.

A third collaborative study, involving a wild troop that was captured and removed from Cayo Santiago in 1984 and subsequently moved into a 2-acre enclosure at the CPRC, was continued in FY88. In the previous year we obtained blood samples and behavioral observations on all members of this troop when they were captured and given standard veterinary examinations over a 1-day period, and this past year we were able to gather

equivalent data when the monkeys were once more captured for their annual veterinary check-up. Preliminary analyses of the data collected to date suggest strong year-to-year continuities in individual response patterns. In particular, strong positive correlations were found between levels of plasma ACTH obtained in each year's samples, and between measures of self-directed behavior displayed following each year's capture. Thus, the preliminary results from this study of wild-born monkeys living in a natural troop are consistent with previous laboratory findings of stable long-term patterns of individual differences in response to environmental challenge.

Vocalizations recorded from the above wild-born rhesus monkeys when they were briefly separated from their social group during the annual veterinary examinations were subjected to detailed acoustical analysis in collaboration with the LCE's Unit on Neuroethology. The rationale for this study was that individual differences in reactivity to the stress of social separation would be reflected in the character of their vocal behavior. The subjects were divided into 2 groups on the basis of blood ACTH values sampled within 30 min. after separation from their group. Ten measures of duration, amplitude, frequency, and change in amplitude of frequency over time were generated by computer. A correlation analysis between each acoustic variable and ACTH group revealed that 2 measures, call duration and pitch instability, had correlations of 0.89 and probability values that approached statistical significance.

A second study of the possible relationship between adrenocortical response to brief separation and vocal patterns was also carried out by Dr. Newman's group in FY88. This study attempted to find physiological correlates associated with individual differences in rate of isolation call production by adult squirrel monkeys during a standardized 15-min. social separation test. Plasma ACTH and cortisol were assayed in this group of animals on 2 separate occasions, once immediately after removal from the home cage, and 1 month later, after a second 15 min. period of social separation. Overall, there was a significant increase in cortisol and ACTH levels with separation, but neither of the 2 assays in either home or separated condition differentiated between the monkeys divided into vocal and nonvocal groups. Heart rate samples were also collected via telemetry from 2 of the nonvocal monkeys and compared with samples from 2 robust vocalizers in the social separation paradigm. Ongoing behavior was also recorded. Mean heart rate did not differ significantly between the 2 groups. However, there was evidence for greater periodic variability in heart beat interval in the Vocal group, suggestive of higher "vagal tone" in these animals.

Another project initiated by the Unit on Neuroethology during FY88 involved testing novel drugs with suspected clinical value in the treatment of anxiety, depression, and other behavioral disorders. The drug of primary interest was milacemide (2-(pentylamino)-acetamide), already shown to have therapeutic value in treating epilepsy in humans and to have low toxicity in animals. In our initial study, 8 adult male squirrel monkeys (4 shown to be reliable vocalizers and 4 poor vocalizers based on prior screening results) were administered doses of milacemide (100-400 mg/kg, i.m.) and tested in the social separation paradigm one hour after drug administration. Milacemide produced a selective, dose-dependent reduction in the isolation calling rate of the "reliable vocalizer" group, without affecting motor behavior, but it did not change the behavior of the non-vocal group. We initially hypothesized that the principal mechanism of milacemide-related reduction in isolation call production was through a GABA-mediated pathway. However, since milacemide is also known to inhibit the activity of monoamine oxidase (MAO-B), we subsequently tested the same group of reliable vocalizers with L-deprenyl, a drug known to irreversibly inhibit action of the MAO-B enzyme in brain. Since we found a significant reduction in isolation call production at high doses (2.5 and 5.0 mg/kg), MAO inhibition cannot be discounted as

a possible mechanism for milacemide's action on vocal behavior. A complimentary approach to investigating the effects of peripherally administered drugs on vocal production is to study the effects of vocal behavior of chemicals introduced directly to the brain. As an initial step in this direction, Drs. Winslow and Newman, in collaboration with Dr. Tom Insel (LCS, NIMH), investigated the effects of corticotropin-releasing hormone (CRH) and an atagonist (alpha-helical CRH) introduced into the cerebro-spinal fluid of adult male squirrel monkeys through cannulae implanted in the cerebral ventricles. CRH produced dose related increases in motor activity, but not the increase in vigilance. The antagonist administered alone increased aggressive behavior directed at the subject's reflection in a mirror.

A final set of studies carried out in FY88 in the Unit on Neuroethology was directed at identifying and differentiating heritable influences on vocal development in primates. Work involving comparisons of the behavior of the "gothic arch" subtype of squirrel monkey in Costa Rica with captive social groups of the same subtype originating from South America identified vocal characteristics common to both Costa Rican and South American groups, as well as other vocal attributes found only in the Costa Rican population. Other work analyzed the development of the isolation call of infant common marmoset twins. Twins separated from their parents called together on nearly the same pitch, producing a unique acoustic signal that was readily identified and distinguishable from the isolation calls of either twin alone. Analysis of the isolation calls from the adult members of our marmoset colony revealed that each adult was very stable in its calling behavior over weekly 15 min. separations. Related work analyzed the temporal fine structure inherent in the serial production of calls by separated marmosets. Both common and pygmy marmosets produced isolation calls that were grouped together in a sequence of 2-10 closely spaced units, with a significant positive correlation between call duration and interval to the preceding unit. However, in the pygmy marmoset intervals and durations increased with sequence position, whereas in the common marmoset the opposite rule was followed. This is the first demonstration in any nonhuman primate of a rule of temporal ordering in a complex vocal sequence, and it suggests a fine degree of genetic programming in regulating in regulating the vocal output of these species.

Parallel studies of vocalization patterns utilized by squirrel monkeys living in complex social groups were carried out in the LCE's Section on Brain, Behavior, and Communication (BBCS), focusing on calls used in social contexts characterized by quiet affiliative and caregiving behavior. This past year Drs. Symmes and Biben recorded the vocal behavior of 6 infants born in our colony during the first 3 months of life, using longitudinal time series sampling with close-in videotaping. Infant vocal behavior during the first month was very limited, restricted to simple tonal or pulsed calls associated with nursing, but even at this early stage other adult female monkeys (including those carrying their own babies) and juvenile females in the group interacted with infants very frequently and in ways which appeared to influence cognitive development, including close inspection with facial approximation, tactile exploration, and vocal exchanges. Both mothers and these other females ("aunts") used a similar call type, the Coax call, but the acoustic details of the Coax call were clearly different in the contexts of nursing and retrieval. Use of this call by aunts seems especially promising for the study of squirrel monkey vocal development because the first identified vocal exchanges involving infant monkeys are with aunts. Moreover, these "dialogues" with aunts appear to differ from those with mothers and to change developmentally as the infants grow and become increasingly independent of the mother.

Continued study during FY88 of vocalizations emitted by juvenile squirrel monkeys

during bouts of active play revealed that the primary function of "play" vocalizations was to alert adult group members to be more vigilant when the young are absorbed in play. Play has been shown to be a risky activity in other primates, exposing the vulnerable young to predation. However, the protection afforded by adults monitoring such activity allows youngers to play with abandon and in large numbers and compensates for what would otherwise be a maladaptive activity where animals crash through the trees, vocalizing loudly and oblivious to predators. This finding is further evidence for both the importance of play and the degree and variety of indirect parental care in this species. The BBCS also continued its collaborative project with Drs. H. and M. Papousek from the Max-Planck Institute of Psychiatry in Munich and with the LCE's Child and Family Research Section investigating the acoustical characteristics of preverbal vocal exchanges between human infants and their caretakers. This research is based on the recent finding that a melodic mode of communication (probably with a genetic basis) is employed by human mothers and fathers in interacting with the prelinguistic infant, as evidenced by recent cross-cultural studies of native Chinese and English speaking mothers. The data for these studies were largely processed on the BBCS sound analysis system. The model provided by this collaborative enterprise is being actively examined at the animal level.

Interactions between human preverbal infants and their caretakers provided the focus of several other major studies carried out in the Child and Family Research Section (CFRS) in FY88. One study investigated the conditional contributions of three domains of maternal activity, including interpersonal affective communication, stimulation of infant attention, and control over object-centered exchanges, to infant language, play and representational competence at 13 months. Naturalistic observations of relevant mother-infant behaviors were conducted in the home and examined in relation to infant language and play competence. Correspondences between infant language and play skills were examined for evidence of an underlying representational competence that might itself relate to conditional maternal activities. Independent associations were found between maternal encouraging attention and infant noun-comprehension, and between encouraging attention and infant representational competence. Two-way interactions between maternal activity domains significantly augmented explained variance in infant skills, in that maternal social stimulation was associated with increased language and representational skills in dyads where mothers, rather than infants, exerted most control over object-centered exchanges, whereas frequent sociability, in the context of frequent maternal encouraging attention, was associated with greater infant play sophistication.

A second major study was designed to replicate and extend these findings by focusing on the extent to which three maternal characteristics (age, employment status, and parenthood status) and type of substitute care experienced during mother's employment can influence the observed relations between caregiver social and didactic stimulation on the one hand and infant social and cognitive competencies on the other. In this study several groups of primiparous mothers and their infants are being observed when the infants are 5 months old; the groups differ systematically in terms of the mothers' mean ages (under 20 years, between 20 and 30, and over 30), employment status (employed vs. homemaker), type of substitute care, and whether the child has been adopted or not. Mothers and infants are being videotaped in their homes in both structured and unstructured interactions with each other and with the substitute caretaker.

A third major study initiated in the CFRS in FY88 has focused on cross-cultural comparisons of mother-infant interactions and caretaking traditions. The purpose of this project is to identify significant similarities and differences in the childrearing

ecologies of Japanese, Israeli, and American infants. It is widely held that Japanese and Americans differ in prominent aspects of their psychological make-ups and that certain social and intellectual distinctions between members of these two cultures arise early in life. Similarly, previous studies on the nature of infant development in Israel: kibbutzim determined that many decisive aspects of infant care -- particularly the close ties between infants and mother -- vary markedly from the American experience. Cross-cultural developmental studies have shown that such rearing differences typically have implications for infants' later cognitive and social behavior and performance. In the present project infants being raised in Tokyo, in urban Haifa, and in a traditional Israeli kibbutzim are being compared with infants reared in New York. Each infant is being observed on 2 occasions, at 5 and 13 months, in the presence of its caretaker. At this point data collection for the Japanese sample is complete, and similarities and differences among Japanese and American infants and mothers have been assessed. In addition, relations among infants' activities within each culture have been evaluated and resultant patterns of relations between the two cultures have been compared. Finally, interactions between mothers and infants in each culture have been studied and patterns of interactions across the two cultures compared. These results will be used to identify activity and interaction patterns which are distinctive to these two disparate cultures as well as patterns which are similar between the two cultures and which may point to processes universal in early development. Data collection has not yet been initiated for the Israeli sample. The study promises to be of great theoretical interest because of known differences in Japanese and American children's preschool performance for the Japanese-American contrast and because the childcare arrangement on traditional kibbutzim violates what are often considered to be crucial aspects of infant care -- particularly the close ties between infants and their mothers -- of the Israeli-American contrast.

Cross-cultural comparisons were also utilized in several studies carried out in the Section on Social and Emotional Development (SSED) this past year in order to characterize the ways in which developmental niches can be described by variations in physical ecology, social and parental attitudes, and values and how differences on these dimensions affect children's development.

In one study, SSED staff followed up previous research on the quality of attachment between infants and adults on Israeli kibbutzim. Infants were tested in Ainsworth's Strange Situation procedure for assessing attachment with their mothers, fathers, and metaplot (careproviders) when they were 11 to 14 months old. At age 5, data concerning the functioning of these children were obtained by measuring IQ and empathy and by obtaining reports from preschool teachers and new careproviders concerning their behavior in kindergarten and the peer group using the CCQ and Baumrind's Preschool Behavior Q-sort (PBQ). A significant discovery was that C-type (resistant) attachments were frequently found on Israeli kibbutzim with communal sleeping but the long-term correlates of this "insecure" pattern had not previously been identified. SSED staff found no significant associations between infant-mother and-father attachment classifications and indices of later child development, but infants who had B-type ("secure") attachments to their metaplot were later less ego controlled and more empathic, dominant, purposive, achievement oriented, and independent than C-group ("insecure/resistant") subjects. All these group differences were in the direction predicted on the basis of prior research on the correlates of infant-mother attachment. All the measures of socioemotional development reflected the children's behavior in the children's house but not at home or with their parents, a finding that may explain, in part, the relatively strong predictive power of attachment status with metapelet as opposed to attachment status with mother and father. These results underscore the central importance of the metapelet as a key figure in the early social

life of kibbutz infants. The findings thus raise questions regarding the developmental significance of attachment relations with various significant adults.

Another major project carried out in FY88 involved analyses of data from a longitudinal study in Sweden examining the effects of center day care, family day care, and home care on the development of 145 children recruited at an average of 16 months of age. Multivariate analyses using Wold's Partial Least Squares "soft modelling" procedure indicated that type of care had no reliable impact on the children one and two years post-enrollment. The quality of care received at home and the quality of alternative care had the most consistent and equivalent impact on personality maturity and emergent social skills with peers and adults. Measures of family social support networks, temperament, and child gender had more modest effects. PLS analyses also showed that quality of home care was the most important predictor of intellectual competence one and two years after enrollment. Compliance with maternal requests in a task-like situation was most strongly predicted by the quality of care received at home. The quality and extent of alternative care were also significant predictors of compliance. The significance of these findings lies in their emphasis on the need to consider not only the type but also the quality of out-of-home care, and to consider the role of factors outside the care setting--such as the quality of home care--when evaluating day care arrangements. Other work on this project involves a small but intensive study of family day care in Utah and an exploration of the association between day care and security of infant-mother attachment in nearly two dozen studies conducted by other investigators. Data for both projects are currently being prepared for analysis.

In a third study, SSED staff explored the effects of agreement between Swedish mothers and fathers regarding socialization values. Parental agreement was computed by correlating the responses of 128 mothers and fathers on Block and Block's Q-sort concerning the values and attitudes they bring to the socialization of their preschool-aged children. The children's functioning was assessed using the Griffiths Developmental Scales, and the Blocks' California Child Q-sort (CCQ), yielding a measure of perceived ego resiliency and ego control. Marital quality was assessed using the two parents' independent responses to the Areas of Change Questionnaire. Data analyses completed to date revealed substantial disagreement between spouses in a substantial number of areas, with mothers showing more expressive and fathers more instrumental concerns. There were few differences between the parents of girls and boys. Parental agreement was associated both with marital quality and with contemporaneous and earlier maternal reports of ego resiliency in both boys and girls, as well as with maternal reports of ego control in girls only. There were no significant correlations between parental agreement and measures of intellectual development in either boys or girls, however. The results suggest that parental agreement may have a less general and a less gender-differentiated impact on psychological functioning in contemporary Sweden than was true in the United States when the Blocks' data were gathered 20 years ago, when it was reported that degree of parental agreement had a substantial impact on child development, especially among boys.

Major progress was also made in FY88 in ongoing studies of antecedents, correlates and consequences of adolescent pregnancy and parenthood, utilizing data from two large nationally-representative samples as well as smaller samples. The goal is to describe the psychosocial context of adolescent parenthood and to explore the long-term effects for both mothers and fathers. Analyses completed this past year revealed that regardless of race, adolescent parenthood was found to be but one symptom of a wider variety of psychosocial problems. Compared with nonfathers and nonmothers of

similar ages and backgrounds, adolescent parents, especially the males, were much more likely to have a history of involvement with the police, school problems, and substance abuse. A smaller study showed that adolescent fathers differed in their attitudes and expectations from adult fathers, and that adult fathers with adolescent partners resembled adolescent fathers more than adult fathers with adult partners. Additional analyses revealed that adolescent marriage was associated with deficits in marital stability, income, educational attainment, and occupational prestige through at least 40 years after the marriage. For mothers, both adolescent childbearing and adolescent marriage were associated with higher lifetime fertility, lower income, less prestigious occupational ratings, lower educational attainment, and more frequent marital dissolution. The "best" outcomes were obtained by those women who delayed both childbearing and marriage into adulthood. These findings strongly suggest that adolescent parenthood is not a random event. It may also have long-term effects on the psychological and socioeconomic status of both men and women.

Finally, studies completed in the Unit on Parent and Infant Studies investigated the relationship between emotional factors during the pregnancy period, the infant's temperament, and postnatal parent-infant adaptation. The first of these studies examined reactivity patterns to recorded infant cries that differed in their degree of aversiveness. Expectant mothers, their spouses, and nulliparous married women were compared in order to examine effects of pregnancy status and sex of the respondent. Recordings of normal infant cries and cries that were deviant on the basis of spectographic and clinical criteria were presented to the 3 groups of adults, and the respondents filled out rating scales after each cry to indicate their subjective evaluation while their heart rate and vagal tone were recorded continuously. All three groups made clear-cut distinctions in the cries on the basis of their subjective evaluations, with the deviant cries consistently rated as more aversive. On the physiological measures, however, the expectant mothers did not discriminate between the aversive and nonaversive cries while the expectant fathers and nulliparous women did, consistent with the hypothesis that the discrepancy between cognitive/subjective and physiologic responses evident in the pregnant women may be adaptive during pregnancy, serving as a protective mechanism for the fetus.

A second study concerned infant individuality, as assessed in behavioral observations conducted in the laboratory, physiological measures, developmental tests, and parental reports of temperament in 3-month-old infants. Infants were classified into groups of high or low heart rate variability (vagal tone), a measure that in other studies has been implicated with underlying central nervous system functioning, maturity, and temperament. Infants with high vagal tone scored significantly higher on the Bayley Mental Developmental Index and showed more rapid visual habituation. In contrast, ratings of temperament, whether based on independent observation in the laboratory or parental report, did not discriminate the groups, although there appeared to be more congruence between observational ratings and parental reports in the high vagal tone group, suggesting that the validity of parental reports of temperament may be partly influenced by characteristics of the babies being rated. The relationship between vagal tone and habituation rate at 3 months is noteworthy because, in other research, both measures have been found predictive of later cognitive functioning.

A third study investigating the consequences of pregnancy loss focused on differential effects of early vs. late loss on a measure of grieving and psychological preoccupation with the loss, the Perinatal Bereavement Scale (PBS). Along with other procedures the PBS was administered to expectant mothers and fathers in the third trimester of a pregnancy that was within 2 years of previous loss; procedures were repeated at 6 weeks postnatally and when the infant was 16 months old. Comparisons were made

between families that had experienced an early loss (loss prior to the 20th week of pregnancy) and families that had a stillbirth or neonatal death. Preliminary analyses indicated that grief is greater for late loss than early loss parents, that mothers experienced greater grief as measured by the PBS than did fathers, and that grief over previous loss diminished with time after the birth of a viable baby. The analyses also revealed a significant 3-way interaction among these variables, in that late loss mothers showed greater grief at all time periods, such that by age 16-months, early loss mothers and fathers and late loss fathers were indistinguishable from each other, but late loss mothers still showed elevated grief scores.

A final study carried out in the Unit in FY88 investigated what parents teach pre-school age children about caregiving during the course of play with dolls. Preliminary results yielded relatively few differences related to sex of the parent but more differences in play behavior related to sex of the child. Both older boys and girls enacted more caregiving activities, e.g., feeding, bathing the doll, etc., than did younger children. Mothers and fathers were relatively similar to each other in eliciting play with dolls, but both parents verbalized different messages to boys and girls. Girls were more frequently told that the doll was "their baby" or that they were the doll's "parent." Girls, in turn, verbalized a parental relationship to the doll more often than boys did. Boys often played out a nurturant role while not articulating verbally that they were in such a role. The results suggest that nurturing the young is an internalized role script for both male and female children very early in life, but females receive and develop an overlay of verbal constructions to support such behavior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00054-14 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Structural and Behavioral Analysis of Vocal Communication in Squirrel Monkeys

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

PI:	D. Symmes	Head	LCE, NICHD
Other:	M. Biben	Senior Staff Fellow	LCE, NICHD
	D. Bernhards	Bio. Lab Technician	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Brain, Behavior, and Communication

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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)*

We have continued the study of squirrel monkey calls used in social contexts characterized by quiet affiliative and caregiving behavior. Recent work on the course of vocal development in young squirrel monkeys has been based on our detailed knowledge of the syntactical rules governing adult use of affiliative calls. Because ontogenetic vocal changes in this species promise to be more subtle than obvious, this level of information on adult forms puts us in a good position to study the stages of development leading to the competent, fully socialized adult.

We have recorded (using close-in videotaping) the vocal behavior of 6 infants born in our colony during the first 3 months of life, using longitudinal time series sampling. Infant vocal behavior during the first month is very limited, and restricted to simple tonal or pulsed calls associated with nursing. Even at this early stage, however, "aunts" or other female monkeys in the group exhibit great attention to the infant and direct much vocal behavior to it. The role of the infant is largely passive, although some examples of vocal exchanges between infants and aunts have been observed. The significant role of aunts in early socialization and developing vocal competence is a new finding.

During FY88 we completed analysis of vocal recordings collected from adult monkeys after "lights out." These data were collected with Visiting Fellow P. Goedeking, and a joint manuscript based on this project has been submitted for publication. The results support the conclusion that maintaining social contact through vocal signals during the night is of greater benefit to squirrel monkeys than any potential risk from exposure to nighttime predation.

Collaborative research on human mother-infant preverbal communication has been largely completed (initial studies of H. and M. Papousek), and several manuscripts are in preparation. Some additional data were added and processed in our computer during FY88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00062-12 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Brain Mechanisms of Vocal Production in Primates

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

P.I.:	J. D. Newman	Head	LCE, NICHD
Other:	J. T. Winslow	IRTA Fellow	LCE, NICHD
	S. H. Boinski	NRSA Fellow	LCE, NICHD
	Y. E. Bryan	Visiting Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, NIMH (Insel, Murphy); Laboratory of Neuropsychology, NIMH (Bachevalier); Division of Child Psychiatry, Johns Hopkins School of Medicine (Harris)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics, Unit on Neuroethology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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)*

This project investigates the pharmacological, neural, and physiological control of vocal behavior in well-characterized primate model species. Current work focuses on the expression of the isolation call, a specific vocal pattern used to to re-establish contact with familiar conspecifics.

New findings this year are: (1) a correlation between activity of the pituitary/adrenal system and the behavioral manifestations of social separation was found in both squirrel monkeys and rhesus macaques. In individually housed adult male squirrel monkeys, a 15-min. separation from the colony resulted in significant elevations in ACTH levels in both high- and low-rate vocalizers. Juvenile macaques separated overnight from their troop and divided into high and low ACTH subgroups showed significant differences between the subgropus in several acoustic characteristics of their isolation ("coo") vocalizations; (2) juvenile rhesus macaques with bilateral ablations of the hippocampal gyrus produce isolation coos that show fewer acoustic abnormalities relative to unoperated control subjects than do age-matched monkeys with bilateral amygdalectomies; (3) isolation coos of 4-week old rhesus macaques with prenatally corrected hydrocephalus show no difference in calling rate from unoperated age-matched controls when briefly separated, but do show differences in both the types of vocalization (more shrieks and noisy coo variants) and the structural details of their tonal coos (less pitch inflection); (4) milacemide, a synthetic anti-epileptic, produces a dose-dependent decrease inisolation calling in socially separated squirrel monkeys, but does not alter concomitant vigilance; (5) inhibition of the enzyme monoamine oxidase (MAO) was implicated in control of isolation call production in squirrel monkeys, since L-deprenyl and MAO-B inhibitor, produced a dose-dependent decrease in this vocalization in the absence of any behavioral signs of toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00702-08 LCE

PERIOD COVERED
October 1, 1987 to September 30, 1988

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

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

PI: J. D. Newman Head LCE, NICHD
Other: S. H. Boinski NRSA Fellow LCE, NICHD

COOPERATING UNITS *(if any)*

World Wildlife Fund, Washington, D.C. (Mast)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Comparative Behavioral Genetics, Unit on Neuroethology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	1.3	.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.)*

This project is directed at identifying and differentiating heritable influences on vocal development in primates. Current work involving comparisons of the behavior of the "gothic arch" subtype of squirrel monkey in Costa Rica with captive social groups of the same subtype originating from South America, has identified vocal characteristics common to both Costa Rican and South American groups, as well as other vocal attributes found only in the Costa Rican population. Other current work has analyzed the development of the isolation call of infant common marmoset twins. While accoustically similar to the call given by separated adults, the infant isolation call is simpler consisting of a steady tone lasting about 1 second. Twins separated from their parents will call together on nearly the same pitch, producing a unique acoustic signal that is readily identified and distinguishable from the isolation calls of either twin alone. Analysis of the isolation calls from the adult members of our marmoset colony reveal that each adult is very stable in its calling behavior over weekly 15 min. separations. Related work has analyzed the temporal fine structure inherent in the serial production of calls by separated marmosets. Both common and pygmy marmosets produce isolation calls that are grouped together in a sequence of 2-10 closely spaced units. Analysis reveals an orderly relationship between the sequence position of each unit, its duration, and the time interval between it and adjacent units in the same series. In both marmoset species, there is a significant positive correlation between call duration and interval to the preceding unit. However, in the pygmy marmoset intervals and durations increase with sequence position, whereas in the common marmoset the opposite rule is followed. This is the first demonstration in any nonhuman primate of a rule of temporal ordering in a complex vocal sequence, and it suggests a fine degree of genetic programming in regulating in regulating the vocal output of these species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01106-05 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity

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

PI:	S. J. Suomi	Head	LCE, NICHD
Other:	K. L. Rasmussen	IRTA Fellow	LCE, NICHD
	C. E. Eisele	Research Psychologist	LCE, NICHD
	J. M. Scanlan	Research Psychologist	LCE, NICHD
	M. Champoux	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)* LCS, NIAAA (Linoilla, Higley, Lane); LNP, NIMH (Gault, Wise); LN, NIMH (Murray); Primate Laboratory, Univ. Wisconsin-Madison (Coe, Schneider); Dept. of Obstet. & Gyn., Georgetown Univ. Med. Sch. (Michejda); Yerkes Reg. Primate Ctr. (Nadler, Bard); Istituto di Psicologia, CNR (Visalberghi)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	1.0	2.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project investigates primate biobehavioral development through comparative longitudinal investigations, with special emphasis on characterizing individual differences among rhesus monkeys in response to mild environmental challenge and on determining the long-term developmental consequences for these individuals in different physical and social environments. Studies completed in FY88 refined neonatal measures predictive of these individual differences, characterized long-term influences of different early rearing environments, extended the known period of development for which continuity of these individual differences can be demonstrated, and identified parallel phenomena among wild-born rhesus monkeys living in field settings. More specifically: (1) Measures of infant state throughout the first month of life were found to be highly predictive of behavioral, neurohormonal, and immunological response to separation in both nursery reared and mother reared monkey infants and juveniles, greatly expanding the utility of such early measures for monkeys born and reared in complex social groups. (2) Differential early rearing (mother vs. nursery-peer) of rhesus monkey infants was shown to have significant behavioral, adrenocortical, neurochemical, and immunological consequences that can be detected under diverse conditions of novelty and challenge throughout the childhood and adolescence years in these subjects. (3) Continuity of individual differences in response to challenge among like-reared monkeys from infancy to adolescence and early adulthood, previously demonstrated for behavioral and adrenocortical indices, was shown to extend to measures of central monoamine turnover, with strong circumstantial evidence that such differences were highly heritable. (4) Studies of wild-born rhesus monkey groups living in naturalistic settings revealed that the basic pattern of developmental stable individual differences in biobehavioral response to challenge identified in previous laboratory studies not only generalized to natural groups but also appeared to be of considerable biological significance for these monkeys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01107-05 LCE

PERIOD COVERED

October 1, 1987 to September 20, 1988

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

Adaptation of Laboratory Reared Monkeys to Field Environments

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

PI:	S. J. Suomi	Head	LCE, NICHD
Other:	K. Rasmussen	IRTA Fellow	LCE, NICHD
	P. O'Neill	Research Psychologist	LCE, NICHD
	G. DiGregorio	Research Psychologist	LCE, NICHD
	C. Price	Biologist	LCE, NICHD
	C. McKenna	Psychology Aid	LCE, NICHD

COOPERATING UNITS *(if any)*

VRB, DRS (Bayne); Department of Psychology, Univ. Massachusetts (Novak)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.6	1.0	2.6

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)*

This project investigates how rhesus monkeys born and raised under different laboratory conditions adapt to placement into naturalistic outdoor environments and compares this adaptation process to that seen in natural settings and in indoor environments that contain specific physcial and social features of the monkeys' natural habitat. Adaptation, both short- and long-term, is assessed by examining behavioral repertoires and by monitoring a variety of physiological systems in these subjects, yielding broad-based indices of relative physical and psychological well-being. The project centers on longitudinal study of a group of 15-year-old rhesus monkeys and 2 generations of their progeny, all of whom live year-round in a 5-acre outdoor enclosure on the grounds of the NIHAC. Despite the facts that the 15-year-old adults were all laboratory born and hand-reared in a nursery, and that these adults and their progeny have never had physical exposure to any other monkeys, all members of this primary study group consistently exhibit the full compliment of species-normative behavioral repertoires, development patterns, seasonal changes (including well-defined breeding and birth seasons), and social organization. During FY88 these species-normative patterns continued to be documented in the primary study group, and comparisons with a second multigenerational group of laboratory-born rhesus monkeys maintained in indoor settings over a comparable period were intiated. The process of adolescent male emigration was also examined in detail in the primary study group and compared with the phenomenon as observed in wild-living rhesus monkey troops. Finally, two studies investigating the effects of differing forms of "enrichment" of the physical environment on behavioral and physiological processes displayed by member of captive monkey groups were begun following the completion of construction of new indoor-outdoor facilities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01108-04 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Comparative Studies of Play Behavior

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

PI:	M. Biben	Senior Staff Fellow	LCE, NICHD
Other:	D. Symmes	Head	LCE, NICHD
	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Brain, Behavior, and Communication

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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.)*

Vocalizations Used in Play. Our previous studies of play behavior and the vocal activity accompanying it established that play is a robust and important behavior in development. One aspect was puzzling, however: squirrel monkeys were very noisy during play and this, coupled with the fact that play was intense and absorbing, would seem to expose playing youngsters (and perhaps the whole troop) to a greater risk of predation during play. Most animals are silent or nearly so when they play, and prior work in our lab had ruled out the possibility that this unusual noisiness was communicating anything of significance between the playing animals themselves. We used a group of four young monkeys housed separately but within earshot of a group of adults to test an alternative possibility that such calls act as signals to nearby adults instead. We found that adult females significantly increased their vigilance for predators during times when the youngsters emitted play vocalizations. This response was obtained whether or not the adults could see the younsters. . We conclude that one function of play vocalizations is to alert adults to increase their vigilance to protect themselves and/or the vulnerable young who are preoccupied in play.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01110-03 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Intuitive Parenting of Infants in Comparative Perspectives

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

PI:	S. J. Suomi	Chief	LCE, NICHD
Other:	H. Papousek	Guest Researcher	LCE, NICHD
	M. Papousek	Guest Researcher	LCE, NICHD
	C. Rahn	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
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.)*

Inactive

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01111-03 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Factors Affecting Nurturant Behavior Toward Infants

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

PI:	F. A. Pedersen	Head	LCE, NICHD
Other:	Y. Bryan	Visiting Fellow	LCE, NICHD
	L. Huffman	NRSA Fellow	LCE, NCIHD
	S. Theut	Guest Researcher	LCE, NICHD

COOPERATING UNITS *(if any)*

Rockefeller Foundation (Berman)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Unit on Parent and Infant Studies

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
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.)*

This project encompasses three studies dealing with the development of nurturant responses to infants. The first study investigates what parents teach pre-school age children about care for the young during the course of play with dolls. It examines whether mothers and fathers communicate differential expectations for boys and for girls regarding nurturing infants. Two additional questions are whether mothers foster stronger nurturant expectations than fathers, and whether fathers differentiate their expectations for male and female children more strongly than mothers do. Data collection has been completed; preliminary analyses suggest relatively strong differences in parental expectations for male and female children, but differences between maternal and paternal behavior were not striking. The second study tests whether a specific psychological stress, previous pregnancy loss (miscarriage, stillbirth, or neonatal death), contributes toward anxiety and depression during a subsequent pregnancy or a dysfunctional adaptation in the postpartum period. Two groups of expectant parents are studied longitudinally, one in which there was a previous pregancy loss and a second group of first-time expectant parents. Data collection has been completed for the early phases, but a follow-up assessment 16 months is still in progress. Among the innovative procedures developed for this study was a measure of grief, called the Perinatal Bereavement Scale. Preliminary findings emphasize more serious psychological sequeli associated with late loss. The third study is concerned with the mother's emotional state during pregnancy, her reactivity to infant cries that show varying degrees of aversiveness, and the unique individuality of her infant as factors that collectively influence the parent-infant relationship in the first year of life. First-time expectant mothers and their spouses are studied during the pregnancy; infants from these pregnancies are studied neonatally, and follow-up studies of parents and infants are conducted at 3, 9 and 12 months. Non-pregnant women also were studied as a control group. The study employs multiple levels of measurement, including observational, self-report, and physiological indices. Data collection is in progress at the 9 and 12 month phases. Preliminary findings from the cry reactivity procedure indicate that pregnancy status attenuates physiological reactivity to cries of differing aversiveness in spite of clear-cut subjective awareness of differences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01112-02 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Effects of Home- and Out-of-Home Care on Child Development

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

PI:	M. E. Lamb	Head	LCE, NICHD
Other:	K. J. Sternberg	Research Psychologist	LCE, NICHD
	R. D. Ketterlinus	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Center for Human Growth and Development, University of Michigan (Bookstein)
Department of Psychology, Goteborg, Swedent (Hwant & Broberg)
Department of Psychology, Catholic University (Prodromidis)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
80	40	40

CHECK APPROPRIATE BOX(ES)

☒ (a) 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 analyses of data from a longitudinal study in Sweden examining the effects of center day care, family day care, and home care on the development of 145 children recruited at an average of 16 months of age.

Multivariate analyses completed in FY88 using Wold's Partial Least Squares "soft modelling" procedure indicated that type of care had no reliable impact on the children one and two years post-enrollment. The quality of care received at home and the quality of alternative care had the most consistent and equivalent impact on personality maturity and emergent social skills with peers and adults. Measures of family social support networks, temperament, and child gender had more modest effects. PLS analyses also showed that quality of home care was the most important predictor of intellectual competence one and two years after enrollment. Compliance with maternal requests in a task-like situation was most strongly predicted by the quality of care received at home. The quality and extent of alternative care were also significant predictors of compliance.

The significance of these findings lies in their emphasis on the need to consider not only the type but also the quality of out-of-home care, and to consider the role of factors outside the care setting--such as the quality of home care--when evaluating day care arrangements. Other work on this project involves a small but intensive study of family day care in Utah and an exploration of the association between day care and security of infant-mother attachment in nearly two dozen studies conducted by other investigators. Data for both projects are currently being prepared for analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01113-02 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood

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

PI:	M. E. Lamb	Head,	LCE, NICHD
Other:	R. D. Ketterlinus	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)* Dept. of Psychology, U of MD-Baltimore County (Teti); Dept. of Pediatrics, U of Utah Med. Sch. (Elster); Dept. of Human Dev., U of MD (Kimmerly); Dept. of Psychology, Catholic U (Hulbert); Dept. of Psychology, American U (Long); Dept. of Psychology, U of VA (Gardner)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.20	1.0	.20

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

This project involves analyses of data from two large nationally-representative samples as well as smaller samples. The goal is to describe the psychosocial context of adolescent parenthood and to explore the long-term effects for both mothers and fathers.

A. Characteristics of adolescent fathers. Regardless of race, adolescent parenthood was found to be but one symptom of a wider variety of psychosocial problems. Compared with nonfathers and nonmothers of similar ages and backgrounds, adolescent parents were much more likely to have a history of involvement with the police, school problems, and substance abuse. The problem behavior syndrome was especially marked among adolescent men. A smaller study showed that adolescent fathers differ in their attitudes and expectations from adult fathers, and that adult fathers with adolescent partners resembled adolescent fathers more than adult fathers with adult partners.

B. Long-term correlates of adolescent parenthood. Adolescent marriage was associated in both men and women with deficits in marital stability, income, educational attainment, and occupational prestige through at least 40 years after the marriage. For mothers, both adolescent childbearing and adolescent marriage were associated with higher lifetime fertility, lower income, less prestigious occupational ratings, lower educational attainment, and more frequent marital dissolution. The "best" outcomes were obtained by those women who delayed both childbearing and marriage into adulthood.

Evidently, adolescent parenthood is not a random event. It may also have long-term effects on the psychological and socioeconomic status of both men and women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01114-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Individual Differences in Physical and Affective Functioning in Infancy

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

PI:	M. E. Lamb	Head	LCE, NICHD
Other:	A. Rosenberg	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Department of Psychology, U of Maryland (Porges); Department of Psychology, Catholic U (Haynie, Scaramella); Ducrey; Revuelta; Miller

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.40	1.20	0.20

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

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

This study is concerned with the ways certain physiological and behavioral signs of arousal or irritability (e.g., heart rate variability, vagal tone, colic, sleeplessness, crying) in the first five months of life are related to measures of the child's temperament, emotional expressiveness, and physiology at later ages. By observing patterns of infant-mother interaction both at home and in laboratory settings, we further expect to determine whether individual differences in maternal behavior interact with early tendencies (as indexed by the signs listed above) in determining patterns of psycho-physiological functioning, emotional expressiveness, attachment behavior, and behavioral inhibition in toddlerhood.

Data collection began in April 1988. No data have yet been analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01115-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Effects of Domestic Violence on Children's Development

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

PI:	M. E. Lamb	Head	LCE, NICHD
Other:	K. J. Sternberg	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)* Ministry of Welfare, Jerusalem Municipality, Israel (Saltzman); Dept. of Psychology, Hebrew U, Jerusalem (Greebaum, Milonek); School of Social Work, U of Haifa (Dahoud); Dept. of Psychology, U of MD (Sandler); Dept. of Psychology, U of Rochester (Cicchetti); Fink; Lowen; Edelstein; Krispin

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.40	1.20	0.20

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [X] (a1) Minors
- [ ] (a2) Interviews

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

This is a new project which staff have been developing since August 1987. The goal of the project is to explore the effects of domestic violence on 10- to 12-year-old children. The study involves four groups of subjects, each comprising 15 boys and 15 girls defined by whether they have been (1) the victims of physical abuse by their fathers; (2) the witnesses of physical abuse of their mothers by their fathers; (3) both victims and witnesses of domestic violence by their fathers; and (4) children from similar backgrounds who have not experienced any forms of domestic violence. Data will be obtained from the children, their parents, and their teachers. The focus will be on the quality of the children's functioning at home, at school, and in the peer group, with attempts made to explore the intrapersonal (temperament, and perceptions of responsibility and control) and exogenous (social support) factors that buffer some children and render others more vulnerable. Data collection takes place (under contract) in Israel in August to October 1988. This is one of the first methodologically sound studies comparing the effects of various types of domestic violence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01116-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Pattern of Childrearing Across Cultures and Ecologies

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

PI:	M. E. Lamb	Head	LCE, NICHD
Other:	A. B. Nsamenang	Visiting Fellow	LCE, NICHD
	K. J. Sternberg	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)* Dept. of Psychology, U of Osnabruck, West Germany (Keller); Dept. of Psychology, Technical U of Darmstadt, West Germany (Voss); Dept. of Psychology, U of Goteborg, Sweden (Broberg, Hwang); Dept. of Child Development and Family Relations, U of NC-Greensboro (MacKinnon); U of MD (Teti, Nakagawa)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.48	1.20	.28

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [X] (a1) Minors
- [ ] (a2) Interviews

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

The work on this project involves a number of studies in a variety of cultures. The overall goal is to explore the ways in which developmental niches can be described by variations in physical ecology, social and parental attitudes, and values and how differences on these dimensions affect children's development.

In one study, SSED staff explored the effects of agreement between Swedish mothers and fathers regarding socialization values. Agreement between parents proved to be much less significant in predicting development outcomes in Sweden than in the USA.

In a second study, SSED staff followed up previous research on the quality of attachment between infants and adults on Israeli kibbutzim. These studies demonstrated that infants form distinctive attachments to mothers, fathers, and professional careproviders, and that the attachments to the careproviders had the greatest impact on subsequent social competence in the peer and preschool contexts.

In another study, SSED staff are planning to explore the perceptions, values, expectations, and practices of West African parents. The goal is to identify the effects of westernization, urbanization, and religion on the ecologies in which children are raised. Such data will be interpreted in the context of information about the varying physical ecologies.

In a fourth study, SSED staff are attempting to assess specific maternal and child attributions about one another in order to identify the extent to which attributions or expectations shape the way that parents and children interact.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01117-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

The Hospitalization Experience: Children's Coping with the Stress of Surgery

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

PI: M. H. Bornstein Head LCE, NICHD

COOPERATING UNITS *(if any)*

Department of Psychology, New York University (Altshuler)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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)*

The purpose of this work is to prepare a critical literature review and to begin data collection, coding, and analysis for a project entitled "The Hospitalization Experience: Children's Coping with the Stress of Surgery." This research is designed to examine age differences in children's understanding of and reactions to a brief stay in the hospital for elective surgery and is based on an integration of the adult stress and coping literature with that on changes in children's cognitive capabilities as they mature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01118-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Latent Behavioral Effects of Diverse Forms of Caretaking in the First Year of Life

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

PI:	M. H. Bornstein	Head	LCE, NICHD
Other:	N. F. Gist	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	.2	1.0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The purpose of this exploratory research study is to learn more about the latent effects of different modes of care in infancy on preschool children whose mothers entered the workforce before their children were 12 months of age. This study is intended to be the first in a series of investigations preliminary to a large-scale effort to document the effects of diverse rearing conditions in the first year of life on children's activities and competences at preschool age. The central purpose of this project is to pinpoint significant variables to be examined in greater detail in the subseqent prospective study. Data are being gathered on a homogeneous, low-risk population and include measures of cognitive, social, and behavioral development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01119-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Specificity of Mother-Infant Interaction

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

PI:	M. H. Bornstein	Head	LCE, NICHD
Other:	J. Suwalsky	Research Psychologist	LCE, NICHD
	P. Ludemann	Research Psychologist	LCE, NICHD
	M. Fivel	Research Psychologist	LCE, NICHD
	C. Rahn	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2	3.3

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

This project investigates environmental factors that contribute to the development of cognitive competencies during the first year of life. Before children are old enough to enter formal social learning situations, nearly all of their experiences stem directly from interactions they have with their primary caretakers. Two conceptually distinct categories of caretaker-child interactions can be identified: social and didactic. These encompass much of the everyday behavior of infants' caretakers. In previous work using samples of convenience, the Principal Investigator linked both of these types of behavior to cognitive development in babies. In the present study set, this work will be replicated and extended by focusing on the extent to which three maternal characteristics (age, employment status, and parenthood status) and type of substitute care experienced during mother's employment influence the observed relations between caregiver social and didactic stimulation on the one hand and infant social and cognitive competencies on the other.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01120-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Observations of Caretaking in Three Societies

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

PI:	M. H. Bornstein	Head	LCE, NICHD
Other:	S. Toda	Visiting Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	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)*

It is widely held that Japanese and Americans differ in prominent aspects of their psychological make-ups and that certain social and intellectual distinctions between members of these two cultures arise early in life. Similarly, previous study on the nature of infant development Israel Kibbutzim determined that many decisive aspects of infant care -- particularly the close ties between infants and mother -- vary markedly from the American experience. Cross-cultural developmental studies have also shown that rearing differences typically have implications for infants' later cognitive and social behavior and performance. The purpose of this project is to identify significant similarities and differences in the childrearing ecologies of Japanese, Israeli, and American infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01121-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Maternal Activities in Children's Language and Play

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

PI: M. H. Bornstein Head LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.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.)*

This study investigates conditional contributions of three domains of maternal activity, including interpersonal affective communication, stimulation of infant attention, and control over object-centered exchanges, to infant language, play, and representational competence at 13 months. Several major data sets have been collected on maternal style, on infant competences, and on the interrelations of maternal style to infant competences. Each of these data sets is highly complex. Each of several infant language and play constructs was assessed in several ways (e.g., concrete versus symbolic play, expression versus comprehension in language), and the degree of independence/interrelatedness among these measures is to be determined in conjunction with an evaluation of the independence/interrelatedness of these measures with maternal activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01122-01 LCE

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Assessment of Children's Mental and Social Abilities

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

PI:	M. H. Bornstein	Head	LCE, NICHD
Other:	C. Tamis-LeMonda	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Program in Applied Child Development, Tufts University (Feldman)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.7	.7	.0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The purpose of this research is to develop new materials, a Project Spectrum Field Assessment Battery, based on the curriculum-oriented Project Spectrum, organized between the Harvard University School of Education and the Eliot-Pearson Department of Child Study at Tufts University. Project Spectrum is unique in the United States for its development of a curriculum that goes far beyond IQ to assess a wide range of preschoool children's interests and capabilities. The 'multiple intelligences' that Project Spectrum's procedures assess include natural science ability, bodily-kinesthetic skills, musical talents, distinctive styles of work, as well as linguistic and logical-mathematical abilities. Because traditional psychometric measures of intelligence at this age sample from a narrow range of mental abilities, such measures are limited in terms of the information they provide about the possible relevance of antecedent variables and are also restricted in terms of the outcome variables they successfully predict. In contrast, the Project Spectrum Field Assessment Battery samples from a wide range of preschoolers' cognitive capabilities and interests and thus affords richer opportunities to respond to many theoretical and pragmatic questions that surround the central issue of stability in mental development.

LABORATORY OF DEVELOPMENTAL AND MOLECULAR IMMUNITY (LDMI)

Z01 HD 00073-17 Regulation of Immune Systems at the Cellular Level
Edgar E. Hanna, Ph.D.

Z01 HD 00920-07 Molecular Structure of Mouse Histocompatibility (H-2) Genes:
Keiko Ozato, Ph.D.

Z01 HD 01301-06 Human Immune Response to Polysaccharide-Protein Conjugate Vaccines
Rachel Schneerson, M.D.

Z01 HD 01304-06 Protective Effect of Vi Polysaccharide Antibodies Against Typhoid Fever
John B. Robbins, M.D.

Z01 HD 01307-05 Pertussis Toxin: An Approach to a New Pertussis Vaccine
Ronald D. Sekura, Ph.D.

Z01 HD 01308-05 Conjugation of Pneumococcal Vi Polysaccharides with Carrier Proteins
Shousun C. Szu, Ph.D.

Z01 HD 01310-02 Developmental Gene Regulation of the Immune System
Keiko Oazto, Ph.D.

Laboratory of Developmental and Molecular Immunity
John B. Robbins, M.D., Chief

Interurine, newborn, infant and childhood is a period of development when there is change in the expression, and eventual adult expression of immunity with its regulatory mechanisms and cell products. The basis for this development and the relation of incompletely express systems to the acquisition of bacterial diseases has been the object of study by this Laboratory.

Bacterial Disease Pathogenesis and Immunity

This Section has been concerned with the specific cell products responsible for virulence and the host immune mechanisms involved in prevention of common and serious bacterial diseases of the neonates, newborns and young children. There a series of invasive diseases, due to encapsulated bacterial pathogens of which Haemophilus influenzae type b is the most common, in which the mechanism of virulence and protective antigen of the pathogen have shown to be its capsular polysaccharide. Synthetic schemes have been developed in order to convert the capsular polysaccharide of H. influenzae type b into a immunogen capable of inducing protective levels of antibodies in infants by covalently binding it to a protein. The development of a conjugate vaccine, composed of the H. influenzae type b capsular polysaccharide chemically bound to tetanus toxoid has been tested and shown to be capable of inducing protective levels of antibodies in infants after the second injection of this vaccine concurrently with DTP. The third injection induces antibodies observed in adults in immune adults. Passively acquired antibodies to the H. influenzae type b capsular polysaccharide and to the tetanus toxoid induce antigens specific suppression despite the fact that both components are on one molecule. Effectiveness studies, to understand the ability of this new vaccine to prevent H. influenzae type b meningitis and other related serious diseases of infants and children are underway. The same technology has been applied to pneumococcus type 6 and pneumococcus type 12 capsular polysaccharides and studies to evaluate the effectiveness of these new vaccines, especially in infants and children with Sickle cell anemia, are also underway. Another serious invasive disease, caused by encapsulated bacteria, is typhoid fever. Two controlled double-blinded field trials have verified the protective effect of the Vi capsular polysaccharide to prevent typhoid fever. The immunogenicity of the Vi has been improved by covalently binding it to a T-dependent carrier protein. In this case, a conjugate of ViTT has been synthesized and its immunogenicity, safety and protective activity is being studied in Nepal. The notion that serum antibodies can prevent invasive diseases caused by enteric bacteria has now been directed toward non-typhoidal Salmonellae and to Shigellae. The O-specific side-chains of these two bacteria have been purified, detoxified, and successfully bound to the β-subunit of cholera toxin, and the β-subunits of the Shigellae toxins. Clinical trials to verify the safety and efficacy of these new vaccines are underway. Bacteremic disease due to Staphlococcus aureus remains a serious and common problem of patients in hospitals. It has been discovered that S. aureus have capsular polysaccharides that are covalently bound to the bacterial cell wall and have unusual chemical properties which require new serologic methods for their detection and new chemical processes for their isolation and characterization. Antibodies to these capsular polysaccharides have been shown to facilitate in vitro

phagocytosis. Conjugates, composed of the S. aureus type 8 and type 5 capsular polysaccharides chemically bound to Pseudomonas aeruginosa exotoxin A have been achieved and these two conjugates are planned for clinical trial. It is hoped that passive immunization of vaccine induced antibodies to these capsular polysaccharides may prevent bacteremic S. aureus disease in hospitalized patients.

Originally postulated by the LDMI, a new vaccine, composed of pertussis toxin inactivated by a novel reagent for preparing vaccines, hydrogen peroxide, has been studied in adults and children. The purified toxoid has been characterized by physical, chemical as well as biologic means and is shown to be biologically inactive and pyrogen-free. Metabolic and clinical studies have shown that the toxoid is immunogenic and safe in adults and children. The levels of antibodies induced by pertussis toxoid vaccines were comparable to or slightly higher than those observed in adults convalescent from disease. Levels of antibodies in infants and children were higher than those induced by DTP vaccine. Clinical trials to assess the safety and immunogenicity of this pertussis toxoid in infants in Boston, Massachusetts and Goteborg, Sweden are underway. It has been postulated that serum antibodies to this toxin confer antibacterial, antitoxin and antipertussis activity to the immunized host.

Immunoregulation and Cellular Control

Dr. Hanna has been studying the effects of bacterial toxins upon cloned lymphocyte hybridomas capable of expressing either precursor or mature T-cell regulatory phenotypes. Immune cytotoxic (CTL) cells have also been studied similarly. Several bacterial toxins, including libopolysaccharide (endotoxin, streptococcal pyrogenic, enterotoxin C, toxic shock syndrome toxin 1, pertussis toxin and streptococcal pyrogenic exotoxin) have been used to probe these precursor cells. The target for the action of these toxins has been studied by examining the effect upon antibody forming cells and cytotoxic T-cells incubated with these toxin treated precursors. Streptococcal pyrogenic exotoxin has been shown to result in decreased suppressor cell function. One of the mechanisms proposed is that the precursor regulatory T-cells, following treatment with this toxin, have lost their capacity to express interleukin-2 receptors. Streptococcal pyrogenic exotoxin was also observed to permanently reduce the amount of CD8, but not CD4 of double expressing (CD4/CD8) precursor clones. Similarily, pertussis toxin treated precursor cells result in a decrease in the cytotoxic T-lymphocyte response of mice. The cellular basis for these toxin-mediated effects upon precursor lymphocytes is under study.

Molecular Genetics of Immunity

Major histocompatibility class I antigens, an essential component of protective immunity, are a highly polymorphic group of cell surface components. Expression of these antigens varies according to developmental stage and type of tissues. Lymphokines, such as interferon, and tumor necrosis factor induce the expression of the antigens. The molecular basis for the regulation of MHC class I antigen expression has been studied at the molecular and cellular level by Dr. Ozato and her colleagues. The DNA sequences upstream of the MHC class I genes have been isolated and some of their functions that could exert regulatory activity have been studied. Two

gene segments, in particular, have been identified. They are the conserved 5' upstream class I regulatory element (CRE) and the interferon consensus sequence (ICS) which are involved in both the developmental expression of and induction of class I MHC antigens by interferon. Site-directed Mutagenesis of these segments have been used to determine the precise sequence required for regulation. The activity of the mutant regulatory sequences has been studied by using chloramphenical acetyl transferase (CAT assay).

The effect of protein binding to the CRE was studied by using undifferentiated F9 embryonal carcinoma cells which do not express MHC genes and L-fibroblast in which these genes are expressed. It was found that only two of three sequences in the CRE are bound from nuclear proteins extracted from F9 embryonal cells. In contract, all three regions of CRE were bound by nuclear proteins extracted from the L-fibroblasts. Protein binding to the CRE correlated well with the stage of development in that binding proteins are found only in tissues that express MHC class I genes. Mutants that have altered binding sites within the CRE failed to interact specific binding proteins. Enhanced MHC class I gene expression was related to the binding to each of the three CRE regions. Binding the nuclear factors to the interferon consensus sequence also exerts effect upon expression of class I MHC genes. Interferon treatment induced binding of new proteins to this sequence, which correlated with enhanced MHC class I genes.

The c-fos oncogene, which encodes a DNA binding nuclear protein, is another regulatory gene believed to be involved in both differentiation and development of the immune system. The technique of using antisense RNA was used to study the function of c-fos gene upon class I MHC antigens. A c-fos antisense plasmid was prepared and introduced into F9 embryonal carcinoma cells. Such cells, which express c-fos antisense RNA, were unable to synthesize c-fos RNA and c-fos proteins in response to interferons and phorbol esters. The antisense c-fos clones also showed a reduced expression of c-myc oncogene. A novel factor, which may be involved in a global gene activation process at birth, was found to be specifically expressed at the neonatal stage. The neonatal protein binds the enhancer region of the c-fos gene, which may be involved in increased expression of class I MHC genes after birth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00073-17 LDMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Immune Systems at the Cellular Level

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

PI:	Edgar Hanna	Head	LDMI, NICHD
Others:	Michael Walker	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*

P. Arora, LN, NIDDK; K.P. Huang, ERRB, NICHD; C. Hansen, VR, DRS

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Immunoregulation and Cellular Control

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Investigations in this laboratory are directed towards understanding the mechanisms by which microorganisms activate, modulate, or subvert immune systems. We postulate these events are mediated within developmental and regulatory pathways of precursors of regulatory T-cells for antibody forming cells and cytotoxic T-cells. Macrophages and natural cytotoxic (NK) cells may also be effected through modulatory effects upon their precursors of their regulatory cells. We have exploited various bacterial toxins as natural probes to facilitate an experimental delineation of mechanisms in this respect. An in vitro modular immune system ("cell complementation system" involving murine spleen immunocytes) allows us to rearrange the order and relative numbers of toxin treated/or untreated cells when recombined in the immune system. Further progress in constructing and cloning perpetual cell lines possessing the phenotypes of many of the parent regulatory immunocytes is ongoing. These phenocopies of regulatory cells are used as targets of the bacterial toxins and subsequently tested for altered function. They may facilitate production of large amounts of homogeneous gene products and cells; thus, promoting continuity of ongoing experiments in using homogenous cells at the same stage of development. Suppressor-negative clones from one of our suppressor precursor clones (NBP2C2, a CD4/CD8 double-positive clone), in the presence of SPE were detected. Similar selections using Et resulted in subclones retaining parental phenotype. But, cell-free supernatants of Et treated, unfractionated NFR/N or nude mouse splenocytes supported an 80-90% recovery of suppressive activity by the SPE selected subclone. Macrophage-depletion negated this activity of supernatants. Selected clones were observed not to differ from their parent in expression of MHC haplotype and an epitope of the TCR-agn, while there were effects upon expression of IL-2R in the parent clone by SPE and to various magnitudes with TSST-1, Pt, and SE-C. The SPE selected subclone expressed markedly less CD8 than its parent. These results may explain the contrasuppressive activity of SPE and similar bacterial products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00920-07 LDMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Structure of Mouse Histocompatibility (H-2) Genes

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

PI:	Keiko Ozato	Head	LDMI, NICHD
Others:	Paul Driggers	IRTA Fellow	LDMI, NICHD
	Peter Burke	Intramural NRSA	LDMI, NICHD
	Yasuaki Shirayoshi	Visiting Fellow	LDMI, NICHD
	Kazushige Hamada	Visiting Fellow	LDMI, NICHD
	Kira Phimmascone	Bio-Aid	LDMI, NICHD

COOPERATING UNITS *(if any)*
E. Appella, LCB, NCI

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Molecular Genetics of Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.55	4.25	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.)*

Major histocompatibility complex (MHC) class I genes encode highly polymorphic transplantation antigens essential for T-cell immune responses. This program addresses how MHC class I genes are regulated during development and by lymphokines. The current emphasis is the analysis of trans-acting regulatory proteins that bind to the cis-acting DNA sequences of the MHC class I gene. Two highly conserved cis-acting regulatory seqences in the 5' upstream region of the class I gene govern developmentally controlled and IFN-induced expression of class I genes. These designated the class I regulatory element (CRE) and interferon consensus sequence (ICS), respectively. By gel mobility shift analyses and methylation interference experiments, we found at least three sequences in the CRE that bind independent nuclear proteins. These three sequences, region I, II, and III correspond to inverted and direct repeats present in the CRE. During fetal stage when MHC class I expression is extremely low, a protein that binds region I is nondetectable, although a protein for region II is present in a large amount. Concomitant with a sharp increase in class I mRNA levels, region I-binding protein becomes detectable at the neonatal stage, denoting a correlation between class I gene expression and the presence of region-I binding protein. We found that IFN treatment induces binding of at least two new proteins to the ICS is induced. These two proteins differ in requirement of de novo protein synthesis. Mutations in the binding region but not in other parts of the ICS abrogate transcriptional enhancement by IFN. A similar motif occurs in other IFN-inducible genes of mouse and human, and they are capable of competing for the proteins that bind the ICS of class I genes. These results indicate that binding of protein to the ICS represents the basic mechanism of IFN-induced transcriptional activation of not only class I but other genes. Finally, in order to dissect functional significance of protein binding to the CRE and ICS, we developed an assay for in vitro transcription of the class I gene. In this assay DNA templates containing the class I upstream region direct class I mRNA synthesis in a cell free condition. This system should allow us to dissect the mechanism of MHC class I transcription in detail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01301-06 LDMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Human Immune Response to Polysaccharide-Protein Conjugate Vaccines

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

PI:	Rachel Schneerson	Research Medical Officer	LDMI, NICHD
Others:	John B. Robbins	Head	LDMI, NICHD
	Yong-Hong Yang	Visiting Fellow	LDMI, NICHD
	Teresa Lagergard	Visiting Associate	LDMI, NICHD
	Lilly Levi	Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)* G. Schiffman, State University, NY; J.C. Parke, Jr., Charlotte Memorial Hospital, NC; J. Schlesselman, USUHS, Bethesda, MD; B. Trollfors, J. Taranger, B. Claesson, University of Goteborg, Sweden; C. Lowe, OD, NICHD; D. Bryla, EBRP, NICHD.

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The age-related and T-independent properties, of Haemophilus influenzae type b capsular polysaccharide (Hib) and other polysaccharides of invasive organisms, limit their protective actions in infants and children, that age group which the highest attack rate of diseases due to these encapsulated pathogens. Organic synthetic schemes, that bound Hib and other capsular polysaccharides to tetanus toxoid, were devised in order to both increase the immunogenicity of and confer T-cell dependence (booster effect) to these protective antigens. Based upon ours, and others work in the field, a conjugated Hib vaccine, prepared by our original method, was licensed by the FDA for universal use in children older than 18 months of age. The safety and immunologic properties of our Hib-TT vaccine has been investigated in 18 month olds and now to two to three month old infants. The preliminary results show that protective levels of antibodies were induced in the young infants after two injections. Effectiveness study of this vaccine in infants and children in Charlotte, N.C. and Goteborg, Sweden are being planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01304-06 LDMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protective Effect of Vi Polysaccharide Antibodies Against Typhoid Fever

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

PI:	John B. Robbins	Head	LDMI, NICHD
Others:	Rachel Schneerson	Research Medical Officer	LDMI, NICHD
	Shousun Szu	Research Chemist	LDMI, NICHD
	Tod Cramton	Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)* H. Kornhof, African Institute of Research; I.L. Acharya, Infectious Disease Hospital, Kathmandu, Nepal; R. Kumar, All India Institute of Medical Sciences; C. Lowe, OD, NICHD; D. Bryla, EBRP, NICHD; M. Cadoz, Institut Merieux, Lyon, France; F.-Y.C. Lin, Agency for International Development, DC.

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Enteric fevers, of which typhoid fever is the most common, remain a serious and frequent cause of morbidity and mortality in most underdeveloped nations. These group of diseases are caused by the Genus salmonellae. The most frequent and serious of these enteric fevers in underdeveloped nations is typhoid fever caused by the Salmonella typhi. The next most common cause of enteric fevers in underdeveloped nations is Salmonella paratyphi A. Evaluation of vaccines for prevention of these diseases has a long and varigated history because both organisms are inhabitants of pathogens for humans only. Two, double-masked, randomized, controlled evaluations of the Vi of Salmonella typhi has shown its ability to prevent typhoid fever in Nepal and in the Eastern Transvaal of the Republic of South Africa. No significance side reactions were observed and effectiveness rate of 70% has been observed for two years. Surveillance and long-term serologic studies are in progress. Based upon these data, new vaccines for the prevention of non-typhoidal enteric fevers using the Vi capsular polysaccharide O-specific side chain of Salmonella paratyhi A covalently bound to carrier proteins is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01307-05 LDMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Pertussis Toxin: An Approach to a New Pertussis Vaccine

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

PI:	John B. Robbins	Head	LDMI, NICHD
Others:	Rachel Schneerson	Research Medical Officer	LDMI, NICHD
	Teresa Lagergard	Visiting Associate	LDMI, NICHD
	Nathaniel Tolson	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*
J. Shiloach, B. Kaufman, NIDDK; B. Trollfors, University of Goteborg, Sweden; G. Siber, Massachusetts Public Health Laboratories, Jamaca Plains, MA: C. Lowe, OD, NICHD; D. Bryla, EBRP, NICHD.

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The incidence and severity of pertussis have been controlled by widespread immunization with DTP which contains inactivated Bordetella pertussis organisms (cellular vaccine). The identification of pertussis toxin, an extracellular protein of this pathogen, as a major, if not the sole protective antigen, was the basis for producing a new vaccine with improved safety and immunogenicity characteristics. B. pertussis was cultivated in a 100L fermenter and the pertussis toxin extracted from the culture supernant by affinity chromatography. The pertussis toxin was inactivated by hydrogen peroxide treatment under controlled conditions. The resultant toxoid, NICHD-PTxD, had less than 1% of its original binding and enzymatic activities by in vitro assays. In vivo assays, which require binding and enzymatic activity on the same molecule, showed no residual activity. Based upon the clinical satisfactory and serologic response in adults injected with this toxoid, two clinical studies with this pertussis toxoid have been completed in 18 month old children. The first, in Boston, gave one injection into 18 month old children previously immunized during infancy with the recommended three doses of DTP. The second gave two, and in some cases three injections, of this toxoid in Swedish children without previous history of either pertussis or known DTP vaccination. The results of both studies show that the pertussis toxoid has both superior safety and immunogenicity characteristics compared to the cellular vaccine component of DTP. The new batch of pertussis toxoid has been formulated and clinical testing of this material for safety immunogenicity in infants and, hopefully, effectiveness in that age group is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01308-05 LDMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Conjugation of Pneumococcal and Vi Polysaccharides with Carrier Proteins

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

PI:	Shousun Szu	Research Chemist	LDMI, NICHD
Others:	John B. Robbins	Head	LDMI, NICHD
	Ali Fattom	Visiting Associate	LDMI, NICHD

COOPERATING UNITS *(if any)*
J.L. Inman, LI, NIAID; W. Vann, OBRR, FDA; W. Karakawa, Dept. of Biochemistry, Pennsylvania State University, PA.

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	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)*

Covalent attachment of polysaccharides, that are potentially protective antigens, to carrier proteins is dependent upon both the size and structure of the two vaccine components. Methods for conjugating the Vi capsular polysaccharide, and similar polysaccharides of high molecular weight containing carboxyl functions were devised using the heterobifunctional cross-linking agent SPDP. Polysaccharides, such as the O-specific side chains of Salmonella paratyphi A and Shigella dysenteriae type 1 (Shiga) were derivatized by covalently binding the reducing terminal end with adipic acid dihydrazide using the technique of reductive amination with cyanoborohydride. The latter derivatives are coupled to the beta subunit of cholera toxin and of Shigella toxins by the carbodiimide reagent. The efficiency, physical chemical properties, standardization, and immunologic properties of these newly devised conjugates are under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01310-02 LDMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Developmental Gene Regulation of the Immune System

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

PI:	Keiko Ozato	Head	LDMI, NICHD
Others:	Ben-Zion Levi	Visiting Associate	LDMI, NICHD
	Steven Hirschfeld	Medical Staff Fellow	LDMI, NICHD
	Shannon Gleason	NRC Biotechnology Fellow	LDMI, NICHD
	Bonnie Orrison	Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Molecular Genetics of Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD, 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.5	3.5	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 program addresses expression and function of regulatory genes that control development of the immune system. The c-fos oncogene that encodes a DNA-binding nuclear protein is one of such regulatory genes and is implicated to play a role in development and differentiation. To study the function of c-fos gene, we prepared an antisense plasmid that can produce a large amount of c-fos antisense RNA. This antisense plasmid was introduced it into F9 embryonal carcinoma cells. F9 cells that expressed c-fos antisense RNA were unable to induce c-fos mRNA and c-fos protein in response to interferons and phorbol ester and had a reduced basal level of c-fos mRNA. Expression of c-fos gene in these cells could be rescued by cycloheximide treatment, demonstrating that the blockade of c-fos gene expression in the antisense clones is due to inhibition of c-fos message expression by the antisense RNA. Further analyses of the antisense clones showed that the levels of c-myc oncogene is reduced by 5- to 10-fold, indicating a specific linkage between c-fos and c-myc oncogene. Expression of c-fos gene is induced on the day of birth in the mouse; this induction is systemic but transient. We recently found that another immediate early gene, Egr that encodes a Zn finger protein is induced at birth in certain tissues. This, and other reports, indicate that gloval gene activation takes place at birth, which may be responsible for controlling neonatal development. To study the basis of the c-fos gene induction at birth, we searched for a nuclear factor that binds the 5' upstream region of the c-fos gene in a gel mobility shift assay. Nuclear extracts from most adult and fetal tissues that do not express c-fos elicited a slow migrating band, which represents factor binding to the ~20 bp enhancer element. The enhancer controls c-fos induction by serum in tissue culture cells. In extracts obtained at birth, a faster migrating band was detected, which was either absent or very low in the fetal and adult extracts. This neonate-specific band also repsesented a c-fos enhancer binding protein. Additional experiments led us to conclude that the neonatal c-fos enhancer binding activity constitutes a novel factor distinct from the factor present in adult and fetal tissues.

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY (LDN)

Z01 HD 00047-19 Biochemical Studies of Neuronal and Other Cell Types
Douglas E. Brenneman, Ph.D.

Z01 HD 00048-14 Transcriptional-level Control of Neurobiologic and Development Phenomena
Bruce K. Schrier, M.D., Ph.D.

Z01 HD 00064-12 Neurobiologic Studies of Neurons and Glia in Cell Culture
Phillip G. Nelson, M.D., Ph.D.

Z01 HD 00094-18 Pineal Regulation: Environmental and Physiological Factors
David C. Klein, Ph.D.

Z01 HD 00095-18 Pineal Regulation: Transsynaptic and Intracellular Mechanisms
David C. Klein, Ph.D.

Z01 HD 00704-03 Tetanus Toxin Effects and Localization in Neurons
Elaine A. Neale, Ph.D.

ZO1 HD 00705-06 Functional Organization of the Nerve Terminal
J. T. Russell, Ph.D.

Z01 HD 00706-02 Physiological Studies of Nervous System Development In Vitro
(Inactive)

Z01 HD 00707-04 Pharmacological Studies of Synaptic Transmission In Vitro
Mark L. Mayer, Ph.D.

Z01 HD 00708-04 Morphologic Studies of Neuronal and Non-Neuronal Cells in CNS Cell Cultures
Elaine A. Neale, Ph.D.

Z01 HD 00709-02 Prevention of Neuronal Deficits Associated with AIDS
Douglas E. Brenneman, Ph.D.

Laboratory of Developmental Neurobiology

The program of the Laboratory of Developmental Neurobiology (LDN) has been substantially strengthened by changes in personnel and organization made during FY 88.

1. Dr. Mark Mayer has been made a Visiting Scientist and his intent for tenure action approved. He now heads up the Unit on Neurophysiology and Biophysics and has inaugurated a productive and independent program of research.

2. Dr. James Russell has joined the LDN and has been proposed for the position of Head of the Section on Neuronal Secretory Systems.

3. Dr. Douglas Brenneman now has a tenured position in the LDN and is functioning as Head of the Unit on Neurochemistry.

4. Dr. Bruce Schrier is retiring in October '88 and Dr. Andres Buonanno has been recruited to fill the position of Head of the Molecular Neurobiology Unit. He has initiated a program directed at the molecular biological analysis of excitatory amino acid receptors and of axon-oligodendrocyte interaction.

Thus the LDN is currently composed of the following Sections and Units:

1. The Section on Neurobiology headed by Dr. Phillip Nelson is concerned with cellular and molecular mechanisms important for nervous system development.

2. The Section on Neuroendocrinology headed by Dr. David Klein studies the pharmacology and molecular and cell biology of the pineal gland.

3. The Section on Neuronal Secretory Systems headed by Dr. James Russell investigates mechanisms of the nerve terminal important for secretion of peptides and other neurotransmitters.

4. The Unit on Cell Biology headed by Dr. Elaine Neale uses morphological and cell biologic methodologies in analyzing neural function and neurodevelopment.

5. The Unit on Neurophysiology and Biophysics headed by Dr. Mark Mayer is concerned with membrane and molecular mechanisms involved with neuronal excitability and their responses to excitatory amino acids.

6. The Unit on Neurochemistry headed by Dr. Douglas Brenneman investigates trophic interactions between neuron and glia that are important for nervous system development. This work has been extended to the study of mechanisms that may be involved in nervous system pathology in AIDS.

7. The Unit on Molecular Neurobiology to be headed by Dr. Andres Buonanno will continue to use molecular neurobiologic methodologies in analyzing important neurobiologic processes.

Section on Neurobiology

The concept of the "Darwinian synapses", that is, neural connections that survive during development because of their appropriate involvement in activity elicited by environmental stimuli, has received considerable attention recently. In continuation of work of some years standing relevant to this concept, the Section on Neurobiology (including Drs. Nelson and Yu in collaboration with Drs. Neale and Fields) has made progress in addressing the issue of differential synaptic development related to electrical activation of different populations of synapses. A three-chambered tissue culture system allows selective stimulation of one of two populations of sensory neurons from two side chambers forming synaptic connections with ventral horn spinal cord neurons in the central chamber. Using intracellular recording techniques, we can assess the number of axons innervating each spinal cord cell, and the size of the synaptic responses produced by these axons. Stimulation of a given set of sensory axons produces an increase in the numbers of axons maintaining synaptic connection by that set of axons and the unstimulated axons as well. Thus the number of axons connected is relatively unselectively increased by axonal activation. The strength of the synaptic connection is selectively increased, however, so that the stimulated afferents gain a competitive, 'Darwinian' advantage over their unstimulated counterparts. Consonant with earlier observations made in the LDN, our observations suggest that synaptic activity initiates two opposing processes that produce stabilizing, augmenting effects on the one hand and inhibitory or destabilizing effects on the other. We have begun the analysis of these two processes by manipulating the Ca^{++} ion concentrations in the bathing medium and selectively blocking an excitatory synaptic receptor, the N-methyl-D-aspartate receptor, during the period of chronic synaptic activation.

Unit on Neurochemistry

A major effort in the LDN this year has focused on investigating the neuronal deficits produced by the external envelope protein (gp120) of the human immunodeficiency virus. One of the confounding aspects of HIV is the diversity of strains and the problems this poses in efforts to neutralize this virus. Workers in LDN have shown that gp120 from three known strains of HIV and two others which are yet to be characterized all produce cell death in hippocampal cultures derived from fetal mice. They have shown that this cell death is mediated through the mouse homologue of the CD4 receptor. Interestingly, they found that antiserum made against an octapeptide of gp120 from the ARV isolate was able to prevent gp120-induced neuronal cell death from all of the HIV strains mentioned above. These data suggest some hope in fighting the effects of the virus, despite its genetic diversity.

In addition to the gp120/neuronal cell death project, an effort was made in this Unit to examine peptide drugs that may prevent the gp120-mediated effects. Peptide T, an octapeptide sequence found in the external envelope protein, was found to potently and completely antagonize gp120-induced death in dissociated hippocampal test cultures. Analogs of the peptide T sequence found in other isolates of HIV were also shown to be active with this assay. Although the mechanism of action of Peptide T is not discernible from these early experiments, these studies indicate that this drug is effective in preventing neuronal cell death associated with gp120 and that it provides a rationale for peptide T to be tested as a therapy for the neuropsychiatric and neurological sequelae of Acquired Immune Deficiency Syndrome.

The LDN has had a rich history in examining the physiological aspects of excitatory amino acids in the central nervous system. This year, another aspect of the NMDA

receptor has been explored for its role in determining the structure and plasticity of developing neural networks. Brenneman and colleagues have found that NMDA antagonists can accelerate neuronal cell death during a critical period of development in cell culture. In addition, NMDA itself was found to increase the survival of neurons under conditions of electrical blockade. These data suggest that excitatory amino acids may have important "trophic" or developmental roles in addition to their recognized function as mediators of excitatory synaptic transmission.

The cell biology of neurotrophic interaction has been addressed by Dr. Alderson. He has identified two proteins of 15 and 40 K daltons as the major substrate for vasoactive intestinal peptide (VIP) induced phosphorylation in cortical astrocytes. He has shown an interaction between NGF and TPA in their effects on survival of cholinergic neurons in culture from the medial forebrain of fetal mice.

Unit on Molecular Neurobiology

The Molecular Neurobiology Unit has obtained four cDNA clones from differentiated mouse neuroblastoma cells and two from wounded rat cerebral cortex, the fusion proteins of which have neuronotrophic activity in one or more of a variety of assays. Sequencing of the inserts in these clones has not provided an understanding of the nature of their trophic activities. One of these clones with exceptional amounts of trophic activity in the chick sympathetic ganglion assay proved to contain a portion of the small subunit of mitochondrial ribosomal RNA. Experiments to determine the source of the neurotrophic activity of this molecule are in progress. Two cDNA clones from neuroblastoma mRNAs, when their transcripts are introduced into frog oocytes, cause the oocytes to be very sensitive to applied angiotensin II, resulting in the marked inward flow of negatively charged ions. Early sequencing data from one of these show the presence of intracisternal A particle sequences at both ends of the insert. The transcript of another clone from neuroblastoma cells causes oocytes to respond to the application of minute quantities of the head activator peptide (HAP), an undecamer which is known to be excreted by some neuroblastoma cells, perhaps in an autocrine regulatory scenario. We are pursuing this clone as a putative receptor for the head activator peptide. A cDNA clone from rat hypothalamus which bound one of two degenerate oligonucleotide probes we designed for the mRNA for HAP, proved not to encode HAP. A clone from mouse neuroblastoma cells which also binds these oligomers is presently being pursued, as is the production of an antibody to HAP, a very poor immunogen. We designed three oligonucleotide probes for conserved regions of the human alpha-2 adrenergic receptor for use in detecting the receptor from mouse tissues. We presently have one rat genomic clone which binds one of the oligomers and is being sequenced. Sequence collected to date shows a >700 bp open reading frame and hydrophilicity regions which may be consistent with a receptor structure. The mRNAs of several tissues are being examined for the binding of the oligomers, for the purpose of obtaining a cDNA clone of the mRNA for the receptor. The MNU is soon to undergo a significant change in personnel; some of these projects will be continued in collaborative arrangements.

Section on Neuroendocrinology

Under the direction of David C. Klein, the Section on Neuroendocrinology has used the pineal gland to make fundamental advances in signal transduction, both on a molecular-mechanistic basis and on a conceptual basis. In addition, the Section has made important advances towards a better understanding of the neural control of gene expression.

Biochemical "AND" gates: The concept of biochemical "AND" gates has evolved within the Section on Neuroendocrinology within the last year. Biochemical "AND" gates are transmembrane signalling mechanisms which integrate input from two sources. Like the electronic "AND" gates, which allow a signal to pass only if one input and a second are activated, biochemical "AND" gates control biochemical process in a similar all or none manner.

Two examples come from studies on pineal cyclic nucleotides, and a third comes from studies on the efflux of potassium ions from pinealocytes. Knowledge of the regulation of pineal cyclic nucleotides has expanded significantly within the last year as a result of intense and highly productive studies by Drs. Anthony Ho and Constance Chik. Their efforts have allowed the Section to pioneer analysis of dual receptor mechanisms which regulate cyclic AMP and cyclic GMP.

Stimulation of pinealocytes by norepinephrine, the physiological transmitter of the pineal gland, causes 100- to 200- fold increases in both cyclic AMP and cyclic GMP. Norepinephrine is known to act through both α_1- and β-adrenergic receptors. Full stimulation requires activation of cyclases and of protein kinase C. Activation of adenylyl and guanylyl cyclase occurs as a result of receptor occupancy by β-adrenergic agonists or by VIP, or by postreceptor actions of cholera toxin or forskolin. However, these actions produce less than a 7% of maximal cyclic AMP response and less than a 3% of maximal cyclic GMP response. Full stimulation occurs only if protein kinase C is activated, which occurs as a result of α_1-adrenergic stimulation. Studies in the Section have indicated that α_1-adrenergic agonists act primarily through an elevation of $[Ca^{2+}]i$, which alone seems to cause translocation of protein kinase C, which is sufficient to cause full stimulation of cyclic AMP. In the case of cyclic GMP, the elevation of $[Ca^{2+}]i$ has a second effect, one which probably involves arachidonic acid.

The third example of an "AND" gate comes from studies in the Section on K^+ efflux, which were performed by Valentine Cena and David C. Klein. Several years ago Klein and his coworker Joan Weller discovered that norepinephrine produces a marked increase in the release of ^{42}K. Klein has continued to study this with Cena, and during the past year has discovered that this release seems to require the interaction of both cyclic AMP and of Ca^{2+}. As discussed above, the increase in cyclic AMP requires activation of both α_1- and β-adrenergic receptors. Release of K^+, however, does not occur if cells are loaded with cyclic AMP. Elevation of $[Ca^{2+}]i$ is also required. Thus there appears to be an "AND" gate which is regulated by both Ca^{2+} and cyclic AMP. Extensive studies on the identity of the gate points to the likely possibility that it belongs to a class of K^+ channels which is activated by Ca^{2+} and inhibited by charybdotoxin. These studies involved biochemical and patch clamp analysis. The biochemical studies provide the main support for the contention that Ca^{2+} alone is not sufficient to open this channel, but that cyclic AMP also seems necessary.

These studies provide clear models of biochemical "AND" gates. It seems probable that such "AND" gates function throughout the body to integrate input. They could play a critical role in the brain, to both decrease noise and to increase the selectivity of neural interactions, by requiring simultaneous activation of two membrane transduction systems.

An outgrowth of the "AND" gate is the concept of multiheaded silver missiles-modern day versions of the elusive silver bullet. Multiheaded silver missiles would contain agonists which are carefully selected on their ability to operate "AND" gates in specific cells. The agonists chosen would not necessarily represent the physiological regulators

of the cell, but would be able to act in a similar manner. For example, in the case of the pineal gland, the α_1- and β-adrenergic stimulatory action of norepinephrine could be mimicked by VIP and phenylephrine.

Pineal Molecular Biology: The Section has made several important advances towards their goal of understanding how the enzymes in the tryptophan->melatonin pathway are regulated during development and by neural mechanisms. Major advances have been made with tryptophan hydroxylase and hydroxyindole-O-methyltransferase. Joan Weller has isolated several sheep tryptophan hydroxylase DNA clones. Initial studies have indicated that at night the tissue content of one species of tryptophan hydroxylase mRNA increases significantly. Studies performed using a human pineal cDNA library, in collaboration with Ed Ginns (NIMH) is leading towards the isolation of human tryptophan hydroxylase. Helena Illnerova, working with Joan Weller has isolated several human pineal hydroxyindole-O-methyltransferase cDNA clones, which are being characterized. The human pineal cDNA's will be used in collaboration with other groups for genetic analysis.

Unit on Cell Biology

Tetanus toxin alters neuromuscular activity, but does not have a direct physiologic effect at the neuromuscular junction. It acts only after uptake at the nerve ending and retrograde axonal transport to the motor neuron cell body. Tetanus toxin receptors are believed to be gangliosides, although there is indirect evidence that a protein receptor may exist also. Investigators in this Laboratory have been studying various aspects of tetanus toxin-neuron interactions, in neuronal cell cultures, for a number of years. Dr. Elaine Neale has undertaken studies aimed, ultimately, at defining some of the cell biology of tetanus toxin action. These involve following the pathway of endocytosis of the toxin molecule and determining whether the organelles involved are different from those implicated in protein receptor mediated endocytosis. Spinal cord neurons, in culture, exhibit a predictable electrophysiologic response upon exposure to the toxin. Intracellular localization of the toxin at selected times during the course of toxin action might suggest which organelles are involved in the production of paroxysmal depolarizing events and which, in the long-lasting electrical quiescence which ensues. Additional studies of toxin effects on neurotransmitter release, and on the survival of developing neurons, are planned.

Experiments to date have indicated that immunocytochemistry may provide a viable approach to intracellular toxin localization. A number of monoclonal antibodies are available, both neutralizing and non-neutralizing, and specific for known regions of the toxin molecule. Pre-mixing certain of these antibodies with toxin increases the levels of both toxin and antibody bound to neurons. The complex appears to be internalized, and disappears with a half-life that is similar to that of the toxin alone. Additional studies show that one particular antibody, which is non-neutralizing and specific for the Fragment C (binding) portion of the molecule, forms a complex with Fragment C which binds to the neuron surface, is non-toxic, and can be used as a substrate for immunofluorescence of living cultures. This probe can be used on neurons within two hours of plating, and appears to persist for several days. Studies are in progress to define the feasibility of labeling freshly dissociated neurons in suspension (prior to plating) such that they may be identified at some later time in co-culture with unlabeled neurons.

Section on Neuronal Secretory Systems

The nerve terminal is a highly specialized region of a neuron, separated from the

neuronal soma by an axon, whose function is to release neurotransmitter quanta and to regulate the number of quanta secreted. Secretion of neurotransmitters and other biologically active substances from nerve terminals form the fundamental means by which the central nervous system (CNS) operates from the time of development to higher order functions in the adult. Modulation of the quantity of the transmitter released at the terminal may form the basis for all central nervous system functions, including integration of information, and long term information storage, and retrieval. This modulation is achieved by transduction of information content in the action potential train, and by local influences at the nerve terminal via activation of receptors at the terminal and the resultant modification of the responses of the terminal membrane. Because of the complexity (cellular heterogeneity, and their complex organization), and extremely small size, basic understanding of the molecular mechanisms of nerve terminal function in the central nervous system is lacking. The program of the Unit on Neuronal Secretory Systems is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. The nerve terminals of the neurons of the hypothalamo-neurohypophysial system, which secrete vasopressin or oxytocin are discretely localized in the neurohypophysis, where they are accessible to experimental manipulations both in vivo and in vitro. These nerve terminals could be isolated from the neurohypophyses without contamination by the post-synaptic membrane, unlike nerve terminals from other regions in the central nervous system.

Studies on the elucidation of the functional organization of the nerve terminal forms the central theme of the Unit on Neuronal Secretory Systems. The current focus of the Unit is on the investigation of the importance of ionic channels and receptors on the initiation, and modulation of secretion at the nerve terminal. Dr. Carolyn Bondy's experiments have shown that a type of K^+ channels may play a central role in modulation of secretion at the terminal caused by frequency information in the action potential train. Dr. Bondy also showed that this K^+ channel is blocked by dendrotoxin, a polypeptide toxin isolated from the venom of the South African green mamba, Dendoaspis angustyiceps. Kappa opiate receptors present on the oxytocin nerve terminals were shown to be involved in the modulation of oxytocin secretion. In these experiments Dr. Bondy showed that dynorphin cosecreted with vasopressin specifically was involved in down regulation of oxytocin secretion from the neighboring oxytocin terminals. The molecular mechanism by which kappa receptor occupation results in inhibition of oxytocin secretion is currently under investigation.

The neurosecretosome preparation (isolated neuroendocrine nerve endings) has been maintained in culture for long periods of time. These cultured nerve endings are being used to study the dynamics of hormone secretion, its modulation by receptor occupation, and for the identification of ionic channels on nerve terminals, using state-of-the-art biophysical techniques so that the channels, and their modulation by neuropeptide receptors on the nerve terminals could be investigated. In collaboration with Dr. Elis Stanley, patch-clamp techniques are being used to characterize both the Ca^{++}, and K^+ channel types present on the nerve terminals.

The neurosecretosome preparation allowed for the study of the kinetics of secretion in response to depolarizing stimuli with very high temporal resolution. These studies revealed that during prolonged depolarizations, secretion undergoes inactivation even when membrane potential is held at depolarizing levels. Dr. Kemal Payza has found that this inactivation is dependent on extracellular calcium ions and not caused by the membrane potential change alone. Membrane permeable analogues of cyclic GMP markedly alters this rate of inactivation. He has also shown that the inactivation process is highly temperature-dependent, suggesting an enzymatic process. He has used

the nerve terminal preparation to identify intracellular second messengers involved in the modulation of secretion. The effects of both kappa opiate receptors and FMRF-NH_2 receptors on secretion, and their intracellular coupling are being investigated.

The neurosecretosome preparation provides an ideal model to study intracellular reactions involved in triggering and regulation of neurosecretion. The use of toxins that block secretion has been shown to provide a means of identifying cellular substrates important in the exocytosis machinery. Dr. Holly Trenchard has shown that tetanus toxin at nM concentrations completely blocks depolarization induced secretion from isolated nerve terminals. This inhibition is dependent on toxin internalization, and is blocked by tetanus antitoxin. Dr. Trenchard is attempting to reverse the inhibition of secretion by replacement of cellular metabolites to gain insight into the possible cellular locus of action of this toxin.

The high resolution video imaging microscope adds a new dimension in the investigation of the functional organization of the nerve terminal . Preliminary studies indicate that this instrument will be valuable in resolving long standing questions on the kinetics of Ca^{++} concentration increase in the terminal and its homeostasis. Furthermore, it is envisaged as a method to visualize and quantitate intracellular biochemical reactions with high temporal and spatial resolutions.

Dr. Carolyn A. Bondy and Dr. James Garbern have joined NINCDS to continue their postdoctoral work. Dr. Holly I. Trenchard joined the Unit in June, 1987 as an IRTA fellow and is involved in studies on identification of intracellular mechanisms of tetanus toxin action. Dr. Kemal Payza has recently joined the Unit as an IRTA Fellow.

The Unit on Neuronal Secretory Systems was transferred to the Laboratory of Developmental Neurobiology in April, 1987 from the Laboratory of Neurochemistry and Neuroimmunology.

Section on Neurophysiology and Biophysics

An explosive increase in research on excitatory amino acids was noticeable at major scientific meetings, reflecting the now widespread realization that L-glutamate and perhaps related amino acids act as both fast excitatory neurotransmitters, and as modulators of processes as diverse as memory formation, neuronal cell death, and motor pattern generation. An understanding of the physiology, biophysics and cell biology of excitatory amino acid receptors is fundamental to experiments designed to probe these complex processes, and work in the Unit of Neurophysiology and Biophysics is centered on studies of excitatory amino acid receptors in mammalian nerve cells grown in culture. Substantial progress was made in developing a fast perfusion system for rapidly applying excitatory amino acids and antagonists, and this is allowing experiments that were previously impossible for technical reasons, such as brief applications of saturating concentrations of agonists required for constructing dose response curves, and measurement of the rate constants of desensitization, and of the binding and dissociation of antagonists.

The activity of the NMDA subtype of L-glutamate receptor is modulated by physiologically important divalent cations, including magnesium and zinc. Since zinc is present in excitatory nerve terminals, and released into the extracellular space, studies on its physiological action are of great interest. In cultures of hippocampus, Mark Mayer and Ladislav Vyklicky have found zinc to have two major effects on excitability, which are produced via several distinct cellular mechanisms. An increase in excitability

reflects reduction of inhibitory postsynaptic potentials, by 50 μM zinc, coupled with a lowering of the threshold for action potential initiation, reduction of the spike afterhyperpolarization, and block of spike accommodation during prolonged excitatory stimuli. Under voltage clamp, zinc suppresses a transient outward current, and antagonizes responses to the inhibitory amino acid GABA. Zinc also modulates excitatory synaptic transmission, and selectively reduces the slow component of epsps, with no action on the early synaptic response. Responses to NMDA are strongly antagonized by 50 μM zinc, while responses to kainate and quisqualate are slightly potentiated. Zinc antagonism of NMDA receptor responses in only weakly sensitive to the membrane potential, is not strongly sensitive to changes in the concentration of allosteric modulators such as glycine, and is reduced on raising the extracellular calcium concentration, suggesting that zinc binds to a unique site on the NMDA receptor complex.

Mayer and Vyklicky have used fast perfusion of responses to excitatory amino acids to reveal three patterns of response: sustained activation of kainic acid receptors by kainic and domoic acids, slow desensitization of NMDA receptors (time constant 200 ms at 100 μM NMDA or L-aspartate), and very fast desensitization of quisqualate receptors by quisqualate, AMPA and L-glutamate. These kinetically distinct processes should be helpful in determining the pharmacological specificity of agonists acting at non-NMDA receptors, since differentiation between kainate and quisqualate receptors has been difficult prior to this. The lectin concanavalin-A selectively reduces desensitization at quisqualate receptors, but does not alter responses to kainate or NMDA, suggesting that the quisqualate receptor may be a glycoprotein.

Ian Forsythe and John Clements have continued to analyze excitatory synaptic transmission in cultures of hippocampus, and have discovered a presynaptic inhibitory action of L-glutamate on transmitter release, which appears to reflect the action of L-glutamate at a fourth type of receptor classified by activation using the synthetic ligand AP4. Discovery of the inhibitory action of L-glutamate on transmitter release is a major step forwards, and helps to assign function to the AP4 receptor, for which no known function had been discovered. The very high potency of L-glutamate at the AP4 receptor suggests a significant role as an autoreceptor regulating excitability via a presynaptic mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00047-19 LDN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Biochemical Studies of Neurons and Other Cell Types

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

PI:	D. Brenneman	Pharmacologist	LDN, NICHD
Others:	R. Alderson	Staff Fellow	LDN, NICHD
	D. Warren	Bio. Lab Tech.	LDN, NICHD
	I. Forsythe	Visiting Fellow	LDN, NICHD
	E. Butler	Expert	LDN, NICHD
	Z.-W. Hua	Visiting Scientist	LDN, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Cell Biology, NIMH (L. Eiden); Department of Physiology, University College, Cardiff (G. Foster).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.7	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 regulation of neurodevelopment by neuropeptides, trophic factors and electrical activity was studied with cell culture systems derived from the fetal mammalian central nervous system. The mechanism of the neuron survival-promoting effects of vasoactive intestinal peptide was shown to involve a neurotrophic factor releasing action which was mediated through nonneuronal cells. Two proteins (~40k and ~15 k) appear to be major substrates for VIP-induced phosphorylation in cortical astrocytes.

Two cDNA clones have been isolated that when placed in an expression vector produced substances which enhanced tetanus toxin binding in hippocampal cultures, increase neuronal survival in ciliary ganglion cultures and enhance the survival of spinal cord neurons under TTX blockade. Two morphologically distinct cholinergic cell types from the basal forebrain were found to preferentially respond to NGF or phorbol esters.

The decrease in neuronal survival produced by NMDA antagonists was shown to be restricted to a developmentally sensitive period. Neuronal cell death produced by electrical blockade with tetrodotoxin was prevented by NMDA or a calcium ionophore, A23187. These studies suggest the importance of the calcium regulation in determining which neurons survive during development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00048-14 LDN

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

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Transcription-level Control of Neurobiologic & Developmental Phenomena

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

PI:	B.K. Schrier	Medical Officer	LDN, NICHD
Others:	E.T. Butler, III	Special Expert	LDN, NICHD
	T.T. Quach	Visiting Fellow	LDN, NICHD
	M.M. Voigt	NRC-NSF Fellow	LDN, NICHD
	S.K. McCune	NRSA Fellow	LDN, NICHD
	R.M. Alderson	Staff Fellow	LDN, NICHD
	A.L. Buonanno	Visiting Associate	LDN, NICHD

COOPERATING UNITS (if any): Lab. of Biochemical Genetics, NHLBI (F. Sutton, H. Chin, M. Nirenberg), Neuropsychiatry Branch, NIMH (A-M Duchemin, R.J. Wyatt), Dept. Pharmacol., East Carolina Univ. School of Med. (J.W. Paul, J.P. DaVanzo)

LAB/BRANCH: Laboratory of Developmental Neurobiology

SECTION: Unit on Molecular Neurobiology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 5.0	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.)

(1) A library of cDNAs made from differentiated mouse neuroblastoma mRNAs was found to contain at least four different clones whose fusion proteins had neurotrophic activity for rodent and chick neurons cultured from the central and peripheral nervous systems. (2) Three clones from this library appear to encode the angiotensin II (A II) receptor sequence, as determined by examination of the A II-induced ion flux through the membranes of oocytes injected with transcripts of these clones or by mRNAs hybrid-selected by them. (3) The transcript from another clone from this library appears to stimulate oocytes to bind Head Activating Peptide (HAP), an undecamer which is formed by neuroblastoma cells and may function as an autocrine regulator of cell growth. (4) Synthetic HAP was found to promote survival of cultured chick sympathetic ganglion cells with great potency, as did Triap (1,1,3-tricyano-2-amino-1-propene), which also was found to stimulate neurite formation and some enzyme activities in PC-12 cells. (5) The fusion protein of a cDNA clone (I-3) obtained from size-fractionated mRNAs from wounded cerebral cortex of the rat, proved to be a powerful trophic factor. (6) A clone from a rat hypothalamus library which bound one of two degenerate synthetic oligonucleotide probes for HAP was sequenced to reveal a sequence which did not code for HAP or any other known sequence. Other clones from neuroblastoma cells which bind these oligomers are being identified. (7) A project to obtain cDNA and genomic clones for the alpha-2 adrenergic receptor in rodents has been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00064-12 LDN

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

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neurobiologic Studies of Neurons and Glia in Cell Culture

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

PI:	P.G. Nelson	Head	LDN, NICHD
Others:	C. Yu	Visiting Fellow	LDN, NICHD
	E.A. Neale	Physiologist	LDN, NICHD
	D. Fields	IRTA Fellow	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS (if any)

Montefiore Medical Center, NY (J. Moskal)

LAB/BRANCH: Laboratory of Developmental Neurobiology

SECTION: Section on Neurobiology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.5	1.3

CHECK APPROPRIATE BOX(ES)

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

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

The voltage range of activation of voltage-sensitive Ca^{++} channels is affected markedly and in opposite directions by calcium ions and calcium channel agonists. We find little evidence for strongly inactivating calcium channels in the cell body of central neurons in vitro. Uniform responsivity of somatic calcium current to calcium channel agonists, such as BayK 8644, and only occasional effects of these agents in transmitter output, suggest a cell-specific regulation of the localization of different calcium channel species. The pharmacology and physiology of central transmitter release would be importantly affected by such a differential calcium channel localization in the synaptic terminals.

Physiological studies of synapse formation between neurons in different compartments of a three-compartment culture system have begun in conjunction with observations described in Project ZO1 HD 00708-04. Different processes appear to regulate synaptic connectivity (number of axons making contact with a given cell) and synaptic efficacy (the total strength of the excitation produced by a given input). Chronic stimulation somewhat non-selectively enhances connectivity, while changes in efficacy selectively favor the stimulated afferents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00094-18 LDN

PERIOD COVERED

October 1, 1987 - September 30, 1988

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

Pineal Regulation: Environmental and Physiological Factors

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

PI:	D.C. Klein	Physiologist	LDN,IRP,NICHD
Other:	A.K. Ho	Visiting Fellow	LDN,IRP,NICHD
	H.Korf	Visiting Fellow	LDN,IRP,NICHD
	J.El Hage	IRTA	LDN,IRP,NICHD
	V.Cena	Guest Researcher	LDN,IRP,NICHD
	C.Gonzalez-Garcia	Guest Researcher	LDN,IRP,NICHD
	J.A.Reig	Guest Researcher	LDN,IRP,NICHD

COOPERATING UNITS *(if any)*

NIAMMD (V.Cena); NIMH (D.Jacobowitz, S.Markey); Georgetown University (M.A.A. Namboordiri); University of Pennsylvania (R.Janovsky); Massachusetts Gen. Hospital (K.Sweadner) Laboratory of Developmental Neurobiology

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project investigates the environmental and physiological regulation of the pineal gland, exclusive of transmembrane and intracellular regulatory mechanisms (See Z01-HD 00095-18 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. It has been proposed that the SCN pineal circuit passes through the paraventricular nucleus of the hypothalamus (PVN). Recent work was completed which supports this with the demonstration that electrical stimulation of PVN stimulated the production of melatonin at a near physiological rate. In other studies, the regulation of pineal phospholipase C has been studied; and the developmental appearance of Na+/K+-ATPase has been examined. It has been discovered that Na+/K+-ATPase develops after birth, as indicated by both ouabain binding and two indices of enzyme activity, ATP hydrolysis by membrane preparations and uptake of rubidium. Results indicate a high affinity form of Na^+, K^+-ATPase, similar to the $\alpha+$ form which has been described in the brain, is the dominant form present in the pineal gland. This indicates that another mechanism might generate membrane potential before this time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00095-18 LDN

PERIOD COVERED: October 1, 1987 - September 30, 1988

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

Pineal Regulation: Transsynaptic and Intracellular Mechanisms

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

PI:	D.C.Klein	Head	LDN,IRP,NICHD
Other:	A.K.Ho	Visiting Fellow	LDN,IRP,NICHD
	H.Illnerova	Guest Researcher	LDN,IRP,NICHD
	V.Cena	Guest Researcher	LDN,IRP,NICHD
	C.Chik	Guest Researcher	LDN,IRP,NICHD
	J.Weller	Chemist	LDN,IRP,NICHD

COOPERATING UNITS *(if any)*

NCI (W.Anderson, T.P.Thomas); Georgetown University (M.A.A.Namboodiri); NEI (T.Shinohara); AFRI (J.Halperin)

LAB/BRANCH: Laboratory of Developmental Neurobiology

SECTION: Section on Neuroendocrinology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.4	4.3	1.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The goal of this project is to discover the molecular basis of neurochemical transduction mechanisms, using the pineal gland as a model. Efforts are directed at determining the details of the chemical and ionic components of transmembrane signalling processing and in the neural regulation of gene expression. The most important advances made in the first area were those that have clearly indicated that cAMP and cGMP are regulated by a two receptor mechanism which appears to be focused on the regulation of adenylyl and guanylyl cyclases. One leg of this pathway activates these enzymes via GTP binding regulatory proteins, similar to GSα. This leg is controlled by β-adrenergic or VIP receptors; activation of this leg produces only partial stimulation of cAMP and cGMP accumulation. Activation of the other leg is via α_1-adrenergic receptors. This activates protein kinase C which acts, perhaps on the regulatory or catalytic proteins, to increase the activation of adenylyl and guanylyl cyclase. Activation of protein kinase C occurs as a result of an increase in $[Ca^{2+}]i$ and in diacylglycerol production by phospholipase C. In addition, in the regulation cGMP, there appears to be a strong requirement for activation of phospholipase A and for an increase in $[Ca^{2+}]i$. In the area of the neural control of gene expression, advances have been made in purifying N-acetyltransferase and hydroxyindole-O-methyltransferase, and in isolating cDNA clones coding for these enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00704-04 LDN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Tetanus Toxin Effects and Localization in Neurons

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

P.I.:	Elaine A. Neale	Physiologist	LDN, NICHD
Others:	L.M. Bowers	Biologist	LDN, NICHD
	J.L. Koh	Bio-Aid	LDN, NICHD
	S.C. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Cooperating Units: Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration (W.H. Habig)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER.
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Premixing tetanus toxin with the monoclonal antibody, 18.2.12.6, results in a two- to three-fold increase in the amounts of toxin and antibody bound to the neuronal surface when the formed complex is applied to neuronal cell cultures, relative to what is bound when the reagents are added sequentially. The binding portion of the toxin molecule, Fragment C, can be substituted for the intact toxin, providing a non-toxic complex that can be used to label the surface of living neurons. The complex persists on the cell surface for several days, and can be used to identify neurons shortly after plating and to study morphological aspects of early development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00705-07 LDN

PERIOD COVERED
October 1, 1987 to September 30, 1988

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

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

PI:	J.T. Russell	Head	LDN, NICHD
Others:	H.I. Trenchard	IRTA Fellow	LDN, NICHD
	K. Payza	IRTA Fellow	LDN, NICHD
	A.B. Lynn	Technician	LDN, NICHD
	B. Fuentes	Bio Aid	LDN, NICHD

COOPERATING UNITS *(if any)*
Lab. of Biophysics, NINCDS (E. Stanley, G. Ehrenstein; Lab. of Molecular Biology, NIMH (D.M. Neville; CBER, Food & Drug Admin. (W.H. Habig); S. Kruger, NBS; Jean J. Nordmann, INSERM, Strasbourg, France.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Neuronal Secretory Systems

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The research program is directed towards studying the biochemistry and physiology of the nerve terminal. The neurohypophysial nerve terminals are used as the model system to investigate the importance of ion channels and receptors on initiation and modulation of neuronal secretion. These include studies on secretion of vasopressin and oxytocin from isolated intact posterior pituitaries and isolated neurosecretosomes, and ion channels on the nerve terminal membrane. A neurotoxin from dendroaspis angusticeps, which specifically blocks a type of K+ channel, was found to enhance hormone secretion under very low frequency stimulation conditions suggesting that these transient K+ channels may be involved in frequency-dependent facilitation. Secretion from oxytocin terminals in intact neural lobes was found to be inhibited by the opiate kappa receptor agonist, dynorphin, released from vasopressin terminals. The preparation of neurosecretosomes has been maintained under tissue culture conditions and have been used to study the mechanism of calcium-dependent hormone secretion, its inactivation and modulation. This preparation is suitable for high resolution microscopy and patch clamp analysis of ion channels. Secretion from isolated neurosecretosomes was found to be inactivated rapidly under maintained depolarizations. This inactivation was shown to be calcium dependent, and requiring an enzymatic step. Cyclic GMP was found to markedly reduce the rate of this inactivation. Tetanus toxin in nM concentrations blocked secretion of both vasopressin and oxytocin induced by veratridine depolarization. This blockade was not reversed by exogenously added cyclic GMP or cGMP-phosphodiesterase inhibitor. Patch clamp studies on intermediate lobe cells in primary cultures revealed the presence of three different types of Ca^{++} channels based on their inactivation kinetics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00706-02 LDN

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

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Physiological Studies of Nervous System Development in vitro

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

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

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.)*

Inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00707-04 LDN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Pharmacological Studies of Synaptic Transmission In Vitro

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

PI:	M.L. Mayer	Visiting Scientist	LDN, NICHD
Others:	I.D. Forsythe	Visiting Fellow	LDN, NICHD
	K. Sugiyama	Visiting Fellow	LDN, NICHD
	L. Vyklicky	Visiting Fellow	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Neurophysiology, NINCDS (J. Clements): Section in Instrumentation, Research Services Branch, NIMH (B.M. Smith and S.S. Hsiao)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neurophysiology and Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.5	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This Unit investigates the mechanism of action of excitatory amino acids as synaptic transmitters and neuromodulators in the vertebrate CNS, utilizing cell culture and electrophysiological techniques. Substantial progress has been made in developing a fast perfusion system for applying drugs and ions to nerve cells, and this is now used routinely. The divalent cations, zinc and cadmium, have two major effects on hippocampal neurons: (1) block of excitatory responses to NMDA receptors (Zinc Kd = 13 micromolars); (2) an increase in excitability, due to block of postsynaptic inhibitory GABA receptors (Zinc Kd = 11 micromolars), and zinc suppression of a transient potassium current, which normally slows repetitive firing. Zinc block of NMDA responses is reduced on raising the extracellular calcium concentration suggesting competition between zinc and calcium or screening of the zinc binding site by calcium. Low concentrations of zinc (50 micromolars) also potentiate responses to kainate, quisqualate, and responses to glutamate at non-NMDA receptors. Fast application of excitatory amino acids produces three patterns of response: fast (tau = 20 ms) desensitization of quisqualate receptors; slow (tau = 200 ms) desensitization of NMDA receptors, sustained activation of kainate receptors. Concanavalin-A, which binds to glycoproteins, reduces desensitization at quisqualate but not NMDA receptors, and does not alter responses to kainate. Low concentrations of L-glutamate @ 1 micromolar) were found to depress excitatory synaptic transmission via activation of a novel presynaptic receptor; L-AP4 mimics this effect. Neither agonist produces a substantial postsynaptic response in glycine free medium. Culture medium is conditioned by substances secreted into the extracellular space, including L-glutamate, which tonically inhibits synaptic transmission; on the other hand neuronal survival in F-12 medium reflects activity of a glial sink for neurotoxic amino acids, which rapidly reduces the L-glutamate concentration from 100 less than 10 micromolars.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00708-04 LDN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Morphologic Studies of Neuronal and Non-neuronal Cells in CNS Cell Cultures

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

P.I.:	Elaine A. Neale	Physiologist	LDN, NICHD
Others:	P.G. Nelson	Head	LDN, NICHD
	C. Yu	Visiting Fellow	LDN, NICHD
	R.D. Fields	IRTA Fellow	LDN, NICHD
	S.C. Fitzgerald	Biologist	LDN, NICHD
	L.M. Bowers	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Cooperating Units: Division of Bacterial Products, Bureau of Biologics, Food and Drug Admin. (W.H. Habig); Dept. of Biochemistry, Univ. of Texas (L.B. Hersh).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.7	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.)*

Immunofluorescence using a monoclonal antibody directed against the L3T4 receptor has revealed a pattern of neuronal labeling in cultures of fetal mouse hippocampus. Nerve growth factor appears to have neither growth-promoting nor growth-inhibiting effects on neurons from the ventral horn of the fetal mouse spinal cord.

Afferents of DRG neurons converging on spinal cord neurons of mice in vitro form more and stronger synapses when exposed to a phasic pattern of electrical stimulation during development. Synaptic efficacy was diminished in competing afferents which were not provided with electrical stimulation, but the number of axonal inputs was not altered. Activity-dependent synaptogenesis and modification of synaptic boutons, while strengthening some connections, may not progress to elimination of weak convergent afferents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00709-02 LDN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Prevention of Neuronal Deficits Associated with AIDS

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

PI:	D. Brenneman	Pharmacologist	LDN, NICHD
Others:	S. Fitzgerald	Biologist	LDN, NICHD
	J. Buzy	Guest Worker	LDN, NICHD
	E.A. Neale	Physiologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, NIMH (C. Pert); M. Ruff, Peptide Design; Laboratory of Microbial Immunity, NIAID (D. Ennist)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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.)*

Cell cultures from the fetal mammalian central nervous system were used to study neuronal cell death associated with the envelope protein (gp120) from the Human Immunodeficiency Virus (HIV). Previous studies indicated that the purified IIIB isolate of HIV produced neuronal cell death in hippocampal cultures. Similar effects are now reported for purified native RFII and LAV isolates as well as for recombinant gp120 produced by vaccinia virus containing the LAV envelope encoding sequences. A recombinant gp160 from the IIIB encoding sequences expressed in insect cells with recombinant Baculovirus had no effect on neuronal survival.

Two monoclonal antibodies against the mouse homologue (L3T4) of the T4 receptor were found to prevent neuronal cell death associated with gp120 in hippocampal neurons. Immunocytochemical evidence for the presence of L3T4 receptors on neurons in culture was discovered.

The following peptide T sequences were shown to prevent gp120-induced neuronal cell death: TTSYT, TTNYT, NTSYG, SSTYR and ETWYS. Antiserum against ASTTTSYT was also found to prevent gp120-induced death, whereas pre-immune serum or serum from vehicle-injected animals were ineffective.

LABORATORY OF DEVELOPMENTAL PHARMACOLOGY (LDP)

Z01 HD 00136-20 Pharmacogenetics
Daniel W. Nebert, M.D.

ZO1 HD 00504-01 Cloning of the AH Receptor Gene
Alvaro Puga, Ph.D.

NICHD Annual Report
October 1, 1987 to September 30, 1988

Laboratory of Developmental Pharmacology

SUMMARY

The LABORATORY OF DEVELOPMENTAL PHARMACOLOGY studies the molecular mechanisms of gene expression involving drug-metabolizing enzymes. The clinical discipline involving the study of genetic differences in drug metabolism has been termed pharmacogenetics. Cytochromes P450 are enzymes involved in the oxidative metabolism of steroids, fatty acids, prostaglandins, leukotrienes, biogenic amines, pheromones, and plant metabolites. These enzymes also metabolize innumerable drugs, chemical carcinogens and mutagens, chemicals in foodstuff, and other environmental contaminants. The large degree of overlapping substrate specificities, classes of inducing agents, and drug-drug interactions have caused great difficulty in P450 studies at the level of catalytic activities and protein immunochemistry. P450 enzymes represent the classical "Phase I" metabolism in which the substrate is oxygenated. "Phase II" enzymes often use the oxygen as a site for further metabolism (e. g. quinone reduction glucuronidation, and sulfate, glutathione, or glycine conjugation). Detoxification usually requires both Phase I and Phase II enzymes.

Hundreds of drugs and other chemicals are known to stimulate (induce) their own metabolism or the metabolic fate of structurally-related compounds. In addition, steroids, prostaglandins, and small peptide hormones have been found to regulate some of these activities. The mechanisms surrounding the induction of these enzymes and expression of these genes are of central importance to such fields as fundamental molecular genetics, developmental biology, teratogenesis, chemical carcinogenesis and mutagenesis, endocrinology, limology, and drug addiction, tolerance and toxicity. This laboratory presently comprises one Section and one Unit.

A. The Section on Pharmacogenetics, under the direction of Daniel W. Nebert, M.D., is interested in the regulation and expression of genes encoding Phase I drug-metabolizing enzymes, most of which represent the P450 proteins, and certain Phase II drug-metabolizing enzymes. The P450 gene superfamily is presently known to comprise thirteen P450 gene families, eight of which exist in mammals. Several conclusions about P450 gene evolution are apparent. The P450 superfamily is ancient and has expanded via divergent evolution. The ancestral P450 gene, present probably more than two and a half billion years ago, had a minimum of 40 exons. Estimates of the unit evolutionary period (UEP; millions of years required for 1% divergence in amino acid sequence) range between 4 and 14, but are difficult due to several presumed instances of gene conversion between homologous P450 genes. Two mammalian mitochondrial P450 proteins, encoded by nuclear DNA, are more similar than the microsomal P450 proteins are to the prokaryotic P450 protein.

Striking differences in developmental-, sex- and tissue-specific P450 gene expression have been demonstrated by modern molecular biologic techniques. P450 expression vectors have also been successfully transformed into yeast and transfected into mammalian cell cultures.

We have extensively studied the CYP1A1 gene (trivial name, P_1450) in mouse hepatoma Hepa-1 cultures and receptor-defective and P_1450 metabolism-deficient mutant cell lines. These lines have been used for transfecting the reporter gene chlorampherical acetyltransferase (CAT gene) driven by various lengths of CYP1A1 upstream sequences. It can thus be determined which upstream regions require a functional aromatic hydrocabon (Ah) receptor and which regions require P_1 metabolism. Upstream P_1450 regulatory sequences include: (i) the TATA box; (ii) a tetrachlorodibenzo-p-dioxin (TCDD)-inducible enhancer, which appears to include (iii) an element that augments constitutive gene expression; and (iv) a separate endogenous control element that may be involved in a negative autoregulatory loop. A homologous gene in the same subfamily, called CYP1A2 (trivial name P_3450), is under complicated control that is quite different from that for the CYP1A1 gene. Metabolism of substrate(s) by the product of the CYP1A1 gene not only controls its own constitutive expression but regulates the expression of genes encoding at least five other enzymes having coordinate metabolic functions--cytochrome P_3450 (CYP1A2), NAD(P)H:menadione oxidoreductase (NMO1), glutathione transferase (GT1), aldehyde dehydrogenase (AlDH1), and UDP glucuronosyltransferase (UGT1). All six genes have been cloned, are modulated by the aromatic hydrocarbon (Ah) receptor and induced by TCDD, and are defined as members of the [Ah] gene battery. Genes encoding the Ah receptor, the putative repressor, and other trans-acting regulatory factors are being cloned and characterized.

Projects in this Section are divided among (1) basic molecular biology and genetics, (2) evolution of these genes and regulatory regions, including studies involving DNA sequencing, chromosomal walking and mapping, and (3) clinically important applications. Experimental systems include the use of recombinant DNA technology, inbred mouse strains, transgenic mice, and somatic cell genetics in culture. As an example of a clinically important application, the human CYP1A1 and CYP1A2 genes and flanking regions have been cloned and sequenced, and localized near the MPI gene on chromosome 15. Evidence has been presented to suggest that human CYP1A1 and CYP1A2 genes, similar to the orthologous genes in laboratory animals, are important in the activation of inert chemical procarcinogens, promutagens and proteratogens to active metabolites. Restriction fragment length polymorphisms (RFLPs) have been found, and families with high and low cancer incidence are being studied. In the future it should be possible to correlate RFLP patterns of these genes with human disease. Such tests would facilitate the evaluation of cancer and toxicity risk for individuals exposed to foreign chemicals. These assays would aid the individual, employer and physician in decisions regarding life style, cigarette smoking, employment, and prescription drugs.

B. The Unit on Microbiology, under the direction of Alvaro Puga, Ph.D., has been recently formed to coordinate the efforts directed at the identification of the Ah receptor gene and other genes encoding trans-acting factors that affect CYP1A1 gene expression. Indirect evidence indicates that individual differences in the Ah receptor gene in human populations may be clinically relevant to explain genetic susceptibility to environmental carcinogens and mutagens, as well as to drug-induced toxicity and birth defects.

Projects in this Unit are divided among (1) basic and novel molecular biological approaches to cloning the Ah receptor gene, (2) developmental of expression vectors to study cytochrome P450 regulation and the role of aryl hydrocarbon hydroxylase in mutagenesis, and (3) identification of genes essential for development that are involved in detoxification processes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00136-20 LDP

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Pharmacogenetics

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

PI: D. W. Nebert Head LDP NICHD

Others: See ATTACHMENT I

COOPERATING UNITS *(if any)*

See ATTACHMENT II

LAB/BRANCH

Laboratory of Developmental Pharmacology

SECTION

Section on Pharmacogenetics

INSTITUTE AND LOCATION

NIH NICHD, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.60	4.02	1.58

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 P450 gene superfamily is known to contain at least thirteen gene families and most likely many more. Eight of these families exist in all mammals. This laboratory has studied most extensively the tetrachlorodibenzo-p-dioxin (TCDD; in the lay press called "dioxin")-inducible P450I gene family, which has two members, CYP1A1 and CYP1A2, trivial names P1 and P3, respectively. We have examined the P1 gene (CYP1A1) in the pSV0-cat plasmid stably transfected into mouse hepatoma Hepa-1 cultures and receptor-defective and P1 metabolism-deficient mutant cell lines. Upstream P1 regulatory sequences include: (a) the TATA box; (b) a TCDD-inducible enhancer which includes an element that augments constitutive gene expression and (c) a separate control element that involves endogenous signals rather than foreign chemical inducers. This latter element may participate in a negative autoregulatory loop. Both the TCDD-inducible enhancer and the endogenous regulatory element appear to require a functional aromatic hydrocarbon (Ah) receptor. Metabolism of substrate(s) by the product of the P1 gene not only appears to control its own constitutive expression but may also regulate the activities of at least five other enzymes having coordinate metabolic functions--P3450 (CYP1A2), NAD(P)H:menadione oxidoreductase (NMO1), glutathione transferase (GT1), aldehyde dehydrogenase (A1DH1) and UDP glucuronosyltransferase (UGT1). All six of these genes have been cloned, are under control of the Ah receptor, and are defined as members of the [Ah] gene battery. The transcriptional activation unit that up-regulates these genes is believed to include the Ah receptor (with foreign or endogenous ligand) and another protein that confers chromatin binding capacity. The endogenous control element interacts with a P1 metabolism-dependent repressor encoded by the AHN gene. We intend to clone and characterize the Ah receptor gene, the AHN gene, and other genes encoding trans-acting factors. One long-range goal of this laboratory is to develop assays, based on recombinant DNA technology, to assess the human Ah phenotype and other pharmacogenetic disorders. Such assays may predict who is at increased risk for certain types of environmentally-caused birth defects, cancers, and toxicity.

ATTACHMENT I - Others:

Cheryl L. Butler	Biologist (Tech.)	LDP NICHD
Anup Dey	Visiting Fellow	LDP NICHD
Cynthia A. Edwards	Staff Fellow	LDP NICHD
Rene Feyereisen	Guest Researcher	LDP NICHD
Josette Feyereisen-Koener	Staff Fellow	LDP NICHD
Saikh J. Haque	Guest Researcher	LDP NICHD
Kiyoko Ikeya	Visiting Fellow	LDP NICHD
John E. Jones	Guest Researcher	LDP NICHD
Kristi L. Kotz	Federal Junior Fellow	LDP NICHD
Karen Martell	Guest Researcher	LDP NICHD
Cynthia E. McKinney	Guest Researcher	LDP NICHD
Lisa A. Neuhold	Biologist (Tech.)	LDP NICHD
Roland A. Owens	Guest Researcher	LDP NICHD
Daniel D. Petersen	Guest Researcher	LDP NICHD
W. Vincent Picolo	Clinical Staff Fellow	LDP NICHD
Vesna Rapic	Guest Researcher	LDP NICHD
Kalman F. Salata	Staff Fellow	LDP NICHD
Yhun-Y. Sheen	Visiting Fellow	LDP NICHD
Keitarou Suzuki	Visiting Fellow	LDP NICHD
You-Hui Yang	Guest Researcher	LDP NICHD

ATTACHMENT II - COOPERATING UNITS:

M. Adesnik, Department of Cell Biology, New York University School of Medicine, 550 1st Avenue, New York, New York 10016

L. Anderson, Frederick Cancer Research Facility, Frederick, Maryland 21701

H. Autrup, The Fibiger Institute, Laboratory of Environmental Carcinogenesis, Nor. Frihavnsgade 70, DK-2100 Copenhagen 0, Denmark

K. Berg, Institute of Medical Genetics, University of Oslo, Blindern, Oslo, Norway

A. L. Borresen, The Norwegian Radium Hospital, Institute for Cancer Research, Department of Genetics, Montebello 0310, Ullernchausseen 70, Oslo 3, Norway

J. Chou, Human Genetics Branch, NICHD, NIH, Bethesda, Maryland 20892

K. H. Cowan, Division of Cancer Treatment, NIH, Bethesda, Maryland 20892

K. Dixon, Intramural Reschearch Program, NICHD, NIH, Bethesda, Maryland 20892

J. S. Felton, University of California L-523 Lawrence Livermore Laboratory, P.O. Box 808, Livermore, California 94550

A. J. Fornace, Jr, Division of Cancer Treatment, NCI, NIH, Bethesda, Maryland 20892

F. J. Gonzalez, Laboratory of Molecular Carcinogenesis, NCI, NIH, Bethesda, Maryland 20892

J. L. Guenet, Institut Pasteur, 28 Rue Du D^{r} Roux, 75724 Paris Cedex 15, France

O. Hankinson, Laboratory of Biomedical & Environmental Sciences, UCLA, 900 Veteran Avenue, Los Angeles, California 90024

K. Henning, Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

H. Hoffman, Animal Genetic Systems, Inc., 628-G Lofstrand Lane, Rockville, Maryland 20850

R. E. Kouri, BIOS Corporation, 291 Whitney Avenue, New Haven, Connecticut 06511

C. Kozak, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Maryland 20892

O. W. McBride, Laboratory of Biochemistry, NCI, NIH, Bethesda, Maryland 20892

U. A. Meyer, Department of Pharmacology, Biozentrum, Basel, Switzerland

J. von Borstel, Department of Genetics, University of Alberta, G216 Biological Sciences Centre, Edmonton T6G 2E9, Canada

W. W. Weber, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104

C. Weinberger, Laboratory of Endocrinology, NIMH, NIH, Bethesda, Maryland 20892

H. Westphal, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, Maryland 20892

D. Wu, Department of Tumor Research, Fujian Medical College, Central 817 Road, Fuzhou, Fujian, China

H. Yonekawa, Department of Biochemistry, Saitama Cancer Center Research Institute, Ina-Machi, Kitaadachi-Gun, Saitama-Ken 362, Japan

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00504-01 LDP

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Cloning of the AH Receptor Gene

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

PI:	A. Puga	NIH Expert	LDP	NICHD
Others:	B. Raychaudhuri	Visiting Fellow	LDP	NICHD

COOPERATING UNITS *(if any)* OSD, NICHD (K. Dixon);
Washington State University, Pullman WA (R. Hannah);
LDP NICHD (D.W. Nebert & coworkers);
Lab. of Endocrinology, NIMH, NIH (C. Weinberger)

LAB/BRANCH

Laboratory of Developmental Pharmacology

SECTION

Unit on Microbiology

INSTITUTE AND LOCATION

NIH NICHD, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

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

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

The Ah receptor is one of the major components of the transcriptional regulation of the CYP1A1 gene. The inducer TCDD binds to the cytosolic receptor, and the inducer-receptor complex is translocated into the cell nucleus and gains chromatin-binding properties. The complex binds to a well-defined DNA sequence element in the 5' upstream region of the gene and, as a consequence, P1450 mRNA is transcribed at a rate 10- to 50-fold higher than before induction. It is likely that many other factors are involved, directly or indirectly, in this transcriptional activation. One of the main objectives of this Laboratory is the isolation and characterization of the Ah receptor gene and other genes encoding trans-acting factors that affect CYP1A1 gene transcription. We are using various experimental approaches to identify Ah receptor cDNA clones from λgt11 expression libraries. Some of these approaches involve well-established techniques, such as antibody screenings and DNA-mediated gene transfer; others are in the developing stages, such as Southwestern lifts and genetic screening in prokaryotic hosts.

It has been shown that a region of chromosome 7 in the mouse contains genes important in the detoxification process, as well as in UV protection and multidrug resistance. In addition, these genes are essential in development, since their absence is lethal within hours of birth. We have begun to construct subtraction libraries containing this region of mouse chromosome 7 in order to identify these genes.

LABORATORY OF MOLECULAR GENETICS (LMG)

Z01 HD 00066-18 Control Mechanisms in Temperate Bacteriophage Lambda
Robert A. Weisberg, Ph.D.

Z01 HD 00067-20 Integration of Macromolecular Synthesis in E. coli
Michael Cashel, M.D., Ph.D.

Z01 HD 00068-17 Factors Influencing Genetic Transcription-Initiation and Termination
Robert J. Crouch, Ph.D.

Z01 HD 00069-16 Molecular Genetics of Mammalian Retrovirus Replication
Judith G. Levin, Ph.D.

Z01 HD 00071-16 Gene and Transgene Regulation in the Developing Mouse
Heiner Westphal, M.D.

Z01 HD 01001-06 Gene Organization and Expression in Drosophila
Igor B. Dawid, Ph.D.

Z01 HD 01002-06 Gene Expression During Embryonic Development of Xenopus Laevis
Igor B. Dawid, Ph.D.

Z01 HD 01004-05 Regulation of Amino Acid Biosynthetic Genes in Saccharomyces Cerevisiae
Alan G. Hinnebusch, Ph.D.

ZO1 HD 01005-01 Regulation of Cellular Proliferation and Diversity in Drosophila
James A. Kennison, Ph.D.

Laboratory of Molecular Genetics

The best-known experiment in embryology may still be the discovery of the "organizer" by Spemann and Mangold in 1924. Progress in the area opened up by these classical observations was one aspect of the wideranging investigations carried out by Laboratory members during the past year. What Spemann and Mangold found is that the implantation of a piece of tissue from the dorsal lip of an embryo into the ventral region of another gastrula led to the development of a second dorsal axis in the host. Most of this second axis was formed from host, not graft, tissue; thus it was said to have been induced by the dorsal lip which was called the organizer, and the phenomenon was termed embryonic induction. The physical basis of induction was much studied in the following decades with inconclusive and often frustrating results. Eventually, many biologists came to regard studies on induction as futile, almost as suspect - but this was just a response to frustration, not any doubt in the reality or importance of the basic phenomenon. In the past year the field of induction received a major boost by several key experiments, leading quickly to a renewed interest and invigorating pace of research in many laboratories; researchers in the Laboratory of Molecular Genetics have participated in this rejuvenation of research on embryonic induction.

The recent chapter of the induction story derives from three major advances. One is the finding, about 20 years ago, that the earliest induction in the amphibian embryo concerns the specification of the mesoderm during cleavage and blastula stages. These results, due primarily to Nieuwkoop, helped divide the organizer induction phenomenon into simpler sub-effects, mesoderm induction during blastula and neural induction during gastrula; mesoderm induction proved more amenable to study. However, further progress was again slow in subsequent years, in part because the general state of biological research was not ready for the next step. This changed with the introduction of recombinant DNA and monoclonal antibody technologies and their application to questions of development. These methods allowed the generation of molecular markers for specific tissues in the early embryo, thereby making it possible to assay inductive processes by more objective and quantitative methods than before. The third important step was the finding by J. Smith of the inducing factor secreted by the XTC cell line into the medium, providing a convenient source of inducer for a variety of experiments. Several laboratories quickly made the connection between the XTC factor and growth factors, leading to rapid progress in the field.

The group headed by **Igor Dawid** with a major contribution of Frédéric Rosa and in collaboration with Anita Roberts and Michael Sporn of the NCI focused on mesoderm induction in Xenopus laevis. This group confirmed that a factor or factors in XTC medium induces morphological and biochemical differentiation towards mesodermal derivatives in explants (animal caps) that otherwise would become ectoderm. A relationship between the inducing principle in XTC medium and transforming growth factor β-2 (TGF-β2) has been established by showing that mammalian TGF-β2 induces mesoderm in animal cap explants and that this activity is blocked by antibody against TGF-β2. TGF-β1 is not active in this assay. Purification of the active factor or factors from XTC medium is underway.

To analyze the immediate molecular consequences of induction several cDNA clones have been isolated that are rapidly induced in animal caps by XTC factor; some of

these clones also respond to fibroblast growth factor (FGF) which has been implicated in certain aspects of mesoderm induction. Study of these clones may help analyze signal responses in the embryo. Thus, molecular studies of embryonic induction are well underway and promise to illuminate this important and traditional area of biology in a new way.

Beyond the studies on mesoderm induction researchers in this group have isolated a series of cDNA clones that are specifically expressed in the developing nervous system of Xenopus from gastrulation onward. One of these clones is a neurospecific β-tubulin while the nature of the others is unknown. These clones will be very useful in studies on neural induction which have been initiated.

Tom Sargent and his colleagues are interested in the control of gene expression in the early Xenopus embryo with particular emphasis on the question how regional differentiation is established initially. It is clear that information localized in the egg is an important source of such specialization in addition to the role played by inductive interactions. The model system studied by this group concerns the activation of epidermal keratin genes, shown previously to be an early and cell autonomous process. Sargent and collaborators have injected keratin gene constructs into fertilized eggs and regenerated the temporal and spatial controls of expression. The relevant control region has been localized in the 5' upstream sequences of the gene, and efforts to find protein factors involved in this regulation are underway. Such factors may make possible a molecular approach to the question of localization of developmental information in the egg, a classical problem of embryology.

Michael Cashel and his colleagues have continued their efforts to understand how a cell can coordinate the expression of its genes during balanced growth as well as during transient nutritional impoverishment. This group focused on the role played by the regulatory nucleotide, guanosine 3',5'-bispyrophosphate (ppGpp) in mediating these cellular responses in E. coli; ppGpp occupies a central position in the regulatory network of the bacterial cell.

A few years ago, Cashel and collaborators began characterizing the genes and the regulatory elements governing the metabolism of ppGpp. This information has been exploited to disentangle cause and effect relationships by artificially manipulating intracellular ppGpp levels; in normal circumstances nutritional conditions dictate ppGpp levels so that their effects cannot be separated. Previous reports have described the cloning and sequencing of the relA gene (which catalyses ribosome-dependent ppGpp synthesis during aminoacyl tRNA deprivation), the spoT gene (which catalyses ppGpp degradation to GDP), and a low level residual synthetic activity that persists despite deletion of the entire relA gene. In the past year this group has identified genes in the spoT operon that give a relA gene-dependent phenotype when interrupted as well as one that is a probable subunit of RNA polymerase, the omega subunit. Further, these workers discovered cis-dominant complementation functions associated with mutations of these genes that suggests complex interactions at a regulatory or subunit association level. Most surprisingly, the source of residual ppGpp synthetic activity has been localized to the spoT operon. One of the requirements for this synthetic activity is the spoT gene product itself, which normally catalyzes ppGpp degradation.

Artificial induction of high levels of ppGpp under nutritionally sufficient conditions stops cellular growth and exerts regulatory effects on gene expression in a manner that is very similar to the responses seen when ppGpp is induced naturally during nutritional stress. Thus, stopping growth during starvation might not be due to nutritional deprivation per se but instead to a ppGpp-sensitive step that has protective

value for the cell. The isolation of ppGpp-resistant mutants defective in this step is being pursued as a way to analyze the mechanisms involved in these phenomena.

Robert Crouch and his colleagues are interested in RNA processing, and specifically in the structure and function of ribonucleases H, a ubiquitous group of enzymes which degrade the RNA strand in a DNA/RNA duplex. These enzymes may be involved in RNA processing and in recombination and replication. As an approach to structure/function studies ribonucleases H from bacteria and retroviruses have been examined for enzymatic activity when either the amino or carboxyl termini were altered. Addition of residues at the amino terminus of Salmonella typhimurium RNase H had little effect on the specific activity of the protein, as did a small deletion of the carboxyl terminus. In contrast, removal of the carboxyl one third of the RNase H portion of the AKR MuLV reverse transcriptase RNase H dramatically decreased the RNase H activity without any significant alteration of the polymerase activity. Substitution of seleno-methionine for methionine in E. coli RNase H does not seem to alter the activity.

A second related project involves ribosomal RNA processing. Yeast is a favorable eukaryotic cell for such a study since both molecular and genetic tools may be applied. Crouch and his colleagues have isolated the RRP1 gene from yeast; mutations in this gene can result in abnormal processing of ribosomal RNA. The mRNA from the RRP1 gene has three unusual properties: 1) there is a long (for yeast) 3'-untranslated region; 2) the level of mRNA decreases as the cell density increases; and 3) there is an overlap of sequence of the 3'-terminus of the RRP1 mRNA with the 5'-terminus of a more abundant 0.6 kilobase mRNA. The RRP1 protein may be important in regulating the amount of pre-ribosomal RNA converted to mature rRNA.

Igor Dawid and collaborators have continued their studies on developmental genes in Drosophila with the analysis of the maternal locus fs(1)h and the regulatory locus trithorax (trx). The fs(1)h gene has been studied by sequence analysis of overlapping cDNAs corresponding to the major ovarian transcripts of 7.6 and 5.9 kb. The 5.9 kb mRNA sequence predicts a protein of approximately 110 kd, the 7.6 kb RNA a protein of 205 kd. The predicted proteins are very rich in glycine, alanine and serine, some of which occur as clusters. The proteins contain several potential asparagine-linked glycosylation sites and transmembrane domains. Antisera have been prepared against fusion proteins that contain portions of the predicted fs(1)h products; use of these sera supports the view that the fs(1)h products are membrane proteins. Staining patterns in progeny of fs(1)h mutant females show very early defects in the expression of products of the evenskipped, engrailed, and Ultrabithorax genes, suggesting a defect in initial segmental organization.

The trithorax gene, a major regulatory developmental locus in Drosophila, encodes two large RNAs of 12 and 15 kb at all developmental stages tested, but the proportions of these RNAs vary. Fusion protein derived from portions of these RNAs have been prepared and antibodies obtained. Their use promises insights into the nature of the trx protein products.

Proteins complexed with RNA in RNPs are believed to be important in RNA processing, transport, and turnover. Studies of functional properties of such proteins will be aided by genetic analysis, which is not possible in the mammalian systems employed most commonly for RNP protein work. It is therefore useful that **Susan Haynes** has isolated a gene encoding a Drosophila RNP protein; the gene has been sequenced and its chromosomal location determined. A related gene has been isolated by cross-hybridization; it encodes a distinct RNP protein, and is located on a different

chromosome. These studies may open the approach to a genetic study of RNP protein genes in a favorable animal system.

The developmental genetics of Drosophila is also the focus of interest of **James Kennison** who joined the Laboratory recently. Kennison's interest is to expand the horizon of known loci that affect segment identity in this organism. In a novel approach to this subject genes involved in the determination of segmental identity in D. melanogaster have been identified on the basis of dosage-dependent genetic interactions with genes already known to have a role in the process. Of eighteen genes identified by interacting mutations, twelve were not previously known to be involved in the process. Four of the newly-identified genes, the brahma, kismet, osa, and moira genes, were chosen for more extensive genetic and molecular analyses. Cells lacking either kismet or moira gene products in mosaic individuals survive and express homoeotic transformations in some segments of the adult cuticle. These results imply that many of the new loci that were selected by interaction with other loci, have themselves homeotic phenotypes.

Both maternal and zygotic expressions of the brahma gene are required for survival of the early embryo. Lack of functional brahma products at either stage severely disrupts development. DNA from the region of the genome containing the brahma gene has been isolated and two candidates for the brahma transcriptional unit have been identified by insertional mutagenesis. Each of the two candidates for the putative brahma transcriptional unit encodes a single mRNA species present at all developmental stages examined. cDNA clones corresponding to both mRNA molecules have been isolated and sequenced. DNA from the chromosomal region containing the kismet gene also has been isolated. An insertion of P element DNA into the osa gene has been isolated and characterized in order to clone the wild-type osa gene. These studies promise to significantly widen the range of developmental genes that are available for study, and thus will be important in understanding the complex network of interactions that regulate embryogenesis.

The study of the regulation of gene expression by environmental or developmental cues is a key problem in modern biology. Much of this work has focused on control at the transcriptional level, but translational control is equally important in the cell's metabolism. Possibly the best-studied example of translational control in an eukaryotic cell is the regulation of GCN4 in yeast, the subject studied by **Alan Hinnebusch** and his colleagues. The GCN4 protein is a transcriptional activator of many enzymes concerned with amino acid biosynthesis. Through a complex cascade of regulatory loci the cell responds to nutritional conditions by adjusting its capacity for synthesis of amino acids; GCN4 is the proximal regulator in this cascade. While GCN4 mRNA synthesis is constitutive, synthesis of the respective protein is regulated. As Hinnebusch and his colleagues have shown this regulation is mediated through sequences in the 5' untranslated region of the mRNA. The mechanism of this regulation has been studied further in the past year.

Translational control of GCN4 expression is mediated by AUG codons followed by short open reading frames in the 5' leader of the transcript: the third or fourth AUG codon is needed for repression in non-starvation conditions; the first is required for derepression in starvation conditions. Positive (GCN) and negative (GCD) trans-acting factors modulate the interactions between these upstream AUG codons. The following advances in our understanding of this translational control mechanism were made: (1) the regulatory functions of both the 5' proximal and 3' proximal AUG codons can be mimicked by heterologous upstream open-reading-frames (URFs), demonstrating a lack of strict sequence specificity for URF regulatory functions. However, placing URF1

downstream from URFs 3-4 abolishes regulation, showing that important sequence differences exist between these elements and their 5'-3' order is critical. (2) Substitutions at URF1 with sequences found at URF4 show that the coding region and sequences 3' to the stop codon distinguish the functions of URFs 1 and 4. These results suggest that ribosomes must first translate URF1 and resume scanning to move beyond URFs 3-4 in derepressing conditions. (4) lacZ fusions to URFs 1, 3, and 4 are all efficiently translated when no upstream AUG codons are present. Moreover, URFs 1 and 2 have nearly the same weak inhibitory effect on translation of URF3-, URF4-, and GCN4-lacZ fusions, arguing against differential effects of URFs 1-2 on translation of URFs 3-4 versus GCN4.

GCN4 regulation depends on several GCN and GCD factors which act upstream in the regulatory cascade, between the nutritional state-sensing mechanism and GCN4. The following results were obtained. (1) Immunoblotting with antisera raised against GCN3 and GCD1 shows that these factors are expressed constitutively, supporting the notion that their functions are controlled by protein-protein interactions. (2) gcd12 and gcd2-1 mutations were shown to be alleles of the same gene that have allele-specific interactions with GCN3; the carboxyl-terminus of GCD2 was found to be homologous to GCN3. Based on these findings, it is likely that GCD2 contains two domains, one of which competes with GCN3. (3) Complementation mapping and DNA sequence analysis of GCN2 was completed; a mutation in a conserved lysine residue in the putative GCN2 protein kinase domain was shown to completely inactivate GCN2 positive regulatory function. (4) Mutations in the structural genes for two yeast eIF2 subunits (sui) behave like gcd mutations in causing derepressed GCN4 expression independent of the positive regulator GCN2. These results should help unravel the complex interactions of genes and gene products which result in the adaptive response by the yeast cell to changing nutritional environments.

The group led by **Judith Levin** aims to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the organization of the MuLV pol gene and on correlation of genetic structure with pol-associated enzymatic functions. Molecular clones containing MuLV reverse transcriptase sequences are being expressed in E. coli. The enzyme expressed by one of these clones, pRT250, was previously shown to have normal MuLV polymerase activity, but only barely detectable levels of RNase H activity. The deficit in RNase H activity has now been correlated with the absence of almost half the amino acid residues comprising a C-terminal region with homology to the E. coli and yeast RNases H. These results support the idea that the catalytic sites for polymerase and RNase H are localized to the N- and C-terminal portions of reverse transcriptase, respectively. Experiments to explore the functional relationship between these domains and to identify functionally significant sequences within the domains are in progress.

Retroviral mRNAs encode different proteins in adjoining units that are separated by translational stops or frameshifts. Special mechanisms must occur to allow expression of all of the open reading frames from these genomes. Such translational control of viral gene expression is being investigated in a separate project carried out by this group. Efforts are focused on the role of tRNA in readthrough and ribosomal frameshift suppression at retroviral gag-pol junctions. Suppression of the in-frame UAG termination codon separating the MuLV gag and pol coding regions has been demonstrated in an in vitro system. Yeast tyrosine amber suppressor tRNA as well as partially purified tRNA fractions, including glutamine tRNA, from MuLV-infected cells stimulate readthrough. Further purification of the mammalian tRNA species with

suppressor activity is underway. In studies on ribosomal frameshift suppression, the distribution of isoacceptor tRNAs corresponding to amino acids present at or around the frameshift site is being analyzed. The results show that cells infected with HIV, HTLV-1, and bovine leukemia virus differ from control cells by a dramatic increase in the representation of some of these tRNAs in the hypomodified form. These modifications may be causally related to the observed frameshifting.

Robert Weisberg and his colleagues have continued their research into the mechanisms of genetic recombination and transcription of bacteriophages. They have shown that transcription of early genes in the λ-related temperate coliphage HK022 is broadly similar to that of its relatives, but nevertheless differs in an interesting way. HK022 expresses the first gene of its pL operon, which encodes Nun, a highly specific transcription termination factor, in the presence of prophage repressor. It appears to do this by synthesizing a novel repressor-activated transcript that begins immediately downstream of the pL promoter. Since no other pL operon genes are expressed in the presence of repressor, transcripts must terminate after *nun* in the presence of repressor but proceed through terminators in its absence. The diffusible factors assumed to be involved in antitermination of transcription have not yet been identified. If any are encoded by HK022, their genes are not located in the usual place for antitermination genes in other lambdoid phages.

HK022 and λ both encode proteins that promote recombination between special DNA sequences called attachment sites. The mechanism of site-specific recombination in the two phages is very similar, but the sites and one of the proteins (the Int protein) are not interchangeable. In order to localize the determinants that distinguish these two recombination systems, the primary structure of the HK022 attachment sites and recombination proteins were determined and compared to the analogous λ elements. Further, segments of the two phage attachment sites have been interchanged. This analysis shows that the critical determinants of the specificity difference are located in the central 50 bp (the core region) of the phage attachment site. Since Int protein binds to sequences within and outside of this segment, this finding suggests that the domains of Int that recognize the core region lie in the non-conserved regions of the two proteins, and that the domain(s) of Int that recognize the exterior regions lie in the conserved regions. These studies should provide detailed evidence on the structural requirements for Int protein function and thus lead to a better understanding of the mechanism of genetic recombination.

The application of the transgenic animal technique to fundamental questions in biology and to biotechnology have been among the most exciting advances in recent years. Heiner Westphal and his colleagues have utilized these techniques in many imaginative ways. A major study is based on earlier work by these investigators which showed that a short region of DNA from the α-crystallin gene could direct the expression of other genes to the lens. This knowledge was used to study the action of an oncogene (SV40 T antigen), a protooncogene (c-mos), and a DNA replication factor (polyoma virus large T antigen), respectively, in the lens of transgenic mice. Each of these factors affected cell growth in vivo in a distinct way: SV40 T antigen led to lens tumors, c-mos to a lens fiber differentiation defect, and polyoma T antigen to non-malignant hyperplasia. One outcome of these studies is that transgene-mediated cell transformation in vivo is a new and powerful way of immortalizing highly specialized cell systems such as the lens cell.

The part of the transgenic project that addresses the human AIDS disease has produced an unsuspected finding. Of all cell systems in the transgenic mouse able to activate the HIV LTR, the Langerhans cell of the skin appears the most powerful. This

enforces the view of strong macrophage involvement in AIDS pathogenesis. In another experiment of biomedical importance, transgenic animals were shown to be a potential source of human proteins of therapeutic value. Mice were generated that produce large amounts of tissue plasminogen activator in their milk. This protein is used for treatment of clotting disorders, and its production in the milk of transgenic animal may be a practical way of obtaining useful quantities of material.

In a different study tissue specific expression of murine P450 genes was analyzed. In situ analysis of expression of P_1 and P_3, two distinct P450 genes, has pointed out specific cell systems involved in the control of smoking and other environmental noxes.

The developmental role of homeobox-containing genes in mammalian embryogenesis was studied by analyzing the expression of two distinct homeobox genes in the developing embryo. As revealed by in situ hybridization Hox 1.1 and Hox 1.5 are differentially expressed, and their expression coincides with specific events of pattern formation during midgestation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00066-18-LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Control Mechanisms in Temperate Bacteriophage Lambda

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

PI:	Robert A. Weisberg	Head	LMG,NICHD
Others:	Jeff Baron	Medical Staff Fellow	LMG,NICHD
	Kaymeaung Cam	Visiting Fellow	LMG,NICHD
	Jacques Oberto	Visiting Associate	LMG,NICHD
	Nagaraja Ramaiah	IRTA	LMG,NICHD
	Sieghild Sloan	Microbiologist	LMG,NICHD

COOPERATING UNITS *(if any)* Institute of Cancer Research; Columbia University, NY (Dr. Max Gottesman); Department of Biochemistry, University of Arizona, Tucson (Dr. John Little); Department of Biochemistry, Tel Aviv University, Israel (Dr. Ezra Yagil)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.85	4.85	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 have shown that transcription of early genes in the λ-related temperate coliphage HK022 is broadly similar to that of its relatives, but nevertheless differs in an interesting way. HK022 expresses the first gene of its pL operon, which encodes Nun, a highly specific transcription termination factor, in the presence of prophage repressor. It appears to do this by synthesizing a novel repressor-activated transcript that begins immediately downstream of the pL promoter. Since no other pL operon genes are expressed in the presence of repressor, transcripts must terminate after *nun* in the presence of repressor but proceed through terminators in its absence. The diffusible factors that we assume are involved in antitermination of transcription have not yet been identified. If any are encoded by HK022, their genes are not located in the usual place for antitermination genes in other lambdoid phages.

HK022 and λ both encode proteins that promote recombination between special DNA sequences called attachment sites. The mechanism of site-specific recombination in the two phages is very similar, but the sites and one of the proteins (the Int protein) are not interchangeable. In order to localize the determinants that distinguish these two recombination systems, we have determined the primary structure of the HK022 attachment sites and recombination proteins, and have compared them to the analogous λ elements. We have also interchanged segments of the two phage attachment sites. This analysis shows that the critical determinants of the specificity difference are located in the central 50 bp (the core region) of the phage attachment site. Since Int protein binds to sequences within and outside of this segment, this finding suggests that the domains of Int that recognize the core region lie in the non-conserved regions of the two proteins, and that the domain(s) of Int that recognize the exterior regions lie in the conserved regions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00067-20 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Integration of Macromolecular Synthesis in Escherichia coli

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

PI:	C. Michael Cashel	Head	LMG,NICHD
Others:	Sharon Zemel	Medical Staff Fellow	LMG,NICHD
	Kenji Ikehara	Guest Worker	LMG,NICHD
	Hua Xiao	Visiting Fellow	LMG,NICHD
	Miklos Kalman	Visiting Scientist	LMG,NICHD
	Ildiko Szevereni	Guest Worker	LMG,NICHD

COOPERATING UNITS *(if any)*

Dr. Gad Glaser: Dept. Cellular Biochemistry, Hadassah Medical School
Jerusalem, Israel

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
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 goal is to understand how a cell can coordinate the expression of its genes during balanced growth as well as during transient nutritional impoverishment. Our focus continues on the role played by the regulatory nucleotide, guanosine 3',5'-bispyrophosphate (ppGpp) in mediating these cellular responses in E. coli.

A few years ago, we began characterizing the genes and the regulatory elements governing the metabolism of ppGpp. This information has been exploited to disentangle cause and effect relationships by artificially manipulating intracellular ppGpp levels. Previous reports have described the cloning and sequencing of the relA gene (which catalyses ribosome-dependent ppGpp synthesis during aminoacyl tRNA deprivation), the spoT gene (which catalyses ppGpp degradation to GDP), and a low level residual synthetic activity that persists despite deletion of the entire relA gene. This year, we have identified genes in the spoT operon that give a relA gene-dependent phenotype when interrupted as well as one that is a probable subunit of RNA polymerase, the omega subunit. We have also discovered cis-dominant complementation functions associated with mutations of these genes that suggests complex interactions at a regulatory or subunit association level. Most surprisingly, the source of residual synthetic activity has been localized to the spoT operon. We have shown that one of the requirements for this synthetic activity is the spoT gene itself, which normally catalyzes ppGpp degradation.

We have found that artificial induction of high levels of ppGpp under nutritionally sufficient conditions stops cellular growth and exerts regulatory effects on gene expression in a manner that is very similar to the responses seen when ppGpp is naturally induced during nutritional stress. Thus, stopping growth during nutritional starvation might not be due to the starvation per se but instead to a ppGpp-sensitive step that has protective value for the cell. We have begun the isolation of ppGpp-resistant mutants defective in this step.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00068-17 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Factors Influencing Genetics Transcription-Initiation and Termination

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

PI:	R.J. Crouch	Research Chemist	LMG,NICHD
Others:	L. Lempereur	Visiting Fellow	LMG,NICHD
	Y. Shimada	Adjunct Scientist	LMG,NICHD
	Eva Kalman	Visiting Associate	LMG,NICHD
	D. McKelvin	Biologist	LMG,NICHD
	D. Seay	Student Trainee	LMG,NICHD

COOPERATING UNITS *(if any)* Dr. J. Levin, (LMG, NICHD); Dr. M.L. Dirksen, Dermatology Branch, (DCBD), NCI, NIH); Dr. S. Kanaya, Protein Research Institute, Tokyo, Japan; Dr. Wayne Hendrickson, Columbia University, New York

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Formation of RNA

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	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.)*

Ribonucleases H from bacteria and retroviruses have been examined for enzymatic activity when either the amino or carboxyl termini were altered. Addition of amino acids at the amino terminus of Salmonella typhimurium RNase H had little effect on the specific activity of the protein as did a small deletion of the carboxyl terminus. In contrast, removal of the carboxyl one third of the RNase H portion of the AKRMuLV reverse transcriptase RNase H dramatically decreased the RNase H activity without any significant alteration of the polymerase activity. Substitution of seleno-methionine for methionine in E. coli RNase H does not seem to alter the activity.

The mRNA from the RRP1 gene of yeast has three unusual properties: 1) there is a long (for yeast) 3'-untranslated region 2) the level of mRNA decreases as the cell density increases and 3) there is an overlap of sequence of the 3'-terminus of the RRP1 mRNA with the 5'-terminus of a more abundant 0.6 kilobase mRNA. The relationship of these unusual characteristics to the function of the RRP1 protein remain unknown but suggest that the RRP1 protein may be important in regulating the amount of pre-ribosomal RNA converted to mature rRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00069-16 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetics of Mammalian Retrovirus Replication

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

PI:	Judith G. Levin	Research Biochemist	LMG,NICHD
Others:	Ya-Xiong Feng	Visiting Associate	LMG,NICHD
	Klara Post	Biologist	LMG,NICHD
	Steve Joe	SIS	LMG,NICHD
	Hue Nguyen	SIS	LMG,NICHD

COOPERATING UNITS *(if any)* NICHD-LMG (Robert Crouch); NCI (Dolph Hatfield, Don Court, Brenda Gerwin); PRI-FCRF (Martin Zweig); BRI Basic Research Program, NCI-FCRF (Alan Rein)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Unit on Viral Gene Regulation (Developmental Biology Section)

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
3.4	2.0	1.4

CHECK APPROPRIATE BOX(ES)

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

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

The goal of this project is to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulation and expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the organization of the MuLV pol gene and on correlation of genetic structure with pol-associated enzymatic functions. Molecular clones containing MuLV reverse transcriptase sequences are being expressed in E. coli. The enzyme expressed by one of these clones, pRT250, was previously shown to have normal MuLV polymerase activity, but only barely detectable levels of RNase H activity. The deficit in RNase H activity has now been correlated with the absence of almost half the amino acid residues comprising a C-terminal region with homology to the E. coli and yeast RNases H. These results support the idea that the catalytic sites for polymerase and RNase H are localized to the N- and C-terminal portions of reverse transcriptase, respectively. Experiments to explore the functional relationship between these domains and to identify functionally significant sequences within the domains are in progress. In other work, translational control of viral gene expression is being investigated. Efforts are focused on the role of tRNA in readthrough and ribosomal frameshift suppression at retroviral gag-pol junctions. Suppression of the in-frame UAG termination codon separating the MuLV gag and pol coding regions has been demonstrated in an in vitro system. Yeast tyrosine amber suppressor tRNA as well as partially purified tRNA fractions, including glutamine tRNA, from MuLV-infected cells stimulate readthrough. Further purification of the mammalian tRNA species with suppressor activity is underway. In studies on ribosomal frameshift suppression, the distribution of isoacceptor tRNAs corresponding to amino acids present at or around the frameshift site is being analyzed. The results show that cells infected with HIV, HTLV-1, and bovine leukemia virus differ from control cells by a dramatic increase in the representation of some of these tRNAs in the hypomodified form.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00071-16 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

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

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

PI: H. Westphal

Others: L. Crofford, Medical Staff Fellow
A. Dey, Visiting Fellow
E.M. Fuchtbauer, Visiting Fellow
A. Griep, Staff Fellow
E. Lee, Veterinarian
K. Mahon, Staff Fellow
M. Mangano, Medical Staff Fellow
T. Nakamura, Visiting Fellow
U. Tillmann, Guest Researcher
M. Tremblay, Guest Researcher
S. Yu, Visiting Fellow
S.-P. Lai, Chemist
Andra Miller, Biologist

(All listed personnel affiliated with LMG/NICHD

COOPERATING UNITS *(if any)* Integrated Genetics, Inc. (A.E. Smith); NEI, NIH (T. Kuwabara); NIAID, NIH (M.A. Martin); NIDDK, NIH (L. Henninghausen); NICHD, NIH (D.W. Nebert); Max Planck Institute, Gottingen, West Germany (P. Gruss); Smith, Kline and French (M. Rosenberg).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Mammalian Gene Regulation

INSTITUTE AND LOCATION
NICHD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
13.0	10.0	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 spece provided)*

Our laboratory continues to investigate mechanisms of gene control in the mouse, using a variety of approaches. The first of these concerns the expression of distinct homeobox genes in the developing embryo. We find that Hox 1.1 and Hox 1.5 are differentially expressed and that their expression coincides with specific events of pattern formation during midgestation. Another of our studies deals with the action of an oncogene, a protooncogene and a DNA replication factor, respectively, in the lens of transgenic mice. We find that each of these factors affects cell growth in vivo in a distinct way. We also find that transgene mediated cell transformation in vivo is a new and powerful way of immortalizing highly specialized cell systems such as the lens cell. The part of the project that addresses the human AIDS disease has produced an unsuspected finding. Of all cell systems in the transgenic mouse able to activate the HIV LTR, the Langerhans cell of the skin appears the most powerful. This enforces the view of strong macrophage involvement in AIDS pathogenesis. In another experiment of biomedical importance, we have shown transgenic animals to be a potential source for human proteins of therapeutic value. We have generated mice that produce large amounts of tissue plasminogen activator in their milk. This protein is used for treatment of clotting disorders. The final study of this report concerns tissue specific expression of murine P450 genes. In situ analysis of expression of P_1 and P_3, two distinct P450 genes, has pointed out specific cell systems involved in the control of smoking and other environmental noxes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01001-06 LMG

PERIOD COVERED
October 1, 1987 to September 30, 1988

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

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

PI:	I. Dawid	Head	LMG, NICHD
Others:	S. Haynes	Senior Staff Fellow	LMG, NICHD
	B. Mozer	Biologist	LMG, NICHD
	N. Bhatia-Dey	Guest Researcher	LMG, NICHD
	D.-H. Huang	Visiting Associate	LMG, NICHD
	D. Henderson	SIS	LMG, NICHD
	D. Johnson	Guest Researcher	LMG, NICHD
	K. Eichhorn	Summer Student	LMG, NICHD

COOPERATING UNITS *(if any)*

Ann Beyer, Department of Microbiology, University of Virginia

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.3	1.2

CHECK APPROPRIATE BOX(ES)

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

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

The maternal effect homeotic gene fs(1)h of Drosophila has been studied by sequence analysis of overlapping cDNAs corresponding to the major ovarian transcripts of 7.6 and 5.9 kb. The 5.9 kb mRNA sequence predicts a protein of approximately 110 kd, the 7.6 kb RNA a protein of 205 kd. The predicted proteins are very rich in glycine, alanine and serine, some of which occur as clusters. The proteins contain several potential asparagine-linked glycosylation sites and transmembrane domains. Antisera have been prepared against fusion proteins that contain portions of the predicted fs(1)h products; use of these sera supports the view that the fs(1)h products are membrane proteins. Staining patterns in progeny of fs(1)h mutant females show very early defects in the expression of products of the evenskipped, engrailed, and Ultrabithorax genes, suggesting a defect in initial segmental organization.

The trithorax (trx) gene, a major regulatory developmental locus in Drosophila, has been cloned. Two large RNAs of 12 and 15 kb have been identified as major products of this locus in different developmental stages.

A gene encoding a Drosophila RNP protein has been cloned and sequenced, and its chromosomal location determined. A related gene has been isolated by cross-hybridization; it appears to encode a distinct RNP protein, and is located on a different chromosome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01002-06 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Gene Expression During Embryonic Development of Xenopus laevis

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

PI: I.B. Dawid, Head (All personnel listed below are associated with LMG/NICHD)

Others: T. Sargent, Senior Staff Fellow
M. Jamrich, Senior Staff Fellow
E. Jonas, Visiting Associate
G. Michaels, Staff Fellow
S. LaFlamme, Guest Researcher
D. Henderson, SIS
M. Rebbert, Chemist
S. Sato, Senior Staff Fellow
K. Richter, Visiting Fellow
F. Rosa, Visiting Fellow
A. Snape, Visiting Fellow
P. Bray, IRTA
P. Good, IRTA

COOPERATING UNITS *(if any)*

L. Charnas and H. Gainer, HGB, NICHD, & LNN, NICHD, & LNC, NINCDS
A. Roberts & M. Sporn, LC, DCE, NCI
B. Brooks and R. Feldman, DCRT

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12.5	11.0	1.5

CHECK APPROPRIATE BOX(ES)

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

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

This work aims to elucidate molecular events during early amphibian embryogenesis. To this end molecular markers specific for early differentiation events are being isolated and studied. Recent work has focused on two systems: (1) Reconstituted expression of keratin genes injected into the embryo. (2) Embryonic induction. Keratin genes have been shown previously to be expressed in the ectoderm only. Constructs of keratin genes have been injected into fertilized Xenopus eggs, resulting in temporally and spatially regulated expression of these introduced genes. Induction of mesoderm has been studied with the aid of culture fluid of the Xenopus cell line named XTC. A factor or factors in this medium induces morphological and biochemical differentiation towards mesodermal derivatives in explants (animal caps) that otherwise would become ectoderm. A relationship between the inducing principle in XTC medium and transforming growth factor β-2 (TGF-β2) has been established by showing that mammalian TGF-β2 induces mesoderm in animal cap explants; this activity is blocked by antibody against TGF-β2. Purification of the active factor or factors from XTC medium is underway. Further, several cDNA clones have been isolated that are rapidly induced in animal caps by XTC factor; some of these clones also respond to fibroblast growth factor (FGF) which has been implicated in certain aspects of mesoderm induction. Study of these clones may help analyze signal responses in the embryo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01004-05 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Amino Acid Biosynthetic Genes in Saccharomyces cerevisiae

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

PI:	Alan G. Hinnebusch	Research Microbiologist	LMG, NICHD
Others:	Paul Miller	NRC Fellow	LMG, NICHD
	Norma Williams	Guest Researcher	LMG, NICHD
	Ernest Hannig	IRTA Fellow	LMG, NICHD
	Chris Paddon	Visiting Associate	LMG, NICHD
	Belinda Jackson	Biologist	LMG, NICHD
	Deborah Crouch	Guest Researcher	LMG, NICHD
	Ronald Wek	IRTA Fellow	LMG, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Molecular Genetics of Lower Eukaryotes (Section on Developmental Biology)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.4	5.6	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.)*

Translational control of GCN4 expression is mediated by AUG codons present in the 5' leader of the transcript: the third or fourth AUG codon is needed for repression in non-starvation conditions; the first is required for derepression in starvation conditions. Positive (GCN) and negative (GCD) trans-acting factors modulate the interactions between these upstream AUG codons. We have made the following advances in our understanding of this translational control mechanism: (1) the regulatory functions of both the 5' proximal and 3' proximal AUG codons can be mimicked by heterologous upstream open-reading-frames (URFs), demonstrating a lack of strict sequence specificity for URF regulatory functions. However, placing URF1 downstream from URFs 3-4 abolishes regulation, showing that important sequence differences exist between these elements and their 5'-3' order is critical. (2) Substitutions at URF1 with sequences found at URF4 show that the coding region and sequences 3' to the stop codon distinguish the functions of URFs 1 and 4. These results suggest that ribosomes must first translate URF1 and resume scanning to move beyond URFs 3-4 in derepressing conditions. (4) lacZ fusions to URFs 1, 3, and 4 are all efficiently translated when no upstream AUG codons are present. Moreover, URFs 1 and 2 have nearly the same weak inhibitory effect on translation of URF3-, URF4-, and GCN4-lacZ fusions, arguing against differential effects of URFs 1-2 on translation of URFs 3-4 versus GCN4. (5) Immunoblotting with antisera raised against GCN3 and GCD1 shows that these factors are expressed constitutively, supporting the notion that their functions are controlled by protein-protein interactions. (6) gcd12 and gcd2-1 mutations were shown to be alleles of the same gene that have allele-specific interactions with GCN3; the carboxyl-terminus of GCD2 was found to be homologous to GCN3. Based on these findings, it is likely that GCD2 contains two domains, one of which competes with GCN3. (7) Complementation mapping and DNA sequence analysis of GCN2 was completed; a mutation in a conserved lysine residue in the putative GCN2 protein kinase domain was shown to completely inactivate GCN2 positive regulatory function. (8) Mutations in the stuctural genes for two yeast eIF2 subunits (sui) behave like gcd mutations in causing derepressed GCN4 expression independent of the positive regulator GCN2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01005-01 LMG

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Cellular Proliferation and Diversity in Drosophila

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

PI:	J. Kennison	Senior Staff Fellow	LMG, NICHD
Others:	B. Judge	Biologist (Tech.)	LMG, NICHD
	E. Kasper	Summer Student	LMG, NICHD

COOPERATING UNITS *(if any)*

Dr. John Tamkun and Dr. Matthew Scott, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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.)*

Genes involved in the determination of segmental identity in Drosophila melanogaster have been identified on the basis of dosage-dependent genetic interactions with genes already known to be involved in the process. Of eighteen genes identified by interacting mutations, twelve were not previously known to be involved in the process. Four of the newly-identified genes, the brahma, kismet, osa, and moira genes, were chosen for more extensive genetic and molecular analyses. Cells lacking either kismet or moira gene products in mosaic individuals survive and express homoeotic transformations in some segments of the adult cuticle.

Both maternal and zygotic expressions of the brahma gene are required for survival of the early embryo. Lack of functional brahma products at either stage severely disrupts development. DNA from the region of the genome containing the brahma gene has been isolated and two candidates for the brahma transcriptional unit have been identified by insertional mutagenesis. Each of the two candidates for the putative brahma transcriptional unit encodes a single mRNA species present at all developmental stages examined. cDNA clones corresponding to both mRNA molecules have been isolated and sequenced. DNA from the chromosomal region containing the kismet gene has been isolated. An insertion of P element DNA into the osa gene has been isolated and characterized in order to clone the wild-type osa gene.

LABORATORY OF NEUROCHEMISTRY AND NEUROIMMUNOLOGY (LNN)

Z01 HD 00056-13 Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones
Yoke Peng Loh, Ph.D.

ZO1 HD 01202-01 Regulation of Expression and Function of Neuropeptides During Development
Yoke Peng Loh, Ph.D.

NICHD ANNUAL REPORT
October 1, 1987 to September 30, 1988
Laboratory of Neurochemistry and Neuroimmunology

This Laboratory is concerned with the development, functional organization and interactions between two major integrative systems in the body - the central nervous system and the endocrine system. The approach of the Laboratory is cell biological in nature, and hence utilizes a wide variety of techniques and concepts from a number of disciplines, e.g. physiology, biochemistry, morphology, immunology, and molecular biology. In particular, we study various neuropeptides, intracellular membrane systems and receptors which are found in these organ systems and which are essential to their functions (i.e., regulation of neuropeptide gene expression, neuropeptide processing, neuronal morphology and function, etc.). A special emphasis is placed on the study of the cellular development of these systems and the role of precociously expressed neuropeptides on embryogenesis and neurogenesis.

The activities of the Laboratory are carried out by one Section (Section on Cellular Neurobiology) which will join the Laboratory of Developmental Neurobiology in FY89. This Laboratory is projected to terminate at the end of FY88.

I. Section on Cellular Neurobiology

The research goal of this Section is to study brain and pituitary peptides which are involved in intercellular neurocommunication and neural development. The emphasis has been on the pro-opiomelanocortin (POMC, ACTH/endorphin/α-MSH) family of peptides and more recently the enkephalins. Endorphin and enkephalins are opiate peptides found in brain and pituitary and have been shown to have effects on nervous system development. α-MSH is present in brain and pituitary, but in humans it is present in the pituitary only during pregnancy and in the fetus. This peptide has been implicated to play a role in osmoregulation, fetal growth, neuronal differentiation and neurite regeneration. ACTH is a pituitary peptide which stimulates steroidogenesis and is a mediator of stress. Recently, another member of the POMC family, (N-POMC 1-49), has been shown to be a potent mitogen for adrenal cells. All these peptides exhibit various central nervous system effects and are thought to act as neurotransmitters and neuromodulators. The major focus has been to continue to study the enzymology and regulation of biosynthesis, packaging and secretion of the POMC family of peptides. In addition, the developmental expression of the POMC and enkephalin genes, anatomical distribution and role of these peptides in the developing and adult nervous system were investigated.

The ACTH, α-MSH and endorphin peptides are synthesized in the intermediate lobe of the pituitary from a common, glycoprotein prohormone (pro-opiomelanocortin, POMC) of about 32,000 daltons in size. We have assayed for several enzymes involved in the processing of this prohormone. These include a carboxypeptidase B-like enzyme, an aminopeptidase B-like enzyme, a paired basic residue-specific prohormone converting enzyme (PCE). This latter enzyme has been purified to apparent homogeneity from secretory vesicles of the bovine pituitary intermediate lobe and neural lobe. PCE from both lobes appear to have very similar characteristics and are likely to be the same enzyme. PCE is a glycoprotein, has a molecular weight of ~70,000 daltons and cleaves several precursors (POMC, pro-vasopressin, pro-insulin, and pro-enkephalin) at paired basic residues to yield products seen in the tissues that synthesize these prohormones

or neuropeptide precursors. Inhibitor studies have shown that PCE is inhibited by two aspartyl protease inhibitors, pepstatin A and diazoacetyl-norleucine methyl ester, but not by thiol or serine protease inhibitors. This finding identifies PCE as an aspartyl protease. Recently, Dr. Nigel Birch has screened a number of aspartyl protease antibodies by Western gel analysis and identified one anti-cathepsin D antiserum which recognizes both Cathepsin D and PCE, indicating structural relationship between the prohormone processing enzyme and Cathepsin D. Based on this finding, Dr. Birch in collaboration with Dr. M. Brownstein (NIMH) has designed a series of oligonucleotide probes to clone PCE. The probes are to specific regions of aspartyl proteases, some of which are conserved across all members of the family and others specific for one particular enzyme. An AtT-20 (anterior pituitary corticotroph) Okayama-Berg cDNA Library has been screened at various stringencies by colony hybridization and clone blotting techniques. Two cDNA's which have strong similarity to human Cathepsin D have been sequenced. The identity of these clones suggests that there are at least two genes expressing Cathepsin-D-like enzymes, a hithertofore unreported observation. This is an exciting finding and raises questions concerning the subcellular distribution (besides the lysosomes) and function of these two Cathepsin D enzymes in the cell. Other cDNA clones which appear to have less similarity to "authentic" Cathepsin D are currently under investigation, one of these may code for PCE.

Further characterization of the cleavage specificity of PCE by Dr. Fernando Estivariz showed that the enzyme, in addition to cleaving POMC to yield ACTH, β-endorphin and 16K glycopeptide, was also able to cleave (N-POMC 1-76), (a fragment of 16K glycopeptide), at an Arg-Lys pair to yield (N-POMC 1-49), the mitogenic peptide and Lys-γ_3 MSH, both of which are found in the pituitary. These products formed were small enough to be accessible for identification by HPLC, immunological cross-reactivity with specific antibodies and amino acid composition, showing unequivocally that PCE cleaved between the Arg-Lys pair of the substrate. He also showed, using the secretory vesicle membrane-associated form of PCE that the enzyme was inhibited by EGTA, a strong chelator of Ca^{++}, indicating for the first time that PCE is a metalloprotease. This is an important finding since it raises the possibility that Ca^{++} may regulate the biosynthesis of POMC-peptides at the post-translational level.

Previous studies of Dr. Stela Elkabes have shown that during salt-loading stress, plasma ACTH and POMC mRNA levels in the anterior pituitary of mice were increased. To determine if two secretogogues of ACTH, CRF and AVP are involved in mediating this response, Dr. Howard Tracer and Dr. Maria Castro have studied the mRNA levels and secretion of these two peptides <u>in vivo</u>, during salt-loading; and the interaction of CRF and AVP at the cellular level, in mediating ACTH secretion and regulation of pituitary POMC MRNA levels. Dr. Tracer, using a quantitative <u>in situ</u> hybridization technique showed that AVP, but not CRF mRNA in the hypothalamic neurons were increased after two days salt-loading. Furthermore, immunoreactive CRF in the median eminence (the release site) was unaltered, but plasma AVP was increased. These results suggest that AVP may play a key role in potentiating ACTH secretion and POMC synthesis in the anterior pituitary during hypertonic stress. This is similar to some other stresses, e.g., when psychiatric patients are given electroshock treatment and during hypoglycemic stress, no change in portal or peripheral blood CRF is observed, but plasma AVP is increased significantly. Why in certain types of stress, ACTH secretion is potentiated by increased AVP levels in the presence of basal CRF levels and in others (e.g. cold, foot-shock stress) by an increase in CRF is an interesting question. Dr. Castro has used mouse, dissociated anterior pituitary cells in culture to study the interaction of AVP and CRF on ACTH secretion to try and understand this issue. She showed that AVP at $\geq 10^{-8}$M in the presence of basal (10^{-10}M) CRF produced an ACTH secretion level equal to 10^{-9}M CRF. Prolonged treatment of the cells at 10^{-9}M CRF resulted in

desensitization of the receptors and decreased ACTH secretion. Such desensitization was less evident when cells were treated with basal CRF and increased AVP. These findings indicate efficiency in the proposed mechanism for regulating ACTH secretion during salt-loading i.e., AVP is the primary regulator of ACTH secretion with CRF playing a permissive role. Such a mechanism may be operative perhaps in chronic stress, whereas an increase in CRF may occur in acute stress paradigms.

Dr. Castro also analyzed the second messengers involved in mediating the CRF and AVP effect. She showed that CRF acts through cAMP/protein kinase A dependant pathway, while AVP act through cAMP independent pathway involving phospholipase C, phosphoinositide turnover and protein kinase C. She found that unlike the rat, when AVP was added with CRF, the potentiation effect on ACTH secretion was not due to an enhancement of intracellular cAMP levels but to other mechanisms, perhaps involving the enhancement of phosphorylation of proteins mediating secretion. Dr. Castro has also demonstrated that AVP and CRF enhanced POMC mRNA levels in anterior pituitary cells. This result suggests that these two peptides (CRF and AVP), in addition to having effects on ACTH secretion play a role in upregulating POMC mRNA levels in the anterior pituitary during salt-loading stress.

Recently, a new project has been initiated to study the developmental regulation of expression of [met]enkephalin and POMC genes, and the role peptides derived from these genes may play in neurogenesis and embryogenesis. Two model systems: the frog (Xenopus laevis) and the mouse were used. Initial studies focussed on defining the temporal/spatial distribution of POMC and [met]enkephalin peptides during development. Dr. Stela Elkabes showed using in situ hybridization histochemistry that POMC mRNA appears very early in development, at embryonic day 10-1/2 (E 10-1/2) in the presumptive arcuate nucleus of the CNS. POMC mRNA did not appear in the pituitary until E 12-1/2 in the anterior lobe and E 14-1/2 in the intermediate lobe. Immunocytochemical studies indicate that the POMC mRNA is translated at E 10-1/2. The POMC neurons appear to mature very rapidly. Neurite outgrowth, arborizations and growth cones were evident at this early stage of CNS development. The POMC system appears to be expressed before other peptidergic systems studied in parallel, (LHRH, oxytocin and vasopressin), suggesting an important role of POMC derived peptides in early neurogenesis. Studies by Dr. William Hayes has shown that POMC [met]enkephalin and TRH mRNAs appear during early development of the frog CNS, at stage 45 tadpoles. In addition, POMC mRNA could be detected as early as stage 33 embryos. This result again indicates the very early expression of the POMC system, as in mammals. He has also mapped the anatomical location of the neurons expressing these mRNAs, both in adult, and in the tadpole brain. He has recently begun studies to test the effect of naltrexone, an opiate receptor antagonist on brain development in early tadpoles. Dr. May Wong has cloned the X. laevis [met]enkephalin gene and is currently mapping the 5' upstream regulatory regions, with the ultimate aim of defining the trans-acting factors which regulate the gene. Expression of these factors may be the key to triggering the tissue specific expression of neuropeptide genes during development. Overall, these studies will lead to a better understanding of the timing and functional interplay of gene, peptide and receptor expression during development, and hence the developmental role of neuropeptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00056-13 LNN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones

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

PI:	Y. Peng Loh	Chief	LNN, NICHD
Others:	Nigel Birch	Visiting Fellow	LNN, NICHD
	Maria Castro	Visiting Fellow	LNN, NICHD
	Toshiyuki Chikuma	Visiting Fellow	LNN, NICHD
	Fernando Estivariz	Courtesy Invest.	LNN, NICHD
	Howard Tracer	Medical Staff Fellow	LNN, NICHD
	Winnie Tam	Microbiologist	LNN, NICHD
	Osvaldo Gigliotti	Stay-in-school Stud.	LNN, NICHD

COOPERATING UNITS *(if any)*

Lab. of Cell Biology, NIMH (T. Zoeller, M. Brownstein, S. Young III & H. Okayama); Lab. of Viral Diseases, NIAID (B. Moss & T. Fuerst); Lab. of Bioorganic Chem., NIDDKD, (F. Gusavsky) Lab. of Biochem. Gene., NIHLB (B. Flucher) Dept. of Psych., Lund Univ. Sweden (R. Ekman)

LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.85	4.15	1.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Pituitary secretory vesicle enzymes involved in the processing of pro-opiomelanocortin (POMC, pro-ACTH/endorphin) and pro-vasopressin were studied. A 70,000 mol. wt., paired basic residue specific prohormone converting enzyme (PCE), previously purified and characterized as an aspartyl protease, has recently been shown to be structurally related to Cathepsin D. PCE was shown to be secreted from bovine intermediate lobe together with α-MSH, in a co-ordinately regulated manner. Pepstatin A, an inhibitor of PCE, blocked processing of POMC in the mouse intermediate lobe further supporting a physiological role of the enzyme in vivo. Cloning of this enzyme is in progress.

Our previous finding that mice, during salt-loading stress, exhibit an increase in plasma ACTH and anterior pituitary POMC mRNA levels, prompted studies to determine the involvement of CRF and vasopressin (AVP) in mediating this response. Quantitative in situ hybridization analyses of CRF and AVP mRNA levels in hypothalamic neurons indicate no change in CRF mRNA, but an increase in AVP mRNA after salt-loading. The content of CRF_i in the median eminence, the site of release of CRF also showed no change after salt-loading. Plasma AVP was increased, and AVP appears to be the primary regulator of ACTH secretion, in the presence of basal levels of CRF, during salt-loading stress. Studies using dissociated mouse anterior pituitary cells reveal that AVP is highly effective in potentiating CRF action at low doses of CRF. Analysis of the molecular mechanism for the signal transduction of CRF and AVP indicate that CRF acts through cAMP-dependent, and AVP through a phosphoinositol degradation pathway. Potentiation of CRF induced ACTH secretion by AVP was not mediated by an increase in cAMP, as in the rat, but perhaps through an increase in the efficacy of phosphorylation of proteins involved in the secretory process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01202-01 LNN

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation of Expression and Function of Neuropeptides During Development

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

PI:	Y.P. Loh	Head	LNN, NICHD
Others:	May Wong	Senior Staff Fellow	LNN, NICHD
	Stela Elkabes	Visiting Fellow	LNN, NICHD
	William Hayes	NRC/Biotech. Assoc.	LNN, NICHD
	Paul Jung	Junior Fellow	LNN, NICHD
	Eric Tamm	Summer Student	LNN, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Neurochemistry, NINCDS (T. Zoeller, S. Wray & A. Nieburg)

LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3.5	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 spece provided.)*

Neuropeptides have been shown to have trophic and mitogenic effects, and have been implicated to play a role in development. The objectives of this project are to study the developmental regulation of expression of two neuropeptide genes coding for the pro-opiomelanocortin (POMC) and the pro-[met]enkephalin family of peptides, and to investigate the role of these peptides in development, particularly in the central nervous system (CNS). Two model vertebrate systems: the frog (Xenopus laevis) and mouse were used. Initial studies focused on defining the temporal and spatial expression of POMC and [met]enkephalin peptides during development. In situ hybridization histochemistry revealed the first appearance of POMC mRNA in the mouse CNS in the region of the presumptive arcuate nucleus, at embryonic day 10-1/2 (E 10-1/2), and in the anterior lobe and intermediate lobe of the pituitary at E 12-1/2 and E 14-1/2 respectively. Immunocytochemical studies indicated that the POMC mRNA is translated at E 10-1/2; the neurons expressing POMC matured rapidly, forming arborizations and growth cones at this stage. The POMC system appears to be expressed before other peptidergic systems studied (LHRH, oxytocin, vasopressin). In the frog CNS, POMC and pro-[met]enkephalin mRNA were present in early tadpoles at stage 45. POMC neurons were observed in the preoptic nucleus, hypothalamus and tegmentum, whereas cells with [met]enkephalin mRNA were present in the telencephalic midbrain and brain stem areas. The early expression of POMC in the developing CNS suggests that this family of peptides may be important in neurogenesis. The frog pro-enkephalin gene including the 5' upstream regulatory region is cloned. Work is now in progress to identify the regulatory elements, a prerequisite to identifying the factors that trigger the activation of the gene during development.

LABORATORY OF THEORETICAL AND PHYSICAL BIOLOGY (LTPB)

Z01 HD 00040-13 Statistical and Mathematical Studies of Molecular Interactions
Peter J. Munson, Ph.D.

Z01 HD 00165-13 Isolation and Characterization of Macromolecular and Cellular Particles
Andreas Chrambach, Ph.D.

Z01 HD 00171-12 Electrophoretic Methodology
Andreas Chrambach, Ph.D.

Z01 HD 00189-07 Computer Programs for Analysis of Laboratory and Clinical Data
David Rodbard, M.D.

Z01 HD 01400-06 Clinical Applications of Stable Isotopes
Alfred L. Yergey, Ph.D.

Z01 HD 01401-06 Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry
Alfred L. Yergey, Ph.D.

Z01 HD 01404-05 Characterization of Opioid Receptors in Brain and Peripheral Tissues
David Rodbard, M.D.

ZOI HD 01405-04 Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus
David Rodbard, M.D.

Laboratory of Theoretical and Physical Biology

The LTPB has a unique program involving the application of several specialized methodologies to current problems in clinical investigation and fundamental research.

The Section on Theoretical Biology has continued its studies of mathematical modelling, statistical analysis, development of computer programs for biomedical research, and a combined theoretical and experimental approach to the characterization of complex systems of receptors for drugs, hormones and neurotransmitters.

The Section on Macromolecular Analysis has continued its studies of the physical chemistry of proteins, nucleic acids and viruses and development of new methods using polyacrylamide and agarose gel electrophoresis, isoelectric focusing, and related techniques.

The Section on Metabolism and Mass Spectroscopy has pioneered the development of thermal ionization mass spectroscopy, to permit the clinical investigation of calcium metabolism in full term and premature neonates, infants, children, puberty, pregnancy, lactation, aging, osteoporosis, and a variety of disease states. Further, it has developed liquid chromatography - mass spectroscopy methods for study of the kinetics of metabolism of glucose, cortisol, testosterone, progesterone, Vitamin D and other compounds in man in a wide variety of clinical investigations.

Section on Theoretical Biology:

We have continued development of general, flexible methods to describe families of curves, as arise in the context of dose-response curves, calibration curves, and when combining results from multiple experiments, subjects, or treatments. The FLEXIFIT algorithm and program have been entirely reformulated to provide a more efficient procedure with enhanced theoretical properties, to permit objective hypothesis testing and provide confidence limits. This program is now being applied to immunoassays, estimation of molecular weights for proteins and nucleic acids, and to dose-response curves.

The DETECT algorithm and program for analysis of pulsatile hormone release has been extensively tested and validated, and compared with other methods by means of receiver-operating characteristic (ROC) curves to evaluate the relationship between sensitivity and specificity. The DETECT algorithm was shown to provide optimal sensitivity and specificity for a wide range of conditions, e.g., peak height, shape, duration, frequency and signal/noise ratio. The algorithm for calculation of the instantaneous secretory rate has been improved with respect to efficiency. New methods have been developed to provide objective, statistically valid computer analysis of two hormonal time series, to evaluate the degree of coincidence of pulses and estimate the lag period, if any, between the two series. This has been applied to clinical studies of LH, prolactin, beta-endorphin, and cortisol.

New programs have been developed to assist the analysis and interpretation of self-monitoring of blood glucose. These programs utilize data entered automatically from glucose meters equipped with memory and clock-calendar to avoid the tedium of manual data entry. They enable one to visualize the circadian glucose profile in dynamic

display ("glucose profile movie"), provide a report of hypoglycemic episodes, and allow visualization of the profile of insulin action in relation to the average glucose profile. These programs are now undergoing clinical evaluation.

A new program has been developed for optimization of design (e.g., number and choice of ligand concentrations) for ligand binding experiments. This program can handle simple cases (e.g., 1 ligand, 1 class of binding sites), and complex ones, e.g., multiple ligands, multiple classes of sites, dose response surfaces, and use of selective competitive ligands to block undesired classes of sites, i.e., "blocking experiments". This program has been tested, validated, and is now being applied to facilitate studies of dexamethasone binding to hypothalamus (in collaboration with the Developmental Endocrinology Branch), and to studies of phencyclidine binding to receptors rat brain (see below).

Experimental studies of drug-receptor interaction: We have investigated the binding of phencyclidine, N-allyl-normetazocine, and several related compounds, to receptors in membranes prepared from rat and guinea pig brain. Using specialized techniques developed in this laboratory for design and analysis of such studies (e.g., programs DESIGN and LIGAND), we are able to demonstrate two distinct classes of binding sites for phencyclidine in rat and guinea pig brain. Both of these sites are distinct from the "sigma" site for (+)-SKF 10,047. Further, in the guinea pig, the "sigma" site appears to exist in both high and low affinity forms. We have developed assay conditions for each of the four types of sites (PCP_1, PCP_2, σ_1, σ_2) and evaluated the rank order of potency of a large number of drugs. The functional significance of these receptor subtypes will be the subject of future investigations.

Collaborative studies have continued to characterize the receptors for vasopressin and oxytocin in male and female genital tract in several species, including man.

Section on Metabolism and Mass Spectrometry:

This section has made important advances in the application of mass spectrometry to identification of molecules and elements of biological and clinical interest as well as in studies of the metabolic kinetics of several of these materials. Stable isotopes of carbon, hydrogen, calcium and magnesium can best be measured at low levels by mass spectrometry, and are now routinely used as tracers or measurement standards.

A major focus of the activity of this section is the elucidation of the in vivo metabolism of calcium. In several clinical investigations, two distinct calcium isotopic tracers are typically administered simultaneously, one orally and one intravenously. The time-dependent dilution of these by natural calcium is determined by thermal ionization mass spectrometry. These measurements are analyzed using multicompartmental mathematical models. Determination of the fraction of dietary calcium absorption can now be obtained using a two-day protocol instead of a lengthy and costly metabolic balance study. Application of this method to studies of normal and osteoporotic women, in collaboration with workers at the Mayo Clinic, has shown that there is no age dependence to absolute calcium absorption, although kinetics of absorption are age dependent. Studies presently underway with a group at the Medical University of South Carolina are examining the question of ethnic differences in fractional absorption.

Results of calcium kinetic studies extending over six weeks have shown a number of intriguing observations directly relevant to the understanding of skeletal growth and development. First, it appears that the pool of calcium with the most rapid turnover

includes a portion of bone, and that this pool reflects skeletal growth. In ten subjects, ages 2 weeks - 14 years, the pool size is strongly correlated with incremental growth rate taken from standard tables. Preliminary results from studies of patients with osteoporosis suggests that this pool size is reduced compared with normal subjects matched for age and sex. Second, the mean residence time for calcium has been shown to correlate closely with skeletal mass. As a consequence, this work on calcium kinetics may lead to a new method for estimation of the skeletal mass, without dependence on radioisotopes.

A second major focus of this Section is the development and use of instruments and methods for the application of thermospray liquid chromatography/mass spectrometry (ThLC/MS). Two major findings have emerged from this work in the past year. First, daily cortisol production rate in normal subjects has been found to be 9.6 $\pm$ 2.3 mg/day (n=11) and 30.7 $\pm$ 9.3 mg/day in six patients with Cushing's disease. This work, performed in collaboration with DEB, NICHD, suggests that the production rate in the normal subjects is approximately one-half the value presently accepted in clinical practice. This finding is consistent with the clinical observation that cortisol replacement therapy administered to adrenalectomized patients using the previous estimates of production rate tend to result in signs or symptoms of hypercortisolism. This work is currently being extended to studies of adolescents and the potential role of cortisol metabolism in the initiation of puberty.

A second major accomplishment is the development of methods to characterize small peptides produced by tumor cell lines, e.g., bombesin-like growth factors. Leucine-enkephalin, a model compound, has been added to culture media at concentrations similar to those expected of other peptides of this size. The peptide has been recovered and identified by use of successive chromatographic separations, enzymatic digestion and finally ThLC/MS. New methods and apparatus have been developed to improve the efficiency of recovery of peptides in thermospray mass spectrometry.

Section on Macromolecular Analysis:

This section has continued studies toward development of improved techniques for fractionation and characterization of proteins, peptides, viruses, and nucleic acids, using polyacrylamide and agarose gel electrophoresis, isoelectric focusing, and 2 dimensional gel electrophoresis. Emphasis has been placed on the relationship between log-mobility and gel concentration (Ferguson plot), and its interpretation in terms of molecular or particle size, conformation and net charge. New theoretical analyses and computer programs provide quantitative interpretation of non-linear Ferguson plots, and can compensate for incomplete polymerization or abnormalities of gel crosslinking. The dependence of mobility on field strength and duration of electrophoresis have been investigated, and necessary correction factors have been measured. A pore-gradient gel, combined with time-lapse photography, has been used to simplify the construction of Ferguson plots.

An improved compartmentalized thin-layer slab gel apparatus has been designed for use with dried agarose and acrylamide gels in discontinuous buffer systems.

An immobilized pH gradient has been used to achieve steady-state isoelectric focusing, and a series of technical problems has been resolved to make this a practical method, suitable for use in two-dimensional macromolecular mapping.

Several of these new methods are being applied to study of DNA-protein interactions. They are also being applied to the characterization of immunogens for vaccines for H-influenza meningitis, in collaboration with the Laboratory of Developmental Immunology. These studies are designed to examine the relationship between physical properties (aggregation state, net charge) and immunogenicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00040-13 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Statistical and Mathematical Studies of Molecular Interactions

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

PI:	P. Munson	Mathematical Statistician	LTPB, NICHD
Others:	D. Rodbard	Head	LTPB, NICHD
	K. Chen	Visiting Associate	LTPB, NICHD
	E. Rovati	Visiting Fellow	LTPB, NICHD
	R. Jernigan	Volunteer Researcher	LTPB, NICHD
	M. Jaffe	Volunteer Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	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.)*

Continued progress has been made on the algorithm underlying the "universal" curve fitting method, FLEXIFIT. This method combines advantages of empirical, nonparametric methods with those of traditional parametric modeling approaches. It is especially appropriate when quantitative estimates are required based on a family of curves with a common shape not easily described by a simple mathematical expression. The method uses cubic smoothing splines to describe the curve shape, together with four parameters to describe horizontal and vertical shifts and stretches required to superimpose members of the family. A major theoretical advance allows for the calculation of equivalent degrees of freedom for the residual sum-of-squares, and asymptotic standard errors of the shift and scale parameters. This advance is based on a definition of the problem as a penalized sum-of-squares, and a reexpression as a least-squares in a transformed variable. This expression makes possible a more efficient numerical algorithm, and provides a theoretical statistical basis for an otherwise apparently ad hoc method. We have applied the method to numerous data sets arising in immunoassay, bioassay, and pharmacology, using data from experiments in our own laboratory and from a number of others outside the NIH.

The first phase of a study of optimal design of ligand binding was completed, resulting in a computer program for determining these designs, together with an extensive catalog of designs for a variety of commonly used experimental protocols. Use of an optimal design can result in a reduction in variance of the parameter estimates of up to 50% compared with some commonly used designs. This study also produced some general rules-of-thumb for designing binding experiments which are useful even in the absence of a computerized analysis. A second phase of this study has begun to provide optimal design of experiments involving multiple ligands simultaneously ("blocking studies and dose response surfaces").

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-13 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Isolation and Characterization of Macromolecular and Cellular Particles

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

PI:	A. Chrambach	Head	LTPB, NICHD
Others:	D. Tietz	Visiting Associate	LTPB, NICHD
	L. Orban	Visiting Fellow	LTPB, NICHD
	L. Wurts	Volunteer Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)* Lab. Develop. Molecular Immunity, NICHD (R. Schneerson, J.B. Robbins); Human Genetics Branch, NICHD (J. G. Koster); Hematology Branch, NIAMD (E. Mihalyi); Division of Cancer Biology and Diagnosis, Laboratory of Molecular Biology, NCI (C. Zwieb and S. L. Adhya).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

(1) The physical characterization and identification of a representative RNA-virus, turnip crinkle virus, was achieved at the nanogram load level, as required by many clinical and biological applications. The method is based on silver stained electropherograms on thin-layer agarose gel strips.

(2) Meningitis immunogen preparations of varying immunogenicity exhibit populations with an identical surface charge density in 2-dimensional agarose gel electrophoresis. Sizes vary from 10 to several hundred nm in radius. The evaluation of gel electrophoretic patterns by computer methods appears promising as a tool for quality control in the production of a meningitis vaccine.

(3) The proteolytic digestion products of human and bovine fibrinogen are quantitatively and qualitatively distinct by the criterion of their SDS-PAGE patterns.

(4) Three components of a subcellular particle from X. laevis with 5' pre-tRNAase activity were separated by agarose gel electrophoresis and silver staining.

(5) The size and net charge relations between a DNA fragment of 155 bp carrying a central protein binding site and the same fragment with a peripheral binding site were investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00171-12 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Electrophoretic Methodology

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

PI:	A. Chrambach	Head	LTPB, NICHD
Others:	J. S. Fawcett	Visiting Scientist	LTPB, NICHD
	D. Tietz	Visiting Associate	LTPB, NICHD
	L. Orban	Visiting Fellow	LTPB, NICHD
	E. Gombocz	Courtesy Associate	LTPB, NICHD
	M. Butterman	Volunteer Researcher	LTPB, NICHD
	L. Wurts	Volunteer Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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 spece provided)*

(1) A gel electrophoresis apparatus for thin-layer gel strips of seven concentrations was constructed. (2) The adsorption of proteins in excess of 200 kDa onto Immobiline gel matrices was found to be protein-specific. Desorption conditions are also specific, but appear to be most effective not with minimal Immobiline and maximal carrier ampholyte concentrations but rather with intermediate concentrations of each. (3) Immobiline monomers are free of significant concentrations of oligomers under the conditions of Immobiline electrofocusing. They are not considered responsible for the adsorption of large proteins in Immobiline electrofocusing for that reason. Reversible oligomers can however be demonstrated with Immobiline of pI 9.3 at high concentrations and sample loads in gel filtration of Sephadex G-10. (4) A computer simulation method for constructing iso-size and iso-net charge profiles on 2-D agarose electropherograms of subcellular-sized particles was devised. (5) A user-friendly computer method for evaluating particle size and net charge on the basis of linear or convex Ferguson plots was devised. (6) A method of Ferguson plot analysis on the basis of mobilities in pore gradient electrophoresis was developed which abolishes the need for multiple gel concentrations in quantitative gel electrophoresis. (7) A method for determining free electrophoretic mobilities and net charge was developed which avoids the error of extrapolation across the fluid (sol) concentration range of polyacrylamide. (8) The dependence of Ferguson plot linearity in PAGE on polymerization conditions was established. Absolute mobilities of proteins in polyacrylamide at low %T increase and those of polystyrene particles in agarose decrease in proportion to the duration of migration. (9) Polyacrylamide gel sieving of a moving boundary was established. (10) Mobility of proteins in PAGE was found to be proportional to field strength. (11) Noncom-pressible polystyrene size standards were introduced into the agarose gel electrophoresis of subcellular particles. (12) Rehydration of agarose gels after drying at room temperature is feasible without introducing negative net charge into the gel. The rehydration is quantitative for strongly bound water (84%); the loss of weakly bound water (16%) is without effect on the effective pore structure of the gel. By contrast, only a partial rehydration is achieved when gel are lyophilized after freezing in alkane-dry ice or in liquid nitrogen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00189-07 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Computer Programs for Analysis of Laboratory and Clinical Data

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

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	A. Genazzani	Visiting Fellow	LTPB, NICHD
	V. Guardabasso	Institute of Pharmacology "Mario Negri," and "Negri Sud"	LTPB, NICHD
	P. Munson	Mathematical Statistician	LTPB, NICHD

COOPERATING UNITS *(if any)*

University of Virginia School of Medicine (J. Veldhuis); Institute of Pharmacology "Mario Negri," Milan, Italy (V. Guardabasso).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have developed improved methods for detection and characterization of episodic hormone secretion, and to estimate the instantaneous rate of hormone secretion using deconvolution analysis. These methods have been applied to several clinical investigations of the dynamics of LH, FSH, prolactin, ACTH, cortisol and beta-endorphin.

The DETECT program and algorithm have been tested extensively, using computer simulation ("Monte Carlo") studies. The sensitivity and specificity of these methods have been estimated for a wide variety of peak shapes, height, duration, signal-to-noise ratio, pulse frequency, sampling frequency, variability of peak shape, and other parameters. The Receiver-Operating-Characteristics (R-O-C) curves have been constructed for DETECT and for another popular program, CLUSTER. Results indicate that for any desired level of specificity (false-positive rate), DETECT shows better sensitivity (lower false-negative rate), and sensitivity is better than 90% in most cases. A new method for computer simulation assumes that inter-pulse interval behaves as a Poisson process. This analysis was useful to examine sensitivity as a function of sampling frequency and led to the concept of 'visible' or observable peaks.

New methods have been developed to evaluate coincidence of pulses in two hormonal time series. These include the concept of "specific concordance" as a function of threshold levels and adjustable lag times, and also uses the Kappa and McNemar's chi-square statistics. Program DETECT and is being extensively revised to improve speed and user-friendliness. A new algorithm for deconvolution has been developed based on use of digital filters. A new program, EXPFIT, has been developed to analyze exponential decay curves.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01400-06 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Clinical Applications of Stable Isotopes

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

PI:	A. L. Yergey	Head	LTPB, NICHD
Others:	Nancy Vieira	Biologist (Tech.)	LTPB, NICHD
	Ronald Goans	NRSA	LTPB, NICHD

COOPERATING UNITS *(if any)* HGB, NICHD (J. Sidbury); Lab. Math. Biol., NCI (D. Covell); Dept. Ped. U. MO Med. Schol., Columbia, MO (L. Hillman); Dept. Endoc., Mayo Clinic (R. Eastell); Cin. Child Hosp. (B. Specker); USDA, Beltsville, MD (Claude Viello); Dept. of Nutr., U. Conn. Storrs (Linsay Allen); Haifa, Israel (Zeev Hochburg)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic and Mass Spectroscopy

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.5	.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)*

A. Continuing work using thermal ionization mass spectrometric (TIMS) analysis of calcium stable isotopic tracers for the measurement of fractional absorption from diet and the kinetics of whole body distribution have led to a number of clinically significant findings. 1) The mass of calcium in the rapidly exchanging internal pool (MPCa) has been determined from the intercept of the curve showing dilution of an intravenously administered tracer. This pool size has been shown to differ substantially from values expected on the basis of considering the pool to be a fixed fraction of body mass. The differences from the expected values in normal children (age range 2 wks - 14 yrs) correlate significantly ($r=0.89$, $p<0.01$, $n=10$) with incremental growth rate (cm/6 mos.). This suggests that the pool is an indicator of physiologically active bone mass or bone formation. Very preliminary data suggest that this pool is lower than expected in subjects with extensive bone demineralization as well. 2) The mean residence of calcium in the body, a measure of total body turnover, has been shown to relate directly to skeletal mass. This relationship has been shown to hold in normal humans over an age range of 2 wks - 45 yrs. 3) Studies of fractional absorption of dietary calcium in normal adult women have shown excellent agreement between radio and stable isotope tracer methodologies.

B. TIMS has been used to measure magnesium tracer dilution in studies of bidirectional magnesium flux in barnacle fibers in vitro. Feasibility has been demonstrated for application of this technique to studies of flux changes undergone during perturbation of normal electrolyte concentrations in the muscle cell culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01401-06 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry

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

PI:	A. Yergey	Head	LTPB, NICHD
Others:	N. Esteban	Visiting Associate	LTPB, NICHD
	D. Vicchio	IRTA	LTPB, NICHD
	P. Smith	NRC	LTPB, NICHD

COOPERATING UNITS *(if any)* Div. of Ped. Met., Dept., of Ped., Duke Univ., Durham, NC (D. Milligton and C. Roe); HGB, NICHD (J. Sidbury); DEB, NICHD (L. Loriaux and T. Loughlin); F. Casorla, B. Linder, J. Zawadzky); NCI P-Navy MOB (J. Mulshine, T. Treston).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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.)*

(1) a) Continued study of daily cortisol production rates (FPR) in patients and normal volunteers has revealed a number of significant findings. The value of FPR in normal volunteers (n = 11) is 9.6± 2.6 mg/24 hrs (RSD = 27%) is both lower in absolute value and has a smaller range than determinations by other methods. The small range of values is consistent with the remarkably low variation observed in mean daily plasma cortisol concentration, [F], of 6 ± 0.9 mu g/dl (RSD = 15%) determined by our isotope dilution methodology. FPR and [F] values are highly correlated in the normal volunteers. On the other hand, our results indicate substantial intersubject variation in metabolic clearance rate, MCR, of 176 ± 95.9 l/day (RSD = 49%). There was no correlation between MCR and either FPR or [F] in the normal volunteers during any single sampling period; however, on a 24 hr basis, the relationship between MCR and FPR suggests that MCR is either unregulated or regulated by FPR rather than [F]. b) All patients with Cushing's Syndrome (n = 6) demonstrated unequivocal elevation in FPR and loss of circadian rhythm. c) To date 16 studies of FPR have been performed in normal children.

(2) To date four studies have been performed to determine testosterone production rate in women with polycystic ovarian disease.

(3) Several alpha-carboxyamidated peptides that serve as models for peptide autocrine growth factors of small cell lung carcinoma have been characterized using a combination of peptidyl amino acid hydrolase and thermospray LC/MS. Preliminary work suggests that there is some ability to obtain sequence information from this approach.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01404-05 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Characterization of Opioid and Peptide in Brain and Peripheral Tissues

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

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	G. Z. Zhou	Visiting Associate	LTPB, NICHD
	A. Katki	Chemist	LTPB, NICHD
	A. Genazzani	Visiting Fellow	LTPB, NICHD
	S. Schwartz	Visiting Scientist	LTPB, NICHD
	A. Ilani	Visiting Scientist	LTPB, NICHD

COOPERATING UNITS *(if any)*

Dept. Endocrinology, U. Florence, Florence, Italy (M. Maggi)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have used quantitative ligand binding studies, to characterize the receptors for (+)-N-allyl-normetazocine (designated (+)-SKF 10,047), phencyclidine (PCP), and several phencyclidine analogs in membranes from rat and guinea pig brain. We found that rat brain has one class of sites for SKF 10,047, and two for PCP. In guinea pig brain, there appears to be 2 classes of sites for SKF (only one is suppressible by haloperidol), and 2 classes of sites for PCP.

Demonstration of these sites required the use of computer modelling, using the LIGAND program, to examine dose-response surfaces, blocking experiments, and for simultaneous analysis of results from multiple curves, multiple experiments, and multiple labeled ligands.

We have continued studies of subtypes of mu opioid receptors in brain, and of kappa opioid receptors in bovine adrenal medulla. We have demonstrated that the vasopressin receptors present in high concentration in porcine seminal vesicles are indistinguishable from the V2 receptors of porcine renal medulla, and these receptors have been localized to the epithelium and not to the musculature. We are now examining the vasopressin and oxytocin receptors of male and female genital tract in the human.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01405-04 LTPB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus

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

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	M. Berger	Volunteer Researcher	LTPB, NICHD
	M. L. Jaffe	Volunteer Researcher	LTPB, NICHD
	P. J. Munson	Statistician	LTPB, NICHD

COOPERATING UNITS *(if any)*

CSL, DCRT (D. Syed, D. Farre); Kantonsspital Basel, Basel Switzerland

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.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.)*

We have developed computer programs to assist in the analysis and interpretation of blood glucose data obtained by self-monitoring of blood glucose by patients with insulin-dependent or non-insulin dependent diabetes mellitus. These programs allow for manual data entry by keyboard, or by automatic electronic transfer of data from glucose meters equipped with clock-calendar and memory. The data are analyzed graphically and statistically. One can obtain a series of standardized reports (summary, glucose-vs.-time, glucose by time of day, glucose in relationship to meals, or by day of the week). Alternatively, one can analyze the data interactively, examining the glucose profile for a single day, or the average glucose profile for a "window" or range of time. The time course of insulin action for several of the most commonly used types of insulin, can be displayed on the same axes as the glucose profile. This can assist the physician in making decisions to adjust appropriate insulin doses or alter insulin regimens, and is also potentially useful for education of both the patient and physician.

OFFICE OF THE SCIENTIFIC DIRECTOR (OSD)

Z01 HD 00093-14 Mechanism of Action of Nerve Growth Factor
Gordon Guroff, Ph.D.

Z01 HD 00137-14 Regulation and Expression of the UDP Glucuronosyltransferase Gene Family
Ida S. Owens, Ph.D.

Z01 HD 01500-06 Adenovirus (AD) and SV40: Molecular and Cellular Biology
Arthur S. Levine, M.D.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00093-14 OSD

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Mechanism of Action of Nerve Growth Factor

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

PI: G. Guroff Head OSD, NICHD

Others: See Attached

COOPERATING UNITS *(if any)* Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel (E. Yavin); Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan (Y. Matsuda); Laboratory of Cell Biology, National Institute of Diabetes and Digestive and Kidney Diseases (P. Lelkes).

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Growth Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.5	9.0	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.)*

Nerve growth factor (NGF) is required for the survival and development of sympathetic, sensory, and certain central nervous system neurons. It binds to specific cell surface receptors on these neurons, initiates a chain of intracellular events and controls the expression of specific genes. The molecular mechanism(s) by which NGF controls gene expression are not known, but the intracellular events mediating the actions of the factor are becoming clear. We have found that the binding of NGF to its receptor is followed rapidly by a calcium-dependent activation of phosphoinositide metabolism and a calcium-independent increase in the release of arachidonic acid. These and other changes in second messenger levels lead to alterations in a number of protein kinases and, in turn, to changes in the phosphorylation of key cellular proteins. Among these are EF-2 and the S6 protein of the ribosomes. In this latter case, it has been found that NGF activates a different S6 kinase than does epidermal growth factor (EGF), a mitogen. This suggests that S6 plays a role in whether the cell divides or differentiates. Also altered is the phosphorylation of a nuclear protein (SMP) perhaps involved in regulating the transcription of specific genes. Work with cell-free systems has suggested that there are a number of parallel, but largely independent phosphorylation cascades, all emanating from the receptor. A major effort now is to find that single biochemical reaction coupling the receptor to these several cascades. The search for this reaction is being conducted using a new tool, K-252a, developed in this laboratory. K-252a is a kinase inhibitor specific for the effects of NGF on PC12 cells. Other studies have focused on the changes in the expression of specific gene products that underlie NGF-induced differentiation. We have found that the expression of the cell recognition molecule Ng-CAM, known to be increased by NGF treatment, is prevented by the presence of glucocorticoids. The functional consequences of the absence of this cell membrane constituent on what are otherwise fully differentiated neurons are currently being explored. Finally, we are exploring the molecular basis of the NGF induced down-regulation of receptors for EGF, a mitogen for these cells. Our present data suggest that the decrease in mitogen receptors could be due to an NGF-induced alteration in the phosphorylation state of these receptors. This study may yield an important insight into the overall control of differentiation and cell division.

Others:	G. Dickens	Biological Laboratory Technician	OSD, NICHD
	B. Rudkin	Staff Fellow	OSD, NICHD
	B. Nikodijevic	Visiting Scientist	OSD, NICHD
	P. Lazarovici	Visiting Associate	OSD, NICHD
	M. Contreras	IRTA	OSD, NICHD
	S. Koizumi	Visiting Fellow	OSD, NICHD
	M. Tocco	Visiting Fellow	OSD, NICHD
	T. Mutoh	Visiting Fellow	OSD, NICHD
	D. Fink	PRAT	OSD, NICHD
	S. Doll	Biotechnology Fellow	OSD, NICHD
	M. Oshima	Adjunct Scientist (Courtesy)	OSD, NICHD
	K. Fujita	Adjunct Scientist	OSD, NICHD
	J. Tanner	Federal Junior Fellow	OSD, NICHD
	M. Sutphin	Federal Junior Fellow	OSD, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00137-14 OSD

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Regulation and Expression of the UDP-Glucuronosyltransferase Gene Family

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

PI:	I.S. OWENS	Head	OSD, NICHD
Others:	O. Michioka	Visiting Fellow	OSD, NICHD
	J. Ritter	Award Fellow (IRTA)	OSD, NICHD
	Y.H. Sheen	Professional	OSD, NICHD
	C. Edmond	Biological Aid	OSD, NICHD
	V. McCauley	Biological Aid	OSD, NICHD

COOPERATING UNITS *(if any)*

Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, Scotland (G. Hawksworth)

LAB/BRANCH

Office of Scientific Director, NICHD

SECTION

Section on Drug Biotransformation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	3.3	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 regulation and genomic organization of the family of UDP-glucuronosyltransferase enzymes are being studied at the RNA, DNA and protein levels in mice and human. An undetermined number of transferase activities is involved in detoxifying through the glucuronidation of numerous lipophiles. Induction of certain activities is known to be effector-specific. An expression system developed in Saccharomyces cerevisiae (pEVP11 vector) shows that sequenced and full-coding mouse transferase cDNA encodes a 51 KDa transferase protein. The endoplasmic reticulum-bound enzyme specifically glucuronidates naphthol, estrone, p-nitrophenol, phenolphthalein 4-methylumbelliferone, dihydrotestosterone, androsterone, and testosterone. Upon removal of the membrane-targeting signal peptide coding region of the cDNA, the truncated insert expresses a cytosolic protein which catalyzes the glucuronidation of naphthol primarily and that of 3-hydroxybenzo(a)pyrene only weakly. A human full-coding transferase cDNA was sequenced and determined to be polymorphic containing six amino acid differences (including a Stu I restriction site change when compared to a sequenced, but otherwise, uncharacterized human transferase cDNA recently published). This form hybridizes to both a 2600- and a 3600-base human mRNA species. Furthermore, mRNA isolated from the excised liver of a Crigler-Najjar patient (who successfully underwent a liver transplant) has reduced hybridization to this form. Yeast transformed with pAAH5 containing this human cDNA insert synthesizes a 50 KDa transferase protein. Studies are underway to determine substrate specificity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01500-06 OSD

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adenovirus (Ad) and SV40: Molecular and Cellular Biology

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

PI:	A.S. Levine	Head			OSD, NICHD
Others:	C.T. Patch	Sr. Investigator	K. Murai	Visiting Fellow	OSD, NICHD
	K. Dixon	Sr. Investigator	E. Roilides	Visiting Fellow	OSD, NICHD
	M. Protic-Sabljic	Visiting Assoc.	M. Carty	Visiting Fellow	OSD, NICHD
	J. M. Hauser	Microbiologist	E. Kajiwara	Visiting Fellow	OSD, NICHD
	A. Razzaque	Sr. Staff Fellow	A. Roy	Guest Researcher	OSD, NICHD

COOPERATING UNITS *(if any)* Lab. of Immunopathology, NIAID (A.M. Lewis, Jr., & M. Carbone); Lab. of Theoretical & Phys. Biol., NICHD (P. Munson); Lab. of Develop. Pharm., NICHD (J. Gielen & D. Nebert); Lab. of Develop. and Molec. Immunol., NICHD (S. Hirschfeld); Lab. of Molec. Genet., NICHD (R. Miskin)

LAB/BRANCH
Office of the Scientific Director

SECTION
Section on Viruses and Cellular Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.5	7.5	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.)*

Chromosomal mutations are the underlying cause of most inherited diseases and many developmental abnormalities. Mutations can also lead to alterations in gene expression in somatic cells, leading to loss of the normal differentiated phenotype and ultimately to cellular transformation. We are studying the mechanism of mutagenesis and DNA repair using SV40-based vectors as a probes to investigate the molecular mechanisms by which agents that damage DNA induce mutations in mammalian cells and how these mutations may be prevented by cellular DNA repair processes. Through use of the pZ189 shuttle vector, we have extensively characterized the types of mutations that occur in mammalian cells either spontaneously or in response to DNA damage. Analysis of the sequence specificity of these mutations has led to models which explain how the mammalian DNA polymerase introduces errors during DNA synthesis, causing mutations. Studies with the vector in an in vitro DNA replication system indicate that cellular factors, in addition to DNA polymerase, appear to influence replication fidelity. Further studies should allow a characterization of these factors on the biochemical level. We are also using the SV40-based shuttle vector system as well as CAT expression vectors to assess the effects of cell wide responses to DNA-damaging treatments. We are also using in vitro DNA repair systems to investigate DNA repair at the molecular level.

Understanding the mechanisms of regulation of cellular proliferation and differentiation is basic to understanding development of multicellular organisms. For the past several years, we have been studying an antimitogenic growth factor secreted by hamster cells transformed by SV40. This mitogenic inhibitor (MI) strongly inhibits a proliferative response in untransformed hamster cells and normal rat cells stimulated with serum mitogens. MI also inhibits a mitogenic response by normal hamster spleen lymphocytes stimulated with lectins that activate T cells (concanavalin A) or B cells (pokeweed mitogen). We have proposed that MI might contribute to the high oncogenicity of the SV40-transformed cells by interfering with mobilization of immune effector cells at the site of tumor growth. We are also using SV40 to study the genetic basis of viral tissue tropism. We find that subcutaneously injected small t-antigen mutants of SV40 often induce abdominal lymphomas in hamsters, rather than the subcutaneous fibrosarcomas induced by wild-type SV40. The mutants may fail to produce a growth factor required for the in vivo transformation of non-proliferating cells.

PREVENTION RESEARCH PROGRAM

BIOMETRY BRANCH (BB)

Z01 HD 00801-13 Studies Based on the Medical Birth Registries of Norway and Sweden
Howard J. Hoffman, M.A.

Z01 HD 00802-13 Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States
Howard J. Hoffman, M.A.

Z01 HD 00803-04 Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors
Howard J. Hoffman, M.A.

Z01 HD 00813-07 Biostatistical Methods for Laboratory Research Studies
George F. Reed, Ph.D.

Z01 HD 00818-07 Research in Developing Nonparametric Methods for Biomedical Applications
George F. Reed, Ph.D.

Z01 HD 00820-07 Statistical Methods for Epidemiologic Data
Daniel W. Denman, M.A.

Z01 HD 00821-06 Development of New Graphical Methods for the Analysis of Biomedical Data
Daniel W. Denman, M.A.

Z01 HD 00840-07 Statistical Discriminant Methods with Applications to Alcoholism Screening
Barry I. Graubard, M.A.

Z01 HD 00841-07 Methods for Comparing and Analyzing Data from Several Complex Surveys
Barry I. Graubard, M.A.

Z01 HD 00842-06 Development of Statistical Methods to Analyze Cluster Samples
Barry I. Graubard, M.A.

Z01 HD 00843-05 An Investigation of Matched Analysis in Case-Control and Cohort Studies
Barry I. Graubard, M.A.

Z01 HD 00850-12 Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia
Dolores A. Bryla, M.P.H.

Z01 HD 00853-04 Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine
Dolores A. Bryla, M.P.H.

Z01 HD 00854-04 Analysis of MCH Data from the National Longitudinal Youth Survey
Dolores A. Bryla, M.P.H.

Z01 HD 00860-08 Analysis of Biomedical Time Series Data
Howard J. Hoffman, M.A.

Z01 HD 00861-06 Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth
Howard J. Hoffman, M.A.

Z01 HD 00871-03 Clinical Trial of New Drug Therapy for Cystinosis
George F. Reed, Ph.D.

Z01 HD 00872-03 Factors Associated with Premature Births: Missouri Follow-back Survey
Dolores A. Bryla, M.P.H.

Z01 HD 00873-02 Relationship of Mother's Prepregnancy Size to Pregnancy Complications and Outcome
Barry I. Graubard, M.A.

ZO1 HD 00874-01 Research on Racial Differences in Pediatric Measures of Gestational Age
George F. Reed, Ph.D.

NICHD ANNUAL REPORT
October 1, 1987 through September 30, 1988

Biometry Branch

The Biometry Branch research activities are structured along three lines: (1) provision of statistical analysis and consultation to NICHD Intramural and Extramural investigators; (2) pursuit of individual and collaborative research in biometry, including both mathematical and biostatistical theory and applications; and (3) support of clinical trials initiated by the NICHD. The Branch maintains strong ties to both the Intramural and Extramural research programs of the Institute. Also, the Branch has supported a number of cooperative studies, including projects supported solely by NICHD and those receiving joint funding from other agencies within the U.S. Public Health Service.

The following review of Biometry Branch research activities is organized by subject matter, rather than by the statistical or mathematical methods utilized in the planning, design, conduct, or analysis phases of these research efforts.

Perinatal Morbidity and Mortality

Perinatal morbidity and mortality are key outcome variables for several studies being performed by the Biometry Branch. A major effort has been devoted to studies comparing United States data with that of developed countries in Europe, Asia and Australia.

Recent publications have compared the birth weight-specific perinatal mortality rates for U.S. Black, U.S. White, Japanese, Norwegian, and Swedish births. These population groups differ in the occurrence of low weight and/or preterm births. U.S. Blacks have the largest number of small, preterm babies and the Norwegian and Swedish populations have the fewest such babies. These studies have shown that birth weight-specific perinatal mortality rates (below 3,000 grams) are affected by the incidence of low weight births. Thus, Norwegian births have a higher birth weight-specific perinatal mortality rate among low weight births than do either the U.S. whites or blacks. The interpretation of birth weight-specific perinatal mortality rates must, therefore, be altered to reflect the need for further standardization. In spite of the difficulty in comparing birth weight-specific perinatal mortality rates, it has been shown that U.S. White, Norwegian, and Japanese births were almost identical in terms of the crude perinatal mortality rate for the decade of the 1970's. However, U.S. Black perinatal mortality rates were generally much higher than that of any other population group. Data from Israel, England, Germany, Scotland, and Sweden have been added to that of Norway, Japan, and the United States for the comparison of time trends in perinatal mortality rates. Only Sweden appears to have had a markedly and consistently lower rate of perinatal mortality during the decade of the 1970's.

Data from the current NICHD Study of Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates will also be used in the assessment of recent time trends. This latter study is being carried out through the research contract mechanism with the Departments of Health of five U.S. States--Michigan, Missouri, New York (Upstate), North Carolina, and Utah--and in four foreign countries--Australia (three States), Japan (Osaka province), Norway, and Scotland. A uniform data tape format has been developed for the years 1980-84 and each participant has prepared their data according to this format.

One of the principal aims of this study is to compare the perinatal mortality attributable to "preterm" low birth weight infants with that attributable to small-for-gestational age (SGA) low birth weight infants in the U.S. and each of the foreign countries. In a study published this year, comparisons were made of standards used in defining SGA births for different racial and national groups. Birth weight percentile curves were calculated for gestational ages from 24 to 44 weeks for different racial and national groups to determine the feasibility of developing a uniform definition of small-for-gestational age. The 5th, 10th, 25th, and 50th percentiles of birth weight for each week of gestational age were computed separately for the interval 24 to 33 weeks of gestation and for 34 to 44 weeks of gestation. Regardless of which of these two gestational intervals were considered, there were significant differences among the five racial and national groups examined in detail (U.S. black, U.S. white, Australian Aborigine, Australian white, and Japanese). At term, 38 to 41 weeks gestation, U.S. white and Australian white births had very similar birth weight percentiles and, also, these percentiles were significantly heavier than those for the other racial or national groups. Australian Aborigine, Japanese, and U.S. blacks had similar 10th and 50th percentiles at term. It was concluded that in order to compare SGA births across racial or national groups, appropriate standards must be defined for each racial or national group. This work is continuing with further publications planned for next year.

Intrauterine Growth Retardation

One research project that has emerged out of this general interest in perinatal morbidity and mortality is a prospective study to delineate risk factors for fetal growth retardation. Using the research contract mechanism, this prospective study is being conducted at two locations: the University of Alabama in Birmingham and the University of Trondheim, Norway.

The aim of this research project is to determine risk factors which will distinguish mothers who have repeated SGA births from those mothers who have a single, unexpected SGA birth. Symmetric and asymmetric forms of intrauterine growth retardation are being assessed prenatally via diagnostic ultrasound measurements and at delivery with standardized measurements. The study protocol includes recruitment of pregnant women before 17 weeks gestation

and subsequent enrollment of women with high risk pregnancies through 33 weeks of gestation. Those enrolled in the study are being carefully monitored throughout the remainder of their pregnancy. Pregnant mothers were enrolled in this study from November 1985 through March 1988. Approximately 2,000 women were enrolled into this prospective study in both Alabama and Scandinavia. It has been estimated that approximately 300 SGA births will occur at the Alabama and Scandinavian sites, separately. Study infants will be followed-up throughout the first year of life to assess catch-up growth, to monitor breast or bottle feeding patterns and occurrence of illnesses, and to assess the achievement of developmental milestones.

Preliminary results from this study were presented at a Symposium held in conjunction with the Nordic Congress of Obstetrics and Gynecology in June in Trondheim, Norway. The high-risk study population at all sites consists of para 1 and 2 mothers with one or more of the following high risk characteristics:

1. previous low birth weight delivery;
2. previous perinatal death;
3. serious renal disease or hypertension during pregnancy;
4. low maternal pre-pregnancy weight (<50 kg);
5. cigarette smoking during the first trimester of pregnancy.

In addition to the above-listed risk factors, the University of Alabama protocol includes the following additional criteria as well:

6. previous spontaneous abortions (2 or more);
7. previous preterm delivery (<37 weeks);
8. low maternal height (<157 cm);
9. alcohol drinking during pregnancy;
10. late onset of first prenatal care visit (26-32 weeks).

The most common risk factor was smoking during pregnancy which occurred in 51% of the Alabama high risk sample and 54% of the Scandinavian high risk sample. The next most common risk factor was having delivered a previous low birth weight baby which occurred in 42% of the Alabama high risk sample and 34% of the Scandinavian high risk sample. However, the comparison of baseline rates of risk factors in the two study populations indicates several differences. For example, in Alabama, approximately 40% of women smoked during pregnancy and 21% had a previous SGA birth. In Scandinavia, 33% of women smoked during pregnancy and slightly less than 11% had a previous SGA birth. Another important difference between the two baseline populations emerges in the comparison of the percentage of para 1 or 2 mothers who had none of the common risk factors: 29% in Alabama and 57% in Scandinavia.

Serial ultrasound measurements during the second and third trimesters of pregnancy from the Alabama study demonstrated systematic differences by sex and race. Black infants had longer femur lengths as measured by ultrasound at all gestational weeks

(16 through 37 weeks) assessed, and males had both larger abdominal circumferences and larger biparietal diameters from the beginning of the third trimester (28 through 37 weeks). Sex and race specific standards for serial ultrasound measurements have not been available for use in the U.S.

The preliminary data analyses have pointed to differences occurring during the index pregnancy for mothers having a single SGA birth (unexpected by history) compared to "repeater" mothers. The mothers with a single SGA birth were more likely to have experienced an unusually stressful pregnancy, whether associated with additional medical complications or with particular social or work-related stresses, compared to the "repeater" mothers.

Also, the question of symmetry versus asymmetry of growth retardation at birth was analyzed based on the Scandinavian study sample. The results suggest that the majority of SGA births in Scandinavia are asymmetrical, i.e., with relatively long crown-heel length for a given low birth weight. Preliminary analyses have been performed on developmental outcome measures during the first year of life with respect to the Fagan Test of Visual Novelty Discrimination at six months of age and, also, the Bayley Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) at one year of age. Significant differences were found in the preliminary data on two of these three tests. SGA infants scored lower on both the Fagan exam and the Bayley MDI compared to normal birth weight control infants. No differences were noted in the preliminary data analysis for the Bayley PDI scores.

Gestational Age Determination and Prematurity

Biometry Branch staff have also participated in the analysis of data obtained through the study of Vaginal Infections in Prematurity (VIP). This is a multicenter, randomized, placebo, controlled trial designed to evaluate the effectiveness of oral antibiotic intervention in reducing premature birth and/or low birth weight. For example, a quantitative measure of bacterial vaginosis was developed in the course of the study in order to standardize the diagnosis across participating centers. Staff provided methodological expertise to determine the accuracy of the proposed measure and its reliability. Also, a separate investigation was begun regarding the question of bias existing in pediatric assessments of gestational age when applied to racial groups different from the reference sample employed to construct the assessment instrument. Of special interest is the Ballard examination, which is an abbreviation and modification of the Dubowitz examination. Since the developmental schedule of many of the items on the examination may well differ on racial lines, gestational age estimates for blacks and hispanics based on the experience of a sample of whites may be less accurate and precise than for whites. Study data will provide obstetric gestational age assessments and Ballard assessments for the three racial groups, so that the Ballard examination can be re-validated for whites and, separately, for the other groups. Comparison of the separate

constructions will reveal the existence of any bias and suggest a means of correction for it, if required.

The Biometry Branch staff have also helped develop the study design and forms for the 1988 National Maternal and Infant Health Survey (NMIHS) being conducted by the National Center for Health Statistics (NCHS) with assistance from the Census Bureau. Also, a followback survey is being funded using the research contract mechanism with the State of Missouri. Information will be obtained through mailed questionnaires to study mothers including all mothers of VLBW infants (<1500 grams), all mothers of fetal deaths, a sample of mothers with moderately low birth weight infants (between 1500-2499 grams), and a sample of mothers with normal birth weight infants (>2500 grams). Data will also be obtained from vital records and medical records abstraction. Study infants will be assessed with a developmental screening test at one year of age, and the VLBW infants will also receive Bayley exams at one of the state's regional perinatal care centers.

Phototherapy Treatment for Neonatal Hyperbilirubinemia

Since 1974 the Biometry Branch has actively participated in the conduct of a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing treated with untreated infants under specific conditions.

During this year intensive effort has been exerted by a special working group to determine if there are any significant differences between the children treated with phototherapy and those that did not receive this treatment. Analyses at six years were conducted both for all centers combined and the two centers with the highest return rate (71%). The rates of mortality and diagnosed medical conditions were not different between the two groups. The rates were similar between P and C groups for cerebral palsy (7.6% vs 6.2%), other motor abnormalities including clumsiness and hypotonia (13.4% vs 14.7%), and sensorineural hearing loss (4.6% vs 2.4%). The WISC-R scores overall were not different for the two groups (verbal, 96.8[P] vs 94.8[C]; performance 95.8[P] vs 95.1[C]). Phototherapy effectively controlled neonatal hyperbilirubinemia without evidence of adverse outcome at six years of age and was at least as effective as management with exchange transfusion alone. It was concluded that phototherapy effectively controlled neonatal hyperbilirubinemia without evidence of adverse neurological or developmental outcome at six years of age.

Sudden Infant Death Syndrome (SIDS) Risk Factors

A major effort of the Branch has been invested in support of the NICHD Cooperative Epidemiological Study of Sudden Infant Death Syndrome (SIDS) Risk Factors. This study is a multicenter, population-based, case-control study of over 800 SIDS cases and 1,600 control infants using data collected at six study centers across the United States. This broadly-based study was designed to

identify new risk factors for SIDS and to confirm or reject several previously claimed risk factors. A critical consideration in developing the study design was the need to determine new risk factors which were specific to SIDS, over and above the risk factors which were generally associated with race and low birth weight. With this goal in mind, two living control infants were chosen for each SIDS case, and one of the control infants (Control B) was explicitly matched for race and low birth weight.

Important differences between mothers of SIDS cases and control infants have been summarized in a publication which will appear later this year. This paper will be published together with other recent findings in SIDS research by the New York Academy of Sciences. Based on the NICHD SIDS Cooperative Study, there was an increased incidence of urinary tract infection, venereal disease, cigarette smoking during pregnancy, illicit drug use, anemia during pregnancy, low pre-pregnancy weight (<110 lbs.), and weight gain <20 lbs. at delivery. Also, associations with a number of maternal variables which were previously suggested in the literature were not found. Thus, no significant differences were found in C-section rates, vaginitis, use of maternal anesthesia and/or analgesia, or in the length of stages 1 and 2 of labor. There were no differences in the incidence of delivery complications, placenta previa, or in mean 1 and 5 minute Apgar scores. When compared to Control A infants, SIDS infants did have an increase in a number of nonspecific symptoms, including: respiratory distress, tachypnea, apnea of the newborn, tachycardia, cyanosis, pallor, irritability, poor feeding, jaundice, vomiting, abnormal cry, lethargy and tremors. After comparison to the race and low birth weight matched Control B infants, only tachycardia and cyanosis remained highly significant ($p<.01$).

In terms of post-neonatal illnesses, SIDS cases did not differ from Control B infants in the number of colds, either "since birth," or in the last two weeks before death or interview. This result was contrary to the expectation from the literature and points out the value of having a well-controlled study. However, SIDS cases did have significantly more diarrhea and/or vomiting during the last two weeks before death or interview ($p <.001$). This gastro-intestinal illness was frequently associated with fever and colds, suggestive of a viral origin. Breastfeeding during the first three months of life (significantly less common among SIDS cases) was also found to be "protective" against diarrhea and/or vomiting. Finally, a listless or droopy appearance within the last 24 hours before death or interview was shown to be highly significant for SIDS cases versus Control B infants (7.8% vs. 0.7%, $p<.001$).

It was concluded that none of the risk factors documented in this paper were of sufficient strength to identify SIDS infants prior to their death. Instead, the profile which emerges is that of suboptimal <u>in utero</u> environment for SIDS infants in addition to an increase in some postneonatal illnesses and less than optimal medical care for SIDS infants.

Biometry Branch staff have also participated in the analysis of cardiac and respiratory patterns in SIDS and normal infants based on a prospective study of 6,914 full-term infants in Britain (16 of whom subsequently died of SIDS). Two papers describing these results have been accepted for publication. There were 22 recordings from the 16 SIDS infants. For comparison, 66 recordings of control infants matched on post-natal age, gestational age, birth weight, and sex were randomly selected. The object of the analysis of these data was to partition heart rate differences by state, e.g., waking, quiet sleep, active or rapid eye movement (REM) sleep, and indeterminate state. One-way analysis of variance was performed on median cardiac and respiratory rate and variability (interquartile range) separately from infants under one month of age and for infants over one month. Heart rate was found to be significantly higher in SIDS victims under 1 month of age compared to the matched control infants during all three sleep-waking states. No differences were found between case and control infants in the distribution of time spent in each of the three sleep-waking states. Above one month of age higher heart rate among SIDS victims persisted only in REM sleep. The importance of these results are twofold: first, they suggest that SIDS cases do differ physiologically from matched control infants and, secondly, since these differences are apparent in the first month of life they may offer clues as to the etiology of SIDS.

Childhood Diseases or Disabilities

The University of California at San Diego has been contracted to conduct a randomized clinical trial to evaluate the effectiveness of phosphocysteamine relative to cysteamine on at least 80 patients to be enrolled in a 3-4 year period. This study, which is to identify and develop new drug therapies and to test them against cysteamine as a standard, quickly identified phosphocysteamine as the only practical alternative cystine-depleting agent. It was later found that oral phosphocysteamine is biologically equivalent to cysteamine within minutes of ingestion. The trial phase of the study is now under way with the purpose of comparing a standard dose treatment with a higher dose treatment of the patient's choice of cysteamine or phosphocysteamine. Treatment assignments are randomized, and changes in renal function are the principal outcome measures. There are now 90 patients enrolled in the study.

The measurement of creatinine clearance presents an ancillary problem that will be addressed with the use of data from the Cysteamine Study and the current trial. Clearances are obtained from 24 hour urine collections, which are difficult to draw reliably and accurately from young patients. A surrogate measure, which employs the patient's height and serum creatinine, has been developed for the general population of pediatric renal disease patients, but a method specific to cystinotic nephropathy is necessary in this case. Analysis on a small set of data has shown that a linear regression predictor may adequately substitute for actual creatinine clearance.

The Biometry Branch is also participating in the planning and coordination of a clinical trial involving pediatric AIDS patients. The Prevention Research Program has funded a data center for a clinical trial to evaluate the efficacy of intravenous immunoglobulin (IVIG) to suppress bacterial infections in children with HIV infection. Staff has advised on sample size requirements, rules for early trial cessation, and other design issues. Another planned trial of azidothymidine (AZT) therapy will require special experimental design implementation in order to satisfy the ethical compunctions on the use of placebo controls while preserving the ability to draw unbiased and unequivocal conclusions from the data.

Since 1985 the Biometry Branch has collaborated with the Laboratory of Developmental and Molecular Immunity, IRP on the Vi Polysaccharide Vaccine Trial in Nepal. Staff participated in the training of field staff in Nepal, and analyzed the data of the pilot study for safety and immunogenicity. In March 1986, 6,912 participants were vaccinated with either the Vi vaccine or a polyvalent pneumococcal vaccine in double blind format, using syringes filled according to a random number program and coded by the Institute Merieux. At the end of the first year of surveillance, 26 confirmed cases of typhoid have been diagnosed in the participants. An independent monitor for this study determined that six of the typhoid cases were given the Vi vaccine and the other 20 received the pneumococcal vaccine. This is significant with a $p < .001$. This trial will last until the end of August which is the end of the monsoon season, the period when the largest number of typhoid cases are observed. The randomization codes will be broken in September in order to do cross-over immunization in October, 1988.

Biometry Branch staff have also worked collaboratively on other research projects with staff of the Laboratory of Developmental and Molecular Immunity, IRP. For example, there is joint work underway to analyze antibody titer levels for a number of potential vaccines. The analysis of antibody titer levels on normal adult volunteers who received pertussis toxin "toxoid" has been completed and the results will soon be published. This is phase one of the study to assess the safety, immunogenicity, duration of synthesis and protective actions of pertussis toxin "toxoid" induced antibodies. Staff have also been involved in the testing and evaluation of a haemophilus influence type b capsular polysaccharide-tetanus toxoid conjugate vaccine for infants under 18 months and a pneumococcus capsular polysaccharide-diphtheria toxoid conjugate vaccine.

Another study, based on the 1981 Child Health Supplement, has included collaborative data development and analysis with the National Center for Health Statistics to produce reliable national descriptions of children's health. One paper entitled: "The Health Status of Low Birth Weight Children in the U.S." will soon be published. Future analysis plans include a more detailed

analysis of the low birth weight children in terms of significant prenatal events and the childrens' later health outcome.

Growth and Development

A significant amount of Branch staff effort has been in the nutrition and growth area. These efforts first began with the analysis of infant feeding data from the Pima Indian Reservation and the George Washington University Study, and have continued with the analysis of the Bedouin Arab Infant Feeding Study. The Pima Indian and the Bedouin Arab data sets were cluster samples including data on all the children in the family. The proper analysis of clustered data where binary observations within each cluster may be correlated is a statistical problem that has been investigated by Branch staff.

The development of statistical methods to understand the complex relationships between growth, development and nutrition from NHANES has also been an active research area of the Biometry Branch. A contract was completed by Research Triangle Institute (RTI) in North Carolina that developed new stochastic regression methods and computer software for analyzing complex designed survey data. This contract work resulted in a report which illustrated the regression methods by reanalyzing the relationship between blood lead levels and blood pressure among NHANES II adults. Analyses are in progress to examine the relationship between blood lead and stunted growth in children using NHANES II data.

Biometry Branch staff also have been involved in the analysis of pregnancy outcomes from the Diabetes in Early Pregnancy Study. The results of this study are described in detail in the Epidemiology Branch summary. Also, staff of the Biometry Branch have participated in a study undertaken by the Epidemiology Branch for the evaluation of the long-term effects to children exposed in infancy to chloride-deficient formula. The full description of this study is provided in the Epidemiology Branch summary.

Biometry Branch staff have also been involved with the Epidemiology Branch and Mental Retardation and Development Disabilities Branch, CRMC, in the planning, development, and conduct of the Chorionic Villus Sampling and Amniocentesis Study. This multicenter clinical trial began in March, 1985. Analyses are being performed on fetal loss rates and time to fetal death.

The Normal Range Study is a collaboration with the Clinical Pathology Department of the Clinical Center designed to establish reference standards for certain blood chemistries such as SED rate, hematocrit, and white blood cell counts. The Branch is currently analyzing the data provided by 1146 normal volunteers at six month intervals over a 2 1/2 year period. For example, staff have developed the methodology for estimating and studying the variability of the various analyte measurements over the time period of the study. It is hoped that results will provide clinically important estimates of a normal person's variability in

certain diagnostic indices over periods of 6, 12, and 24 months. Also, new nonparametric techniques in exploratory data analysis are being employed in order to characterize the various distributions more completely than the usual normal theory approach would allow. Graphic methods, families of transformations, and g- and h-distributional families all are providing insight into the non-Gaussian nature of these variables. This unusually complete data set will allow for analysis by covariates such as race, gender, age, smoking, drinking, and level of exercise as well as for estimation of the within-person variability over the 2 1/2 years of data collection. Results are currently being prepared for a series of articles characterizing the normal ranges of these measures in the medical literature.

Presentations:

Brock MA, Denman DW, Hoffman HJ, van der Vate J. Comparisons of human daily temperature and pulse rate measurements to clinical observations obtained annually in the Baltimore Longitudinal Study on Aging. Contributed paper for the Society for Research on Biological Rhythms. Charleston, South Carolina, May, 1988.

Denman DW. Introduction to SASGRAPH. Invited presentation for the Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences. Bethesda, Maryland, January, 1988.

Denman DW, Hoffman HJ, Rothwell CJ. Indicators of perinatal outcomes using multinational matched file data. Invited presentation for the 115th annual meeting of the American Public Health Association. New Orleans, Louisiana, October, 1987.

Eyster JT, Hoffman HJ. Multinational comparisons of birthweight and gestational age specific perinatal mortality rates. Invited presentation for the 115th annual meeting of the American Public Health Association. New Orleans, Louisiana, October, 1987.

Graubard BI. Effects of cluster sampling on epidemiological analysis in population based case-control studies. Invited presentation for the Biostatistics and Epidemiology Branch, Division of Cancer Etiology, NCI. Bethesda, Maryland, November, 1987.

Hoffman HJ. Chairperson--Multinational comparisons of birth weight-specific perinatal mortality rates, 1980-84. Invited session for the 115th annual meeting of the American Public Health Association. New Orleans, Louisiana, October, 1987.

Hoffman HJ. Multinational comparisons of perinatal and infant mortality rates, 1980-84: An overview. Invited presentation for the 115th annual meeting of the American Public Health Association. New Orleans, Louisiana, October, 1987.

Hoffman HJ. Design considerations for the 1990 longitudinal followup to the 1988 National Maternal and Infant Health Survey (NMIHS). Invited presentation for the Planning Conference for the 1990 Longitudinal Followup to the 1988 NMIHS jointly sponsored by The Ford Foundation and the National Center for Health Statistics. Bethesda, Maryland, April, 1988.

Hoffman HJ, Denman DW, Brock MA, van der Vate J. Seasonal changes in a 38-year record of human daily temperature and pulse rate measurements. Contributed paper for the Society for Research on Biological Rhythms. Charleston, South Carolina, May, 1988.

Hoffman H.J. Design of the prospective study on risk factors for successive small-for-gestational age births. Invited presentation for the Symposium at the Nordic Congress of Obstetrics and Gynecology on Successive Small-for-Gestational Age Births--A Longitudinal Study of Fetal Growth and Perinatal Outcome. Trondheim, Norway, June, 1988.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00801-13 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Studies Based on the Medical Birth Registries of Norway and Sweden

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

PI:	Howard J. Hoffman	Chief	BB PRP NICHD
Other:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)* Inst. of Hygiene & Soc. Med. & Dept. of OB/GYN, Univ. of Bergen, Norway (P. Bergsjø and L. Irgens); Dept. of Community Medicine, Univ. of Trondheim and Nat'l Inst. of Public Health, Oslo, Norway (L. Bakketeig, A. Arntzen); Dept. of OB/GYN and Social Med., Uppsala Univ. (G. Lindmark, S. Cnagtingius, O. Meirik).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.4	.2	.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway and Sweden, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, (3) perinatal mortality in relation to order of birth and size of sibship, (4) epidemiologic risk factors for preterm birth, and (5) epidemiologic risk factors for small-for-gestational age births.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00802-13 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States

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

PI:	Howard J. Hoffman	Chief	BB PRP NICHD
Other:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)* EB, PRP, NICHD (G.G. Rhoads, M.D. Overpeck); CRMC, NICHD (A. Willoughby); EB, BRAP, NIEHS (A.J. Wilcox); Departments of Health in the following states: Michigan, Missouri, New York State, North Carolina, and Utah; Office of International Statistics, NCHS (R. Hartford).

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NICHD, NIH, Bethesda, Md. 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives are to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. Similar information regarding fetal deaths, based on reports filed for fetuses of at least 20 weeks gestation, will also be studied. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g., birth weight, gestational age, maternal age, race, parity, etc.). The information on fetal or infant death records includes immediate and underlying cause-of-death categories corresponding to the International Classification of Diseases (ICD), based on either the eighth or ninth revision of the ICD codes. Some additional data are available from selected states regarding: smoking during pregnancy, maternal prepregnant weight and height, weight-gain during pregnancy, occupation of parents, and the levels of obstetric and pediatric care available to mother and infant.

Several research contracts have been jointly funded by NICHD and NIEHS to provide data from selected U.S. States (listed above) to compare with data from other developed countries (Australia, Japan, Norway and Scotland) for the time period, 1980-84. This study is entitled: Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00803-04 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors

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

PI:	Howard J. Hoffman	Chief	BB PRP NICHD
Others:	Karla H. Damus	Consultant	BB PRP NICHD
	Jehu C. Hunter	Consultant	BB PRP NICHD
	Daniel W Denman III	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)* U. Wash., (D. Peterson; G. van Belle); Loyola U. (J. Goldberg); UCLA (R. Harper and J. Kraus); Columbia U. (J. Parker, E. Krongrad); N.Y. State Health Dept. (S. Standfast); U. Mo. (L. Hillman); U. London, U.K. (D. Southall); U. Miami (M. Dapena); U. NM (P. McFeeley); AFIP, Washington, D.C. (T. Stocker).

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.4	.3	.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 NICHD Cooperative SIDS Study was designed to enable identification of risk factors which could differentiate SIDS infants from non-SIDS infants. The design is that of a multicenter, population-based, case-control study with a sample of 838 SIDS cases (800 singleton and 38 multiple birth SIDS cases) ascertained under a common necropsy protocol. There were 1,600 matched living singleton control infants and 40 co-multiple birth control infants recruited into the study. It is the largest detailed epidemiological study of SIDS ever undertaken. Data were collected for babies who died over a 15-month period from October, 1978 through December, 1979. Every infant death was autopsied in accordance with a common necropsy protocol developed specifically for the study. Twenty-six different slides of tissues were preserved for detailed examination by a panel of three SIDS pathology experts. Under an Inter Agency Agreement with the Armed Forces Institute of Pathology (AFIP), technical support is being provided for the preparation of a SIDS Histopathology Atlas and "study sets" to be used for the education of practicing forensic pathologists or pathology students.

In another SIDS risk factor study, techniques of time series analysis are being used to examine potential abnormalities in the development of negro-physiological and cardio-respiratory control mechanisms in the first three months of life. The study materials consist of computerized data sets from long-term electrophysiological recordings of infants from three earlier SIDS research studies. Comparisons will be made among the following groups of infants: subsequent siblings of SIDS infants, "near-miss" infants, twins, matched controls, and infants who later died of SIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00813-07 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Biostatistical Methods for the Analysis of Laboratory Research Studies

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

PI:	George F. Reed	Mathematical Statistician	BB PRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD
	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)*

CPD, CC, NICHD (R. Elin and M. Ruddell); IRP, NIAID (D. Alling); Dept. of Statistics, Harvard U. (D. Hoaglin).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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.)*

Research in design and analysis problems arising from laboratory studies on: (1) dose-response relationships, (2) bioassay and potency estimation, (3) time to event, life table analyses, and (4) other investigations of the effects of external stimuli.

In addition to work on techniques for estimating tolerance limits for chemical residue depletion in animals, a major effort in this research area has arisen in the analysis of data from the Clinical Center's Normal Range Study. This study has resulted in the collection of a large number of biochemical and clinical measurements taken serially for 2½ years from "normal" volunteers. The object of the analysis is to characterize the distribution of each variable in order to determine values that can be considered normal. Some of the statistical techniques to be applied will be exploratory data analysis methods, including graphical techniques and outlier detection, transformation of variables, analysis of variance components, and serial correlation. The results of this project will appear in several published reports of quantitative characterizations with special reference to factors that may affect these distributions, such as smoking, drinking, and eating habits, and other demographic or socio-economic factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00818-07 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Research in Developing Nonparametric Methods for Biomedical Applications

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

PI:	George F. Reed	Mathematical Statistician	BB PRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD
	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)*

LCDB, NIDDK (L. Amende and J. Blanchette-Mackie).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.2	.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective is to investigate and develop distribution-free methods in areas of application for which standard parametric techniques are inappropriate or too insensitive to violations of underlying assumptions.

Much of the work of the Branch lends itself to the nonparametric approach. In sample size studies involving analysis of 2x2 tables, the determination of the minimum detectable risk for a given sample size is often required. Although techniques based on asymptotic results for this have been developed within the Branch, they must ultimately be validated by comparison with an exact technique which is based on the theory of randomization testing. This technique has been developed as part of this project. Another common statistical problem that arises from the work of the Branch is the examination of residuals in linear regression to assess goodness of fit. A test based on the distribution of the variance of the size of runs of positive and negative residuals is a potentially apt instrument for such assessment; a computation based exact distribution of the test statistic has been developed and compared to the existing approximate distribution based on asymptotic results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00820-07 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Statistical Methods for Epidemiologic Data

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

PI:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD
Others:	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD
	Howard J. Hoffman	Chief	BB PRP NICHD
	George F. Reed	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)*

Biomathematics Department, School of Medicine, UCLA (E. Korn).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.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.)*

Since many epidemiologic problems cannot be solved by standard techniques, new methods are required to extract more complete answers from research data. The objective of this project is to use mathematical theory and computer simulations to develop and evaluate statistical methods appropriate to data arising in epidemiologic research, and to carry out the statistical programming needed to make these methods easily available to other researchers. This may include evaluating outside computer software, using standard programs in novel ways, and writing special purpose programs.

Further study will continue in then use of influence statistics and regression diagnostics, in particular using the SAS procedures for regression and generalized linear models. Methods appropriate to categorical data and contingency tables will also be given special attention. Useful techniques will be presented in seminars and publications in statistical journals, as well as applied to data analysis within the Branch.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00821-06 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Development of New Graphical Methods for the Analysis of Biomedical Data

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

PI:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD
Others:	Howard J. Hoffman	Chief	BB PRP NICHD
	George F. Reed	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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

Statistical graphics are an integral part of the analysis and presentation of data. Rapid development in this field is evidenced by an extensive research literature and a host of new computer graphics technologies.

The objective of this project is to draw from current literature and computer demonstrations in order to develop graphical methods for: (1) more effective statistical analysis, particularly of multi-dimensional data sets and time-dependent variables; and (2) for more easily understood summaries in finished presentations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00840-07 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Statistical Discriminant Methods with Applications to Alcoholism Screening

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

PI: Barry I. Graubard Mathematical Statistician BB PRP NICHD

COOPERATING UNITS *(if any)*

Alcohol, Drug Abuse and Mental Health Administration (R. Rawlings, S. Teper, V. Fadden and M.J. Eckardt).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.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.)*

This study investigates the statistical properties of a variety of discriminant functions and determines how well they differentiate between alcoholic, other diseased, and normal populations using standard batteries of blood chemistries. Blood chemistry variables that are used to discriminate between diseased and normal groups have been found to have skewed distributions. Using computer simulations, the properties of parametric (linear and quadratic) and nonparametric (fixed and variable kernel) discriminant methods have been investigated when the data comes from a skewed multivariate lognormal distribution. In addition, rank and inverse normal score transformations were applied to the data from the simulation in order to determine if they could improve upon the accuracy of the discriminant functions. It was found that the nonparametric methods were less accurate than the parametric methods when the data came from a multivariate lognormal distribution. The rank and inverse normal score transformations greatly improved the classification accuracy of the parametric methods.

The rank and inverse normal score transformations have been applied to data from multivariate repeated measure designs in order to remedy the effect nonsphericity and non-normality has upon classical repeated measure analyses. It was shown through simulations that the inverse normal scores does improve the performance of certain classical tests used with repeated measures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00841-07 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Methods for Comparing and Analyzing Data from Several Complex Surveys

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

PI:	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)*

EDB, NIA (D. Brock; T. Miles); Research Triangle Institute (B.V. Shah); Biomathematics Department, School of Medicine, (E. Korn).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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

This study will develop statistical methods for the analysis of data from complex designed surveys and test them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). Existing multiple linear regression methods for the analysis of data from complex surveys are compared to newly developed regression methods. These regression methods will be applied to the NHANES data sets to determine if they can be used to provide new information on the complex relationships of growth and nutrition. The preliminary results from this research indicate that the newly developed regression models can better describe complex relationships in the data. This research is being pursued in part through a research contract with the Research Triangle Institute to work in collaboration with NICHD to carry out this study. Over the course of this contract, manuscripts will be prepared for publication which will present the results of the study along with the development of computer programs for applying the methods to real data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00842-06 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Development of Statistical Methods to Analyze Cluster Samples

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

PI:	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)*

BB, PRP, EMS, NCI (M. Gail and T. Fears).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.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.)*

This research project will study statistical methods for analyzing categorical data that comes from cluster samples where the observations within each cluster may be correlated and where the observations may be selected with unequal probabilities. In particular, the analysis of cluster samples from population-based case-control studies and cross-sectional and longitudinal health surveys is examined. Research has concentrated on developing modifications to logistic regression and Mantel-Haenzel and Wolf-Haldane procedures that would account for the complex sample design. Computer simulations are used to validate statistical approximations used in the development of modified methods. Preliminary results from this research indicate that the modified methods for analyzing data from cluster samples appropriately take into account the intra-cluster correlation structure and the unequal weighting of the observations. These methods will be useful for analyzing infant feeding studies and repeat pregnancy studies where the family constitutes the cluster.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00843-05 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

An Investigation of Matched Analysis in Case-Control and Cohort Studies

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

PI:	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD
	George F. Reed	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)*

Biomathematics Department, School of Medicine, UCLA (E. Korn).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.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.)*

This study will investigate the validity and efficiency of neighborhood matching for case-control and cohort studies. The National Health and Nutrition Examination Survey II data were used in conjunction with neighborhood codes (i.e., specifying which individuals in the sample lived close together) to empirically determine the effect neighborhood matching would have upon validity and variance of estimates of risk of various conditions with respect to differing exposures. It was demonstrated that for some types of exposure-condition relationships, neighborhood matching was useful for controlling for confounding. However, there was a loss in efficiency due to a reduced number of matchable observations and a smaller number of degrees of freedom in the test statistics. These empirical examples can provide some guidance to researchers who contemplate neighborhood matching for an observational study. This project is one of the first known attempts of investigating the effect neighborhood matching has upon the analysis of observational data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00850-12 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

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

PI:	Dolores A. Bryla	Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)* Office of the Associate Director, PRP, NICHD (H. Berendes); Human Learning and Behavior Branch, CRMC, NICHD (P. Scheidt); Intramural Research, Neuroepidemiology Branch, NINCDS (K. Nelson and D. Hirtz); Computing Sciences Consultant (K. Fetterly).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

This study, which began in 1974, is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000-2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants, 2,000 grams and above, were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants were followed through six years of age.

The Biometry Branch served as a data center for this study and was the focal point for receipt of examination forms. The master files for each year's follow-up were edited for keypunch and coding errors and for internal consistency. The Branch is now analyzing the data in cooperation with the principal investigators from the cooperating units. The results of the newborn data were published in a supplement to Pediatrics in February 1985. It is anticipated that manuscripts on the follow-up data will be submitted for publication by the end of 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00853-04 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine

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

PI:	Dolores A. Bryla	Statistician	BB PRP NICHD
Other:	George F. Reed	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)*

Office of the Director, NICHD (C.Lowe); Laboratory of Developmental & Molecular Immunity, NICHD (J. Robbins); TEKU Hospital, Nepal (I. Acharya).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

The study is a cooperative, randomized trial to determine the efficacy of Vi polysaccharide in preventing typhoid fever in Nepal. The Biometry Branch's involvement in this study is to design data collection forms, and assist in the data management and the analysis with the study investigators from NICHD and Nepal.

In March 1986, 6,912 volunteers from five villages in Nepal were randomly vaccinated with either the Vi polysaccharide or pneumonococcal vaccine. These volunteers will be visited every three days for the next two years to verify their health status and to detect any typhoid cases prior to treatment. Blood cultures will be done on anyone with a fever of three days duration. The results of the randomization will not be available until late in 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00854-04 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Analysis of MCH Data from the National Longitudinal Youth Survey

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

PI:	Dolores A. Bryla	Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)*

Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis); Ohio State University (F. Mott).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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.)*

This project has as its primary objective to analyze and publish data based on a series of annual interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. In addition, a wealth of other data have been collected on the youth cohort sample in relation to their employment and work history, military service, educational attainments, etc.

The collection of data on pregnancy outcome and the first year of life of the child began in 1983 and is continuing. With this five year data base, analysis of trends over time in the maternal and child health can be done.

The Biometry Branch has joined in the funding of the data collection effort together with the Demographic and Behavioral Sciences Branch, Center for Population Research, NICHD. The mechanism of support for the field study is through an Inter Agency Agreement with the Department of Labor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00860-08 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Analysis of Biomedical Time Series Data

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

PI:	Howard J. Hoffman	Chief	BB PRP NICHD
Other:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)* CI, CP, GRC, NIA (M. Brock); Dept. of Pediatrics, Univ. of South Florida College of Medicine, St. Petersburg, Florida (B. Bercu); Pediatric Nutrition, Mead Johnson Company (J. Hansen); Univ. of Cambridge, England (K. Dalton and G. Breborowicz); Univ. of Alabama in Birmingham (C. Lowery and R. Goldenberg).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of this project are: (1) to characterize developmental patterns from daily measurements of gonadotropins and for estrogens in premenarchial girls and pubescent boys based on radioimmunoassay methods for measuring urinary luteinizing hormone, urinary follicle stimulating hormone, and urinary estradiol, estriol and estrone hormones; (2) gonadotropins in both castrated and intact male monkeys of different ages; (3) growth hormone in normal and precocious pubertal children; (4) to assess circadian and other rhythms in heart rate, temperature and other serial data collected from long-term studies in humans; and (5) to perform analysis of these serial measurements using methods of statistical time series analysis, including autoregressive filtering, auto- and cross-spectrum analysis, and robust smoothing procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00861-06 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

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

PI:	Howard J. Hoffman	Chief	BB PRP NICHD
Other:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)* PRP, NICHD (H. Berendes); CRMC, NICHD (D. McNellis); Univ. of Trondheim, Norway (G. Jacobsen, L. Bakketeig); U. of Bergen, Norway (P. Bergsjø, T. Evans, T. Markestad); Uppsala Univ., Sweden (G. Lindmark); Bell Communications, Livingston, N.J. (G.W. Reed); U. of Alabama in Birmingham (R. Goldenberg).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.3	.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 project has been expanded to encompass two related research studies. The first study has analyzed data derived from a randomized clinical trial of diagnostic ultrasound use during pregnancy conducted by the team of Norwegian investigators in Trondheim, Norway. The purpose of the analysis is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyseal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound; and (4) maternal hemoglobin level. Regression models have been fit to the serial measurements for each mother. The coefficients of the regressions have been analyzed in relation to various indicators of birth size such as weight, crown-heel length, ponderal index, and birth weight-for-gestational age percentile. Using an analysis of covariance procedure, additional factors (e.g., cigarette smoking, alcohol intake, low maternal prepregnancy weight, etc.) will be tested for significance in modifying intrauterine growth patterns.

In addition to the study described above, a prospective study to determine risk factors for intrauterine growth retardation, or small-for-gestational age birth, was begun in 1984 through the research contract mechanism with both the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala). The study protocol includes recruitment of pregnant women before 17 weeks gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally and at delivery. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00871-03 BB

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

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Clinical Trial of New Drug Therapy for Cystinosis

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

PI:	George F. Reed	Mathematical Statistician	BB PRP NICHD
Other:	Daniel W. Denman III	Mathematical Statistician	BB PRP NICHD

COOPERATING UNITS *(if any)*: HGB, IRP, NICHD (W. Gahl); Univ. California, San Diego (J. Schneider); Univ. of Michigan Medical School (J. Thoene); Univ. of Texas Health Science Center, Dallas (J. Reisch).

LAB/BRANCH: Biometry Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	.0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

The Cysteamine Study provided answers to the question of the drug's efficacy with some inferential difficulty, since cysteamine's unpleasant taste and smell rendered it unpalatable to many patients, who subsequently did not receive effective amounts of the drug. The design of the study itself, with no randomized concurrent control group, obscured effects and required a good deal of reliance on adjustment techniques in the final analysis.

The object of the current study is to improve treatment of cystinosis and determine more of the effects of cysteamine. In the drug development phase of the trial, investigation of a cysteamine analog, phosphocysteamine, revealed that it converts rapidly to cysteamine in the bloodstream, so that the two drugs are effective equivalents. Moreover, since the taste and smell of phosphocysteamine are less obnoxious to some patients, it serves as an alternative treatment that may improve patient compliance. The current study randomizes patients to a low dose of cysteamine (or phosphocysteamine as the patient chooses) or to a high dose; so designed the trial is an optimal vehicle for ascertaining the best course of treatment.

Patient recruitment and treatment is coordinated at contracted study center at the University of California, San Diego. Data center functions are the responsibility of the University of Texas Health Science Center at Dallas. The study will encompass 3-4 years of enrollment and treatment of at least 90 patients. The treatments will be evaluated on the basis of renal function as measured by serum creatinine levels and creatinine clearance, as a surrogate of glomerular filtration rate, at the end of the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00872-03 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Factors Associated with Premature Births: Missouri Follow-back Survey

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

PI:	Dolores A. Bryla	Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)*

Missouri Division of Health (G. Land, W. Schramm, and J. Stockbauer

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

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

The objective is to obtain more accurate information relating to the very low birth weight (VLBW) infant, <1500 grams, for calendar year 1987 than is now available from the United States vital records. This objective will be accomplished by the following: (1) to design and administer a mail questionnaire to mothers of VLBW infants, mothers of all fetal deaths, and a sample of mothers of LBW infants (1,500-2,499 grams) and normal birth weight infants (≥2,500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods; (2) to design and conduct telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on mail questionnaires; (3) to develop and conduct procedures for ascertaining from hospital and physician records unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and (4) to prepare and deliver an edited data tape to NICHD. In addition, mortality will be ascertained throughout the first year of life for this birth cohort. This information will help to answer the question: Has there been a reduction in neonatal mortality at the expense of an increase in post-neonatal mortality for these infants?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00873-02 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Relationship of Mother's Prepregnancy Size to Pregnancy Complications and Outcome

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

PI:	Barry I. Graubard	Mathematical Statistician	BB PRP NICHD
Other:	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)* EB, PRP, NICHD (J. Mills).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.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)*

This project will study the relationships between the prepregnant body mass size of a woman and the risk of adverse pregnancy complications and pregnancy outcomes. The Kaiser-Permenante Walnut Creek malformation data set will be used for the analysis. The results from this study could help obstetricians to inform prospective mothers about the potential dangers that obesity and underweight can have upon their fetuses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00874-01 BB

PERIOD COVERED

October 1, 1987 to September 30, 1988

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

Research on Racial Differences in Pediatric Measures of Gestational Age

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

PI:	George F. Reed	Mathematical Statistician	BB PRP NICHD
Others:	Howard J. Hoffman	Chief	BB PRP NICHD

COOPERATING UNITS *(if any)* EB, PRP, NICHD (M. Klebanoff); Research Triangle Institute (V. Rao).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.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 Dubowitz Examination and its derivative Ballard Examination are instruments for estimating gestational age at the time of birth on the basis of observed physical and neurological maturity. Since racial differences in the distribution of some developmental indices are acknowledged or suspected, it is hypothesized that the current pediatric assessments (i.e., the Dubowitz and Ballard tests), which were constructed and validated on a sample of white babies, may effect a bias in the estimation for other racial groups.

The Vaginal Infections in Prematurity (VIP) Study offers data to test the hypothesis. If it is found that differences do exist, the study will also provide the wherewithal to produce a modified pediatric assessment which is proper for the racial group in question.

EPIDEMIOLOGY BRANCH (EB)

ZO1 HD 00318-08 A Prospective Study of the Frequency and Duration of Infant Feeding Practices
Natalie Kurinij, Ph.D.

ZO1 HD 00323-08 District of Columbia Perinatal Study
Heinz W. Berendes, M.D., M.H.S.

Z01 HD 00325-07 Neural Tube Defects and Folate
James L. Mills, M.D., M.S.

Z01 HD 00329-06 Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.
Mary D. Overpeck, M.P.H.

Z01 HD 00331-05 Diabetes in Early Pregnancy Project (DIEP)
James L. Mills, M.D.

Z01 HD 00332-05 The Risk of Adverse Pregnancy Outcome Following Cervicitis During Pregnancy
Robert P. Nugent, Ph.D.

Z01 HD 00333-05 Congenital Anomalies and In Vitro Fertilization (IVF)
James L. Mills, M.D.

Z01 HD 00334-05 Low Birth Weight Across Generations
Mark A. Klebanoff, M.D., M.P.H.

Z01 HD 00340-05 Ethnic Differences in Birth Weight and Length of Gestation
Patricia H. Shiono, Ph.D.

Z01 HD 00344-05 Long Term Effects of Infant Formulas Deficient in Chloride
Michael H. Malloy, M.D., M.S.

Z01 HD 00346-04 Time Trends in the Incidence of Biliary Atresia
Mark A. Klebanoff, M.D., Ph.D.

Z01 HD 00352-03 Studies of Human Immunodeficiency Virus - Related Problems
George G. Rhoads, M.D., M.P.H.

Z01 HD 00360-02 A Prospective Study of 1st Trimester Use of Bendectin and Malformations
Patricia H. Shiono, Ph.D.

EPIDEMIOLOGY BRANCH (EB)
(continued)

Z01 HD 00361-02 Child Health Supplement to the 1988 National Health Interview Survey
Mary D. Overpeck, M.P.H.

Z01 HD 00362-02 Nutritional Aspects of Perinatal Epidemiology in Central America
Jose Villar, M.D.

ZO1 HD 00363-01 NICHD Smoking Trial of Pregnant Women (STOP)
Leslie C. Cooper, M.P.H.

ZO1 HD 00364-01 Syrup of Ipecac Usage in the General Population
Michael H. Malloy, M.D., M.S.

ZO1 HD 00365-01 A Randomized Clinical Trial of Umbilical Artery Catheter Placement
Michael H. Malloy, M.D., M.S.

ZO1 HD 00366-01 Survey of Pregnancy Outcomes Among Medical Residents
Patricia H. Shiono, Ph.D.

ZO1 HD 00367-01 Follow-up of the 1988 Child Health Supplement to Investigate Accidents and Injuries
Mary D. Overpeck, M.P.H.

ZO1 HD 00368-01 Vaginal Delivery of Very Low Birth Weight Infants: Association with Day 1 Deaths
Michael H. Malloy, M.D., M.S.

Z01 HD 00832-05 Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities
Leslie C. Cooper, M.P.H. and Mary D. Overpeck, M.P.H.

NICHD Annual Report
October 1, 1987 to September 30, 1988

Epidemiology Branch, Prevention Research Program

The Epidemiology Branch conducts a broad research program addressing the distribution and determinants of health conditions in mothers and children. The single largest focus of branch effort is in the area of low birth weight, preterm birth, and infant mortality. Other areas of inquiry concern teratologic and genetic problems, nutrition, and human immunodeficiency virus infection.

Low Birth Weight and Infant Mortality

Descriptive Studies: Washington, DC and certain other cities have been cited for unusually high rates of infant and perinatal mortality. However, concern with reliability of reporting of early fetal deaths and ambiguous classification between live births and fetal deaths has led to uncertainty in comparing fetal and infant deaths among various jurisdictions. To address this problem Branch staff developed special methods to describe perinatal mortality for 59 U.S. cities over the period 1972-1981. These data allowed analysis based on losses after 24 or 28 weeks gestation, which is more reliable than the usual data on fetal deaths which are based on 20 weeks gestation. By combining late fetal and neonatal deaths, reasonably comparable perinatal mortality statistics can be developed among the 59 jurisdictions. Examination of shifts from neonatal to post-neonatal mortality and correlation with regionalization of care and size of city are in progress.

The Role of Infection: The causes of preterm birth and of intrauterine growth retardation are poorly understood but a number of lines of evidence have suggested that genital tract infection may play a role. Pathologically defined chorioamnionitis is known to be much more common in preterm than in term births, but the literature relating carriage of particular vaginal or cervical organisms to the onset of labor has been confusing. A major project to examine these issues, funded by CRMC and NIAID, is being largely coordinated by Branch staff. More than 12,000 women have been enrolled in seven medical centers across the country with eventual enrollment projected to be between 12,000 and 16,000. Vaginal and cervical cultures are being performed on participants during the second trimester of pregnancy. A variety of organisms are being sought. Outcomes are being monitored in terms of subsequent complications of pregnancy, intrapartum events, and perinatal outcome. Women carrying Group B streptococci, Chlamydia trachomatis, and Ureaplasma urealyticum have been invited to participate in a randomized trial of long-term erythromycin therapy (1 gram daily) in order to assess its prophylactic effect. To date over 2100 women have agreed to be randomized including 1581 with Ureaplasma, 467 with Group B

streptococcus and 203 with Chlamydia. (Some women have more than one organism.) Initial analysis has revealed no beneficial result of erythromycin therapy in 1200 women with Ureaplasma but without Group B streptococci or Chlamydia. Consequently, carriage of ureaplasma is no longer a reason for recruitment into the trial. Enrollment of women with the other two organisms is scheduled to continue into 1990.

In a related but smaller project approximately 800 women attending the Johns Hopkins University prenatal clinic have been enrolled in a study including careful observation and photographs of the cervix in the second trimester of pregnancy. Cultures for multiple organisms were also taken. Follow-up of the women has been completed and the data analysis is nearing completion. Results so far suggest that cervical inflammation is difficult to define in a reproducible way, which is likely to make it difficult to use the concept clinically. Within this inner city population Chlamydia colonization was more common in Black (15.4%) than in other (6.9%) women. A paper presenting the major findings of the study has been submitted for publication. Mycoplasma hominis, Chlamydia trachomatis, heavy smoking and delivery of previous low birth weight infant were associated with preterm birth. Chlamydia, Candida albicans, maternal smoking and drinking were associated with intrauterine growth retardation. Preliminary analyses of Gram stained smears suggest a possible role of bacterial vaginosis in increasing the risk for preterm delivery. The predictive value of increased numbers of polymorphonuclear cells in identifying pregnant women with Chlamydial infection appears to be much lower than in non-pregnant women. The effort required to quantitate PMN's accurately is sufficiently great and the predictive value low enough to make the "swab test" a poor indication of Chlamydial infection in pregnant women.

Intergenerational Studies: It is known that low birth weight tends to recur across generations. Evidence from the linked birth certificates of mothers and children born in Tennessee indicates that the rate of intrauterine growth mediates this effect more strongly than does length of gestation. For example, mothers who weighed 2000-2499 grams at birth are nearly 4 times as likely to have a small for gestational age infant compared to mothers who weighed 4000-4499 grams, but only 1.6 times as likely to give birth to a preterm infant. It was not possible to evaluate the effect of the mother's own gestational age at birth. In order to determine which of these mechanisms is operating, it will be necessary to acquire data sources from the early 1960's in which both length of gestation, birth weight, and other confounding factors were recorded for subjects who can be traced and whose own reproductive performance can be assessed at the present time. Data of this type have been assembled from a health district in Sweden which maintained a low birth weight registry in the 1950's. Preliminary results indicate that women who were small for-dates at birth are at increased risk of giving birth to both small for dates and preterm infants. Women who were preterm at birth are not at increased risk for either outcome. Two ongoing projects

are studying the intergenerational associations of birth weight, gestational age, and possibly other perinatal complications. One contract with the University of Pennsylvania and Brown University will trace girls who were members of the Philadelphia and Providence cohorts of the Collaborative Perinatal Project (1959-66). The other contract with the University of Southern California and the Psykologisk Institut in Copenhagen will locate girls who were subjects in the Danish Perinatal Study (1959-61). In each study all girls who were born preterm or small for gestational age, and a random sample of controls will be located and their reproductive outcomes determined. Subject tracing is currently in progress.

Other Risk Factors: In an effort to elucidate environmental variables associated with low birth weight a case-control study of low birth weight infants born to residents of the District of Columbia has been carried out. The study cases were low birth weight babies (<2500 grams) born in participating hospitals which accounted for 90% of the low birth weight births occurring in the city. Controls were the next infant born within the same hospital of the same race and 'normal birth weight' (>2500 grams). The mothers of the cases and the controls were interviewed on the postpartum units, with data verification obtained through abstraction of the medical records. The factors investigated include: socioeconomic status, past pregnancy history, prenatal care, past gynecologic history, stress, infections during pregnancy, family history of poor pregnancy outcome, paternal factors, environmental exposures, nutrition, and use of non-prescription drugs including tobacco, alcohol and 'street drugs' (illegal drugs). Delays in analyzing these data were occasioned by the failure of the original contractor to complete the work. However, three abstracts summarizing the results of this work have been accepted for presentation and manuscripts are now in the process of being prepared.

The reasons for the large ethnic differences in the incidence of low birth weight and preterm delivery are unknown. Known risk factors such as smoking, level of maternal education, restricted maternal weight gain, and a variety of obstetrical conditions do not explain the two-fold increase in incidence in low birth weight among Black women as compared to White women in the U.S. Nor do they explain why Hispanic women, despite their relatively low economic status and lack of formal education have relatively low rates of low birth weight. The Branch is currently working to find previously undescribed reasons for this discrepancy. At prenatal clinics affiliated with Columbia and Northwestern Universities, data will be prospectively obtained from pregnant women from six ethnic groups: American Black, Chinese, Mexican, Dominican, Puerto Rican, and White. This information includes such topics as social support, level of physical activity, nutrition, stress, beliefs and attitudes about pregnancy, and acculturation. The study instruments have been completed, piloted, and formal data collection is currently in progress.

The effects of employment during pregnancy on rates of low birth weight and preterm delivery are controversial. The effects of stressful employment on pregnancy outcome will be examined in a study of pregnancy outcomes among young physicians. Several studies of paid employment by women during pregnancy have shown an increased risk of both preterm birth and low birth weight associated with strenuous occupations. However, the majority of studies show no increased risk. None of the previous studies were able to control adequately for the socioeconomic status of the women, and in many instances inappropriate controls were used. To address some of these problems the branch is initiating a study of pregnancy outcome in women physicians who become pregnant during residency. These women are in many respects an optimal group in which to study this issue. They are highly educated and of high socioeconomic status, yet their occupation is highly stressful and physically demanding. For this reason, the effects of a mentally and physically demanding occupation can be studied independently of socioeconomic status. Spouses of male residents comprise an appropriate control group, as they are also of high socioeconomic status, but in most cases have less strenuous occupations than do the medical residents themselves. Approximately 10,000 residents in their third post-graduate year (all of the women residents and a 50% random sample of male residents) will be surveyed to determine the pregnancy outcomes of the female residents and spouses of male residents.

Risk Factors in a Developing Country: Perinatal risk factors are being investigated in the Longitudinal study of Perinatal and Nutritional Epidemiology which was conducted in Guatemala City, Guatemala. The study population (n=17,000) was selected from the Guatemalan Social Security Institute's Ob/Gyn Hospital. This is a 230 bed Ob/Gyn hospital with a tertiary neonatal intensive care unit. Participating women were eligible to receive health care at the study hospital because of their own employment or that of their husbands. Between April 1, 1984 and January 10, 1986 women having their first prenatal visit at the hospital clinic were enrolled in the study. Baseline information on a number of obstetric risk factors, parasite infestation, and nutritional status was collected and has been related to the subsequent outcome of pregnancy. The data are being used to produce a simple, empirically developed instrument for the identification of mothers at risk of delivering LBW infants in developing countries. Such an instrument would provide information to detect mothers and children at greater risk of morbidity and mortality associated with LBW. It will also explore the type of medical care that is related with risk level and negative pregnancy outcomes. It is of interest that asymptomatic intestinal parasite infestations among these women were common but were unrelated to the incidence of low birth weight.

Prevention Studies: Maternal smoking has been identified as the most important single risk factor for low birth weight that is potentially modifiable. The Smoking Trial of Pregnancy Project (STOP) will be carried out as a randomized clinical trial to

evaluate different approaches to smoking cessation within physician practice settings. This project is a collaborative effort between the NICHD and the American College of Obstetricians and Gynecologists. The unit of randomization in STOP will be a practicing physician's population of pregnant women who smoke or have recently stopped smoking. The STOP project will be directed into two phases: a pilot study and a formal trial. The objective of the pilot study is to develop the protocol and study materials, assist in the development of all quality control procedures, develop all necessary data management materials (data entry program, SAS data sets, edit specifications for all data, analysis of the data, etc.) and train the contractor selected to run the formal trial in all aspects of the study. All study materials (forms, urine testing, pamphlets, etc.) will be modified to be easily incorporated into the daily routines of private physicians' offices. It is anticipated that the pilot will begin in October, 1988 and continue until spring of 1990.

In an inner city initiative, the Branch has collaborated with several private sector organizations in the Better Babies Project. The project is aimed at reducing the rate of low birth weight infants in a target area in the District of Columbia. Outreach workers are identifying as many pregnant women as possible in the target area and encouraging them to begin prenatal medical care, improve the frequency and total number of their prenatal visits, improve their adherence to health and medical advice and link them with specific interventions designed to reduce prematurity, smoking, and social stress. Branch staff have provided recommendations on study design and types of intervention and will be responsible for evaluating the impact of the project on low birth weight. An extensive pilot project was completed on August 31, 1986. The formal trial began September 1, 1986 and is expected to continue through 1990.

Clinical Management: The Branch has been assisting CRMC with the implementation and coordination of a randomized study of IVIG to prevent infection in low birth weight infants being cared for in the neonatal intensive care units (NICUs) at eight university centers around the country. More than 700 infants of less than 1500 g have been seen at these nurseries and about half are being recruited for the study. Descriptive analyses of the characteristics and outcomes of infants in this network of NICUs and of patterns of care at the several centers are also planned.

Branch staff members are presently designing another clinical trial to determine if very low birth weight infants (VLBW) who receive an umbilical artery catheter placed high in the thoracic aorta are at greater risk for intraventricular hemorrhage than VLBWs who receive a catheter placed low in the abdominal aorta. Piloting of the project should begin in the fall of 1988 with the main project beginning in March of 1989.

Teratologic and Genetic Problems

A study of periconceptional vitamin use in women having fetuses or infants with neural tube defects has been in progress since 1985. The study is examining the question, "does periconceptional vitamin use reduce the risk of neural tube defects?" Data collection is now complete. Data on vitamin exposure have been edited and we will soon begin comparing vitamin use in the NTD case group with the two groups of control subjects (malformed and normal). Data are available on approximately 570 cases, 540 malformed controls and 560 normal controls. We expect to write a final report on this study within the next 12 months. In a related effort an attempt has been made to get serum samples from early pregnancy in NTD cases and controls in Finland. Because of changes in human subjects' protection regulations, it has proved very difficult to obtain information on cases. This issue is being addressed by our collaborator in Helsinki.

Analysis and reporting of the Diabetes In Early Pregnancy (DIEP) Study results are progressing rapidly. The first major paper was published by the New England Journal of Medicine in March 1988. Diabetic women who entered the study periconceptionally had lower malformation rates in their offspring than diabetic women who entered late, but higher rates than control women. Glycemic control did not explain these malformations, indicating that the search for teratogenic mechanisms needs to be widened.

The second major question in the DIEP is now being examined. The risk of pregnancy loss in diabetic and control women has been compared. Overall, the diabetic subjects had no higher loss rates 62/386 (16.1%) than the control subjects 70/432 (16.2%). However, the small subgroup of diabetic women in relatively poor control had significantly higher loss rates. When mean first trimester glycosylated hemoglobin values were above the normal range, each standard deviation increase above the normal control mean was associated with approximately a 3 percent increase in pregnancy losses. Thus, diabetic women in good metabolic control are at no increased risk for pregnancy loss, but those with elevated blood glucose or glycosylated hemoglobin levels in the first trimester are at significantly increased risk. In the DIEP this risk did not appear as an increase in the overall loss rates in the diabetic group because the vast majority of diabetic women were well-controlled.

Another DIEP analysis has shown that placental hormone levels in early pregnancy generally are no different in moderately well-controlled diabetic women than in non-diabetic women. hCG alpha sub-units were an interesting exception. They were significantly lower in diabetic women at multiple points early in pregnancy. This may represent a defect in cytotrophoblast function in diabetic women.

The study of congenital malformations and development in children conceived in vitro is now complete. The in vitro fertilization

(IVF) group did not have a significantly higher malformation rate than the control group. Both groups scored well above average on the Bayley developmental scale. We had anticipated that the IVF group would perform well because of their high socioeconomic status and the "wantedness" of these children. Our study confirmed that the IVF children scored as high as a socioeconomically matched control population. These results indicate that IVF is not associated with a major teratogenic risk, nor does it cause developmental delay.

The Branch has continued its involvement in coordinating the NICHD Chorionic Villus Sampling (CVS) Study. CVS is done between 8 and 12 weeks after the last menstrual period and provides prenatal diagnosis 1-2 months earlier than does amniocentesis. The accuracy of the procedure will be assessed in all consenting patients having CVS at one of the seven participating centers. Those at average obstetric risk who live within 1-2 hours driving distance of the centers and who have a baseline ultrasound showing a viable pregnancy of 49-90 days gestational age will be used to assess the safety of the procedure.

The first paper from the CVS Study is based on this latter group of women. The safety and efficacy of prenatal diagnosis for maternal age was compared in 2278 women undergoing CVS and in 671 undergoing amniocentesis. Subjects in both groups were recruited in the first trimester and verified by ultrasound at one of the seven participating centers to have a viable pregnancy. Cytogenetic analyses were successfully performed in 97.7% of CVS and 99.1% of amniocentesis cases ($p<.05$) and revealed 1.7% and 1.4% aneuploidy, respectively. Patients often reported cramping (22%) and spotting (32%) following CVS whereas these were less common after amniocentesis. After adjusting for slight differences in gestational age and menstrual age at entry, the combined losses due to spontaneous and missed abortion, termination of abnormal pregnancies, still births, and neonatal deaths were 0.7% (80% C.L. -0.7% to 2.0%) higher in the CVS than in the amniocentesis group. Loss after CVS was 10.8% in cases with 3 or 4 transcervical catheter passes compared to 2.9% with one pass ($p<.01$). There were no serious maternal infections in these cases or in an additional 1990 CVS cases being studied mainly for procedure accuracy (upper 95% C.L. for CVS = 0.08%). Recruitment is continuing into a randomized comparison of transcervical and transabdominal CVS which is expected to enroll 4000 patients.

Nutrition

The Branch has continued to be involved in several projects relating to nutrition during pregnancy and childhood. As noted above the Longitudinal Study of Perinatal and Nutritional Epidemiology, conducted in Guatemala, has examined height, weight, and weight gain in 13,000 pregnant women in a developing country setting and has related them to subsequent pregnancy outcome. Multiple skinfold measures were obtained at several points in

pregnancy in a sample of these women and are being analyzed in conjunction with 24 hour recalls. Studies of lactose digestion were examined in another group of these women and suggested that lactose tolerance improves during pregnancy. Studies of calcium and iron absorption in pregnancy have been conducted in a separate group of 400 lower class pregnant women in Baltimore.
Considerable progress was made this year on a population based study of children who ingested chloride-deficient infant formula in 1978-79. Surveys were conducted of children enrolled in the Fairfax, VA, and Montgomery County, MD, school systems to identify these children and controls who were exposed to other soy formulas. About 250 neomullsoy children and 500 control children (matched on race, sex, and maternal education) are scheduled for psychological testing in their homes, representing a response rate of about 70% in both groups. Tests include the WISCR, the Boston Naming Test, the Ray-Osterreith, and sentence imitation and oral direction sub-tests from the Detroit Learning Tests. A test of verbal fluency is also included.

In addition to these school based studies about 30 children from across the country who had documented hypochloremic metabolic alkalosis as a result of defective formula ingestion are being examined in the Washington area and compared to selected, matched control children from the school based study. Fieldwork for these studies is expected to finish in 1988 and a final report is anticipated in 1989.

Analysis of data from the prospective study of infant feeding practices of U.S. women has continued this year. The study was carried out to investigate the underlying reasons for differences in breast-feeding rates between white and black women. Primiparae (n=1179) were interviewed during the first few days postpartum to ascertain their infant-feeding behavior and the factors which led them to choose exclusive breast feeding, breast and formula feeding, or formula feeding. These women were followed through the first year with a series of interviews to ascertain when they actually stopped breast feeding and their reasons for stopping. Ethnic differences in the rate of breast feeding are evident with 84% of white women breast feeding at birth compared to only 49% of black women giving birth in the three hospitals selected for study. The influence of sociodemographic factors on the incidence and duration of breast feeding was examined, and it was found that maternal educational level was strongly associated with breast feeding, whereas the effect of ethnicity was moderate. These results have been published in Pediatrics.

Sociodemographic differences between breast and formula feeders have been extensively studied, yet these factors are not modifiable. Identification of maternal infant-feeding attitudes and mothers' perceptions of social support for breast feeding is important for planning education programs. Three attitudes predictive of breast feeding were identified by factor analysis: "breast feeding is best for the baby," "breast feeding is not socially restrictive," and "maternal confidence in ability to

breast feed." A paper reporting the effect of these factors on the deviations of breast feeding is being prepared.

Studies Related to Human Immunodeficiency Virus

The Prevention Research Program has played a key role in initiating a study of intravenous immunoglobulin (IVIG) in the amelioration and prevention of disease in HIV infected children that is being carried out in collaboration with CRMC and NIAID. This randomized placebo controlled clinical trial began in early 1988. It is hoped that approximately 340 children will be enrolled, some of whom will be symptomatic and some pre-symptomatic. A data center has been recruited to assist with this study and will be supervised by PRP staff. So far 24 centers have entered 115 children into this protocol.

Other Activities

The Epidemiology Branch is participating in the NICHD Cooperative Maternal Fetal Medicine Unit and Neonatal Intensive Care Unit Networks which have been created to evaluate therapeutic modalities in the perinatal period, especially those relating to low birth weight. Both networks employ a distributed data entry system. Information is entered directly on a micro-computer at the study sites eliminating the need for exchange of forms by mail. In addition, the computer will directly aid the collaborating centers in determining eligibility and monitoring protocol compliance. The Epidemiology Branch provides advice to the data center and the Steering Committee of these two networks on epidemiologic and clinical trials issues. The Maternal Fetal Network consists of seven leading obstetrical centers, a data center and representative of the Epidemiology Branch and the Pregnancy and Perinatology Branch. In one study, which began in December 1987, women whose pregnancies have gone beyond 41 completed weeks are being randomized to immediate induction of labor or surveillance and serial tests of fetal well-being with labor being induced only for demonstrated fetal compromise. Neonatal and maternal outcomes are compared between the two groups. Assuming adequate recruitment, results of this study will provide insights on ways to reduce the increased neonatal morbidity associated with post dates pregnancies, and possibly to reduce the high Cesarean section rate seen among these women. A second study, scheduled to begin later this year, will examine the use of low-dose aspirin to prevent pre-eclampsia. Primiparous women will be randomly assigned to receive 65 mg of aspirin or a comparable placebo once a day from the second trimester to term. The incidence of pre-eclampsia in the two groups will be compared.

An analysis of the 1985 Health Interview Survey Supplement on Health Promotion and Disease Prevention pointed out an area for enhancing the prevention of childhood poisoning morbidity. The analysis showed that only a small proportion (25%) of the general population with children less than 10 years of age had syrup of ipecac in their households. In a small telephone survey of

pediatricians in the Washington, DC area we observed that only 7% distributed ipecac from their offices. It appears that a major increase in the availability of ipecac in American homes could be achieved if pediatricians and others providing health care for young children would distribute it as part of their well child care.

A national survey is being conducted as a supplement to the National Health Interview Survey to document the health status of children in the U.S. in 1988. Subjects will include accidents, injuries, poisonings, other childhood morbidity, child care, family relationships, perinatal events, use of health services, school performance and behavior. The survey is a collaborative effort of NICHD, the Health Resources and Services Administration, Child Trends Inc., the National Center for Health Statistics and the U.S. Census Bureau. The Branch took a very active role in developing the instrument, providing analysis plans and reviewing edit specifications. Data should be available for analysis in 1989.

The Branch is collaborating with other units at NIH on the follow-up of children who received human growth hormone for the possible development of Creutzfeld-Jacob disease. The cohort of children who received National Pituitary Agency growth hormone has now been identified. Clearance from OMB has been obtained and subjects are currently being interviewed. Approximately 6000 subjects will be asked to participate. Since this study began, an interesting second issue regarding the safety of growth hormone has arisen. Japanese investigators have identified 5 growth hormone recipients who went on to develop leukemia. This is far above the expected incidence. Initial examination of our U.S. data indicates that we have 3 cases. This may represent an increase over the expected rate. Other cases are being sought.

As therapy for leukemia improves, an increasing number of children are achieving long-term remissions and many are presumed to be cured. These children are now reaching reproductive age. The long-term effects of radiation and chemotherapy on their fertility and pregnancy outcomes need to be addressed. We are collaborating with the National Cancer Institute and the Children's Cancer Study Group to identify and interview a cohort of survivors of ALL and cousin controls. A wide range of reproductive issues will be examined including pubertal development, menstruation, fertility, spontaneous abortion, and congenital malformations in the offspring of survivors. If possible, other potential problems survivors face such as poor growth, psychosocial adjustment problems and other medical complications of cancer therapy will be addressed. Data instruments have been developed and are currently being reviewed.

Presentations:

Mark Klebanoff. Short interpregnancy interval and the risk of low birth weight. American Public Health Association, October 1987.

Michael Malloy. The association of maternal smoking during pregnancy and congenital malformations. American Public Health Association, New Orleans, October 1987.

Michael Malloy. The 1990 Objectives. Invited Presentation: Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas, October 1987.

James Mills. Diabetes in early pregnancy. Albert Einstein Medical College Conference, New York, NY, November 1987.

Patricia Shiono. A prospective study of first trimester use of bendectin and congenital malformations. American Public Health Association, New Orleans, November 1987.

Patricia Shiono. Ethnic differences in low birth weight and preterm delivery. Invited Presentation: Institute for Basic Research in Developmental Disabilities, Staten Island, New York, November 1987.

Jose Villar. The use of epidemiological methods in health services: Validation and data quality control. Spanish National Public Health Congress, Madrid, Spain, November 1987.

Jose Villar. The use of epidemiological methods in health services: Measurement errors in data collection. Spanish National Public Health Congres, Madrid, Spain, November 1987.

James Mills. Diabetes and birth defects. Inter-Institute Genetics Conference, NIH, Bethesda, MD, December 1987.

James Mills. Diabetes in pregnancy. American Diabetes Association, Professional Writers Seminar, Palm Desert, CA, January 1988.

James Mills. Teratogenic effects of diabetes. Center for Environmental Health, CDC, Atlanta, GA, January 1988.

Mark Klebanoff. The epidemiology of low birth weight. Johns Hopkins University, February 1988.

James Mills. Malformations and diabetes. U.S.-Italian Collaborative Diabetes Research Conference, Bethesda, MD, February 1988.

Margarett Davis. Breast feeding and AIDS. Community Pediatrics, School of Medicine/Maternal and CHild Health, School of Public Health Joint Seminar, University of North Carolina, Chapel Hill, NC, February 1988.

Jose Villar. Body composition estimation during pregnancy and its differential effect on birth weight. Society of Perinatal Obstetricians, 8th Annual Meeting, Las Vegas, NV, February 1988.

Jose Villar. The reduction in the incidence of lactose malabsorption during pregnancy. Society of Perinatal Obstetricians, 8th Annual Meeting, Las Vegas, NV, February 1988.

James Mills. Spontaneous abortion, Diabetes in pregnancy. Lectures in Graduate Courses. University of Pittsburgh, Pittsburgh, PA, and Johns Hopkins University, Baltimore, MD, February and March, 1988.

Michael Malloy. Newborn screening. Maternal and Child Health course, Johns Hopkins School of Public Health, Baltimore, MD, March 1988.

Michael Malloy. The high risk infant. Maternal and Child Health course, Johns Hopkins School of Public Health, Baltimore, MD, March 1988.

James Mills. Diabetes in pregnancy. Dept. of Population Dynamics Seminar, Johns Hopkins University, Baltimore, MD, March 1988.

Michael Malloy. Follow-up of the high risk infant. Maternal and Child Health course, Johns Hopkins School of Public Health, Baltimore, MD, March 1988.

James Mills. Invited Guest. FDA Hearings on Accutane, Rockville, MD, April 1988.

Margarett Davis. The role of lactation in vertical transmission of HIV. Pan American Health Organization Rountable on Vertical Transmission of HIV, Washington, DC, April 1988.

George Rhoads. Design issues for a study of the safety of chorionic villus sampling. American College of Obstetricians and Gyencologists, Boston, MA, May 1988.

James Mills. Participant - Lawson Wilkins Pediatric Endocrine Society. Working Group on Growth Hormone and Leukemia, Bethesda, MD, May 1988.

Michael Malloy. Trends in fetal and day 1 deaths in Missouri, 1980-1983. Society for Pediatric Research, Washington, DC, May 1988.

Margarett Davis. Relationship of infant feeding to childhood cancer risk. American Pediatric Society and the Society for Pediactric Research, Washington, DC, May 1988.

Margarett Davis. The role of human milk in HIV transmission. Late consequences of infant feeding type. [2 presentations] NICHD Lactation Workshop, Bethesda, MD, May 1988.

Margarett Davis. Breast feeding and AIDS. Committee on International Nutrition Programs, National Research Council, National Academy of Sciences, Washington, DC, May 1988.

Jose Villar. Role of calcium in pregnancy-induced hypertension. 1988 FASEB Symposium "Maternal Nutrition" Keynote lecture, FASEB Annual Meeting, Las Vegas, NV, May 1988.

Margarett Davis. Infant feeding and childhood cancer risk. Society for Epidemiologic Research, Vancouver, BC, Canada, June 1988.

Jose Villar. Weight gain and body composition during pregnancy: Methodology and preliminary results from the Guatemalan Study. Committee on Nutritional Status during Pregnancy and Lactation, NRC/NAS, Washington, DC, June 1988.

Robert Nugent. Ureaplasma urealyticum (Uu) and pregnancy outcome: Results of an observational study and clinical trial. Society for Epidemiology Research, Vancouver, BC, Canada, June 1988.

George Rhoads. Funding for epidemological research in maternal and child health. Society for Pediatric Epidemiologic Research, Vancouver, BC, Canada, June 1988.

Mark Klebanoff. Distinguishing between maternal preterm and SGA effects on pregnancy outcome. Society for Epidemiologic Research, June 1988.

George Rhoads. Safety and efficacy of transcervical chorionic villus sampling. Society for Epidemiologic Research, Vancouver, BC, Canada, June 1988.

James Mills. What causes diabetes - associated malformations? Symposium, Pregnancy Council, American Diabetes Association, New Orleans, LA, June 1988.

James Mills. Fetal losses in normal and diabetic women beginning within 3 weeks of conception. Society for Epidemiologic Research, Vancouver, BC, Canada, June 1988.

George Rhoads. Principles of nutritional epidemiology. Diet and Behavior: A Workshop on Methodologies sponsored by the International Life Sciences Institute Nutrition Foundation, Toronto, Canada, July 1988.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00318-08 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

A Prospective Study of the Frequency and Duration of Infant Feeding Practices

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

PI:	Natalie Kurinij	Nutritionist	EB/PRP/NICHD
Others:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD
	Patricia H. Shiono	Epidemiologist	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.45	.05	.40

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.)*

Although breastfeeding is generally recognized as the optimal way to feed infants through the first 4-5 months, it is well known that many American women nurse their babies for much more limited periods or not at all. In this prospective study characteristics associated with choice and duration of breast feeding are being investigated. The specific objectives of the study are: 1) to provide detailed information on the change in the infant-feeding pattern over time; 2) to investigate the underlying meaning of the milk insufficiency syndrome; 3) to investigate the relation between maternal employment and choice and duration of breast feeding; 4) to determine the sociocultural differences in infant feeding between two ethnic groups. Approximately 1200 women having their first child in one of three hospitals in the Washington, DC, area were interviewed with respect to factors that may have influenced their infant feeding behavior. Data collection was completed in April, 1986. The initial paper describing socio-demographic factors associated with incidence and duration of breast feeding in black and white women and a paper evaluating the effects of maternal employment on breast feeding have been accepted for publication in Pediatrics. Other analyses are currently underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00323-08 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

District of Columbia Perinatal Study

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

PI: Heinz W. Berendes Director PRP/NICHD

COOPERATING UNITS *(if any)*

Epidemiology Branch, PRP, NICHD (L.C.Cooper)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

The D.C. Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to resident mothers in the District of Columbia. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race matched normal weight infant (= >2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. However, if the hospital medical record did not contain adequate prenatal information arrangements were made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

In September 1985 SRA returned the data instruments to NICHD due to an inability to complete the contract. Raw data was returned as well as data entered on two data tapes and disk through the Division of Computer Research and Technology (DCRT). It was necessary to re-key all of the data originally submitted by SRA Technologies. One hundred percent of the data have now been keyed. Manuscripts are now in the process of being prepared for submission to peer reviewed journals. Three abstracts were accepted for presentation at the American Public Health Associatons 116th Annual meeting in Boston, Massachusetts, November 13-17, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 701-HD-00325-07 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Neural Tube Defects and Folate

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

PI:	James L. Mills	Research Medical Officer	EB/PRP/NICHD
Others:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

Biometry Branch, PRP, NICHD (H.Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.3	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.)*

The Epidemiology Branch (PRP) is conducting a case-control study in Illinois and California to determine whether the use periconceptional vitamin supplements can reduce the risk of neural tube defects. Women having either a fetus or an infant with a neural tube defect have been ascertained through perinatal networks, vital records, and other sources and were matched to two controls on maternal race and geographic locale. One control is a mother with a normal pregnancy, and the other the mother of an infant with a fetus with a major health problem. Cases and controls were interviewed within 3 months of the end of pregnancy to determine whether those having a conceptus with a neural tube defect are less likely to have used vitamins in the periconceptional period. Data collection for this study is now complete. Data will be available on approximately 500 subjects in each of the three groups. We are completing record cleaning, editing and identifying the specific constituents of vitamins reportedly used by study subjects. We hope to complete this process within the next three months following which we will prepare a report of our findings for submission to a medical journal. In addition we hope to write one or possibly more reports from the same data set regarding the epidemiology of neural tube defects and possibly other genetic syndromes associated with neural tube defects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00329-06 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

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

PI:	Mary D. Overpeck	Statistician	EB/PRP/NICHD
Other:	George G. Rhoads	Branch Chief	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Epidemiology Branch, PRP, NICHD (H.W.Berendes): Greater Washington Research Center, Washington, DC (J.Maxwell); Better Babies Project, Washington, DC (D.Coates).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.7	.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 Better Babies Project (BBP) pilot study was a three-year research and demonstration effort funded by private foundations to reduce the rate of low birth weight and associated infant mortality and illness in a specific high risk area of the District of Columbia. The BBP Service Delivery team began collecting data July, 1984, for the project's mini pilot. As a result of the mini pilot findngs a number of revisions were made in the forms and interventions. These revised forms and interventions were then developed and piloted. A four year trial of the project began September, 1986. The Project will attempt to identify all pregnant women in a high risk area, help link them with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

NICHD had funded two contracts for the Better Babies Project to assist with the evaluation. The contract for data management and analysis was readvertised and awarded to Group Operations Inc. in September 1987. The D.C. Department of Human Services, Research and Statistics Division, through a contract with NICHD, is providing us information on all pregnant women delivering in the District of Columbia during the period of the project. Analyses of preliminary data should be completed by June 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00331-05 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Diabetes In Early Pregnancy Project (DIEP)

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

PI: James L. Mills Research Medical Officer EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Cornell Univ.Med.Center, NY (L.Jovanovic); Brigham and Womens Hosp. Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ.of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ. of Washington, Seattle,WA (R.Knopp).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

The Diabetes in Early Pregnancy Project has the following objectives: 1) To examine the relationship between maternal diabetic control during organogenesis and malformations in the offspring. To identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state; and 2) To compare early fetal loss rates in women with diabetes and control subjects. The first objective has now been realized. The report on malformation rates in diabetic and control subjects has recently been published in The New England Journal of Medicine. In brief, we found that diabetic women who came into care before the period of organogenesis achieved better results than those who came in later; but their results were still poorer than our non-diabetic control subjects. Differences in maternal glucose levels during organogenesis did not explain the malformations in the offspring of the women who were followed throughout pregnancy. This leads us to suggest that other factors in addition to glucose may be teratogenic in diabetes. Our second objective, determining pregnancy loss rates in diabetic versus control pregnancies, is now being addressed. The data to answer this question have been generated and a report of our results is currently being prepared. Our results have been presented at several scientific meetings including the Society for Gynecologic Investigation, the American Diabetes Association and the Society for Epidemiologic Research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00332-05 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

The Risk of Adverse Pregnancy Outcome Following Cervicitis during Pregnancy

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

PI:	Robert P. Nugent	Epidemiologist	EB/PRP/NICHD
Other:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

Johns Hopkins University, Baltimore, MD (B.F. Polk, L.Berlin), University of Washington, Seattle, WA (S.Hillier)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

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.)*

All eligible women (age 18 and older) seen in the obstetric clinic at Johns Hopkins University between November 1983 and January 1985 who agreed to participate had their cervix evaluated for signs of inflammation. In addition cultures were taken for a number of aerobic and anaerobic organisms and a sample of cervical mucous was evaluated for the presence of inflammatory cells. The women were interviewed to obtain information on a number of risk factors related to preterm and low birth weight delivery. The women were then followed to delivery to evaluate the effect of cervicitis on preterm or low birth weight delivery. Approximately 800 women participated in this study.

The gram stains have been reviewed by Dr. Sharon Hillier of the University of Washington for signs of cervicitis and bacterial vaginosis. These data are currently being analyzed. A paper presenting the major findings of the study has been submitted for publication. Mycoplasma hominis, Chlamydia trachomatis, heavy smoking and delivery of a previous low birth weight infant were associated with preterm birth. Chlamydia, Candida albicans, maternal smoking and drinking were associated with intrauterine growth retardation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00333-05 EB

PERIOD COVERED October 1, 1987 through September 30, 1988

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

Congenital Anomalies and In Vitro Fertilization (IVF)

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

PI: James L. Mills Research Medical Officer EB/PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

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.)*

In vitro fertilization has become an increasingly popular method of conception over the past few years. To date no controlled study of infants conceived in vitro has been reported to determine if they are at increased risk for congenital malformations or developmental delay. Dr. Mills and the Epidemiology Branch have conducted a historical prospective study of infants who were conceived in vitro and matched controls in order to determine whether IVF carries an increased risk for either malformations or developmental problems. The Eastern Virginia Medical School, Norfolk, VA, is serving as study and data center for this project (Dr. Fred Wirth, Principal Investigator). Extensive investigations have been performed on each in vitro fertilization subject and control subject. These include physical examination, intracranial ultrasound, echocardiography, electrocardiography, and abdominal ultrasound. Patient evaluation has been completed and our goal of 160 participants was slightly exceeded. We reached a total of 83 IVF and 93 control subjects in this project. All data from this study have been computerized, cleaned and edited and the data analysis is complete. A paper is currently being prepared for submission to a journal. Our data indicate that IVF is not associated with a major increase in malformation rates nor is any developmental delay attributable to the procedure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00334-05 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Low Birth Weight Across Generations

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

PI:	Mark A. Klebanoff	Research Medical Officer	EB/PRP/NICHD
Other:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

Office of the Director, PRP, NICHD (H.W.Berendes); World Health Organization, Geneva, Switzerland (O.Meirik); University of Pennsylvania (S.Katz), University of Southern California (B.Mednick)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.65	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 original description of the association of maternal and infant birth weights was followed by the description of the association between large maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Study of other fetal growth parameters, including length and head circumference, demonstrated that infants of low birth weight mothers were both shorter and lighter than infants of larger mothers, but that the infants were normally proportioned.

In a related study, birth certificates of infants born in Tennessee between 1979 to 1984 were matched with those of their mothers, who were born in Tennessee between 1959 to 1966. Maternal and infant birth weights were again shown to be correlated. In addition, women who were themselves of low birth weight were up to 4 times as likely to have a small for gestational age infant as were women weighing 4000-4499 grams, but the low birth weight women were less than twice as likely to have a preterm infants.

Follow-up of girls who were born in the 1960's as subjects in the Collaborative Perinatal Project and Danish Perinatal Study is currently underway in order to examine their reproductive histories. Small for gestational age, preterm and control girls will be located and interviewed. Hospital records of their confinements will also be retrieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00340-05 EB

PERIOD COVERED October 1, 1987 through September 30, 1988

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

Ethnic Differences in Birth Weight and Length of Gestation

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

PI:	Patricia H. Shiono	Epidemiologist	EB/PRP/NICHD
Others:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD
	Natalie Kurinij	Nutritionist	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
.3	.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

A contract to obtain a quantifiable description of behavior and lifestyle differences among pregnant women of different ethnic groups which are known to differ in their rates of low birth weight has been awarded to Columbia University and to Northwestern University. The overall goal of this project is to define previously undescribed risk factors affecting birth outcome from pregnant women in the following ethnic groups: American Blacks, Chinese, Mexican-Americans, Puerto Ricans, and Whites. The work scope of the contract includes development of an extensive questionnaire by a multidisciplinary team of experts, pretesting of the interview instruments, interviewing pregnant women from the five groups noted above, and preparing an edited data tape of all responses. The study is reaching the end of the second year. Study instruments have been developed and piloted and recruitment of pregnant women into the study has commenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00344-05 EB

PERIOD COVERED October 1, 1987 through September 30, 1988

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

Long Term Health Effects of Infant Formulas Deficient in Chloride

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

PI:	Michael H. Malloy	Research Medical Officer	EB/PRP/NICHD
Others:	George G. Rhoads	Branch Chief	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Office of the Director, PRP (H.Berendes); Biometry Branch, PRP, NICHD, (B.I. Graubard); Center for Research for Mothers and Children,. NICHD (A.Willoughby

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

During 1978 and 1979 two infant formulas deficient in chloride were marketed in the United States. It has been estimated that a minimum of 20,000 infant years of these formulas were purchased and more than 100 children were reported to the Centers for Disease Control with metabolic and other abnormalities, principally hypochloremic metabolic alkalosis. In a study of 21 of these children at 2 years of age a significant inverse correlation between length of exclusive use of defective formula and cognitive outcome as measured by the Bayley Scales of Infant Development ($r = -.55$, $p = .01$) was noted. In a population based study which ascertained the infant formulas used by first and second graders attending public school those who were exposed to defective formula scored lower on the general cognitive index and the quantitative scale (McCarthy) than did the children who used other soy formulas.

To substantiate these findings a further study of children is in progress in the metropolitan Washington, D.C. area schools. It is anticipated that about 250 children exposed to deficient formula and 500 matched control children exposed to other soy formulas will be recruited. In addition, approximately 39 children with a documented history of hypochloremic metabolic alkalosis resulting from defective formula use will be brought to the Washington area. The performance of all these children on a battery of psychological tests will be measured and a careful statistical analysis undertaken to look for an effect of exposure to defective formula with and without documented illness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00346-04 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Time Trends in the Incidence of Biliary Atresia

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

PI: Mark A. Klebanoff Research Medical Officer EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Case Western Reserve University (B.Chatterjee)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

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.)*

Extrahepatic biliary atresia is a liver disease presenting in early infancy, manifested by progressive obliteration of the extrahepatic bile ducts. It has been estimated to occur in from one per 8000 to one per 15000 live births, and is the single most common indication for performance of liver transplantation in children. None of the incidence figures is based on a well defined geopolitical region; most estimates of the frequency of this condition are derived from referral centers. Some investigators have suggested a time-space clustering of this condition.

This project has gathered birth certificates and other information on all cases occurring among children born over a period of 12 years in Ohio. Ninety-four (94) cases were identified, corresponding to a rate of 0.5 cases/10,000 births. Cases will be compared to the other births in the state for evidence of changes in incidence and clustering. A number of potential risk factors for the condition will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00352-02 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Studies of Human Immunodeficiency Virus - Related Problems

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

PI:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD
Other:	Margarett Davis	Epidemiology Staff Fellow	EB/PRP/NICHD
	Michael H. Malloy	Research Medical Officer	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Infectious Disease Branch, NINCDS (J.Sever, M.Gravell), Office of the Director, PRP (H.W.Berendes), HRSA (S.S.Kessel), American Academy of Pediatrics (C.Croft, G.Fleming).

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

A breast milk study was begun in 1988 to further knowledge about the role of human milk in HIV infection. Little is known about the frequency, timing and determinants of HIV in breast milk. Paired milk and blood specimens are being collected from HIV-infected women and tested for antibodies, antigen, and virus. The results of this pilot study will be a useful first step in understanding the transmission of HIV into milk.

NICHD/PRP is involved in helping the American Academy of Pediatrics (AAP) develop an education program for pediatricians that deals with developmental sexuality and AIDS. The Academy has designed a program that calls for the development of an educational package by a group of experts in human sexuality, adolescent medicine and human development. The educational package is then to be administered to a randomly selected group of pediatricians. Follow-up of the pediatricians who receive the education program and follow-up of a group of pediatricians who did not receive the protocol will be carried out to determine if the program affected the pediatricians' behavior in the office setting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00360-02 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

A Prospective Study of 1st Trimester Use of Bendectin and Malformations

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

PI:	Patricia H. Shiono	Epidemiologist	EB/PRP/NICHD
Others:	Mark A. Klebanoff	Senior Staff Fellow	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

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

Most previous studies on this topic used a retrospective case-control design or indirect measures of exposure (pharmacy records). In this prospective study, 31,602 women were asked at their first prenatal visit about the use of Bendectin; 2,711 women reported use in the first trimester. The odds ratio (and 95% interval estimates) for major malformations was 1.05 (0.78-1.40). When individual malformations were evaluated, Bendectin use was statistically associated with microcephaly (5.33 (1.61-17.7)), cataract (5.33 (0.98-29.1)), and lung malformations (4.58 (1.76-11.9)). Since it is not clear whether these associations are due to the use of Bendectin or to the indication (vomiting) for which the drug was prescribed, the association between vomiting and these malformations was studied using previously published data from the Collaborative Perinatal Project. In that study, vomiting was associated with microcephaly (3.3 (1.1, 9.8)) and cataract (3.5 (0.8-16.1)). Vomiting was associated with these two malformations only among nonusers of Bendectin. Lung malformations were not associated with vomiting during pregnancy (1.3 (0.8-2.1)). These data strongly suggest that Bendectin is not associated with these malformations, however the possibility that vomiting is associated with microcephaly and cataract is supported.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00361-02 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Child Health Supplement to the 1988 National Health Interview Survey

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

PI:	Mary D. Overpeck	Health Statistician	EB/PRP/NICHD
Other:	George G. Rhoads	Branch Chief	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Biometry Branch, PRP, NICHD (H.J.Hoffman); HLBBranch, CRMC, NICHD (P.C.Scheidt); DBSB, CPR, NICHD (V.S.Cain, W.Baldwin); National Center for Health Statistics; Bureau of the Census; Maternal and Child Health, HRSA; Child Trends, Inc.(N.Zill)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

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

This survey provides data on a nationwide representative sample of 20,000 children. Subjects include child care, family relationships, accidents, injuries, poisonings, other childhood morbidity, perinatal events, use of health services, school performance and behavior. It establishes current normative ranges for the U.S. It will provide data for analysis of trends in the U.S. using the 1981 Child Health Supplement for comparisons. The survey is being conducted by the U.S. Census Bureau for the National Center for Health Statistics during the 1988 calendar year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00362-02 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Nutritional Aspects of Perinatal Epidemiology in Central America

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

PI: Jose Villar Expert EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Computer Sciences Section, PRP, NICHD (E.E.Harley); Biometry Branch, PRP, NICHD (H.J. Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.	PROFESSIONAL	OTHER.
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This study attempts primarily to develop a simple instrument, empirically produced for the identification of mothers at risk of delivering a LBW infant. Longitudinal data are available for selecting variables at different points during pregnancy. Sample size of the total population is 17,000. The risk score is developed in a random sample of 8000 patients and tested in the remaining group. Furthermore, the following projects are performed using this source of data:

- Epidemiology of subgroups of IUGR infants and their neonatal morbidity (data analysis completed).

- Physical activity and work during pregnancy and pregnancy outcome (data analysis completed).

- Protozoan and helminthic infections during pregnancy and its effect on birth weight (data analysis completed - manuscript ready for publication).

- Lactose malabsorption during pregnancy: A longitudinal study (study completed paper published).

- Body composition and physical activity during pregnancy and birthweight(datanalysis in progress).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00363-01 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

NICHD Smoking Trial of Pregnant Women (STOP)

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

PI:	Leslie C. Cooper	Nurse Epidemiologist	EB/PRP/NICHD
Others:	Patricia Shiono	Epidemiologist	EB/PRP/NICHD
	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

Office of the Director, PRP, NICHD (H.W.Berendes)

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	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 STOP Project will be carried out as a randomized clinical trial to evaluate different approaches to smoking cessation within physician practice settings. This project is a collaborative effort between the NICHD and the American College of Obstetricians and Gynecologists. The unit of randomization in STOP will be a practicing physician's population of pregnant women who smoke or have recently stopped smoking. Physicians will be solicited to volunteer to take part in this randomized study.

There will be two major phases to the STOP Project - a pilot and a formal trial. At this time a RFC is being prepared for the pilot portion of the trial only. The objective of this pilot study is to develop the protocol for the STOP study, develop all study materials (pamphlets, study forms, manual of operations, etc.), recruit private physicians who will assist us in finalizing the study protocol and materials, assist in the development of all quality control procedures, develop all necessary data management materials (data entry programs, SAS data sets, edit specifications for all data, analysis of the pilot) and train the contractor selected to run the formal trial in all aspects of the study. All study materials (forms, urine testing, pamphlets etc.) will be modified to be easily incorporated into the daily routines of private physicians' offices.

The desired result of the pilot will be to have all necessary forms, materials, etc. complete and ready for use in the formal STOP study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00364-01 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Syrup of Ipecac Usage in the General Population

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

PI:	Michael H. Malloy	Research Medical Officer	PRP/NICHD
Other:	George G. Rhoads	Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

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.)*

This project was designed to answer the question of whether or not persons in U.S. households with children under 10 years of age were aware of certain poison prevention measures. This information was obtained from the 1985 Health Interview Survey Supplement on Health Promotion and Disease Prevention. In addition, Washington, D.C. area pediatricians were surveyed by telephone as to their poisoning prevention education practices carried out in their offices. The results of the analysis suggest that although the general population is aware of the existence of poison control centers and have the phone numbers of these centers, only one-quarter of houses with children under 10 years actually have syrup of ipecac on hand. From our telephone interview of pediatricians, it appears that pediatricians do attempt to inform their patients of poison control centers, but they do not distribute syrup of ipecac from their offices. We suggest that distribution of ipecac from the offices of pediatricians may enhance the availability of ipecac in the homes of children where it would be available for immediate use.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00365-01 EB

PERIOD COVERED: October 1, 1987 through September 1988

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

A Randomized Clinical Trial of Umbilical Artery Catheter Placement

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

PI:	Michael H. Malloy	Research Medical Officer	PRP/NICHD
Other:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.25	0.25	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.)*

This project was formulated to determine if very low birth weight infants who receive umbilical artery catheters that are placed high in the thoracic aorta (T6-T8) are at higher risk of intraventricular hemorrhage than are infants that receive an umbilical artery catheter placed low in the abdominal aorta (L4-L5). We propose to randomize infants to receive either a high or low catheter and then to review the incidence of intraventricular hemorrhage. The project will enroll a total of 650 infants in several neonatal intensive care units beginning in 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00366-01 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Survey of Pregnancy Outcomes Among Medical Residents

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

PI:	Patricia H. Shiono	Epidemiologist	EB/PRP/NICHD
Others:	Mark Klebanoff	Senior Staff Fellow	EB/PRP/NICHD
	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.10	.10	

CHECK APPROPRIATE BOX(ES)

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

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

The effects of stressful employment on pregnancy outcome will be examined in this study of pregnancy outcomes among medical residents. Several studies of paid employment by women during pregnancy have shown an increased risk of both preterm birth and low birth weight associated with strenuous occupations. However, the majority of studies show no increased risk. None of the previous studies were able to control adequately for the socioeconomic status of the women, and in many instances improper controls were used. Women who become pregnant during medical residency are in many respects an optimal group in which to study this issue. They are universally highly educated and in many respects of high socioeconomic status, yet their occupation is highly stressful and physically demanding. For this reason, the effects of a mentally and physically demanding occupation can be studied independently of socioeconomic status. Spouses of male residents comprise an appropriate control group, as they are also of high socioeconomic status, but in most cases have less strenuous occupations than that of a medical resident.

We hypothesize that the mentally and physically strenuous occupation of residency adversely effects the pregnancy outcomes of female residents, as compared to the pregnancy outcomes of spouses of male residents. The proposed study will examine pregnancy outcomes among a cohort of recent medical school graduates. Approximately 10,000 residents in their third post-graduate year (all of the women residents and a 50% random sample of male residents) will be surveyed to determine the pregnancy outcomes of the female residents and spouses of male residents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00367-01 EB

PERIOD COVERED October 1, 1987 through September 30, 1988

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

Followup of the 1988 Child Health Supplement to Investigate Accidents and Injuries

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

PIs:	Mary D. Overpeck	Statistician	EB/PRP/NICHD
	Peter Scheidt	Medical Officer	HLBB/CRMC/NICHD
Other:	George Rhoads	Branch Chief	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Center for Disease Control (Y. Harel); National Center for Health Statistics (K. Long); CDC Office of Smoking and Health (J. Pierce); National Cancer Institute; American Cancer Society.

LAB/BRANCH

Epidemiology Branch, PRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.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.)*

With support from NICHD, and other agencies, the National Center for Health Statistics is currently conducting the 1988 Child Health Supplement to the National Health Interview Survey. It is a nationally representative sample of approximately 20,000 children to the age of 17. The childhood injury information from this data source has been greatly expanded and for the first time the use of E codes for the cause of injury will be available. This expanded database of information about childhood injuries should generate important new knowledge about the epidemiology, behavior and other factors associated with childhood injury.

Independently of NICHD, the National Center for Health Statistics, CDC's Office of Smoking and Health, National Cancer Institute, and American Cancer Society initiated plans for a telephone follow-up of smoking habits of 10,000 11 to 19 year old participants. Questions to screen this adolescent population for additional injuries in the previous year will be added to the smoking questionnaire. A subsequent 10-15 minute telephone follow-up of those identified will be performed to identify safety habits, behavior patterns, and physical activity related to injuries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00368-01 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Vaginal Delivery of Very Low Birth Weight Infants: Association with Day 1 Deaths

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

PI:	Michael H. Malloy	Research Medical Officer	EB/PRP/NICHD
Others:	George G. Rhoads	Chief, Epidemiology Branch	PRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology Branch

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

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

This project involved the analysis of linked birth and death certificates from Missouri for the years 1980-1984. The 200,000 births occurring during this period are being studied to determine whether or not cesarean sections performed for very low birth weight infants have any advantages over vaginal delivery after adjusting for various complications of delivery. Our preliminary analysis suggests that the vaginal delivery of a very low birth weight infant carries a greater risk of day 1 death than does cesarean section. This was not the case in infants of higher birth weight and the significance of the finding is being explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00832-05 EB

PERIOD COVERED: October 1, 1987 through September 30, 1988

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

Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities

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

PIs:	Mary D. Overpeck	Health Statistician	EB/PRP/NICHD
	Leslie C. Cooper	Nurse Epidemiologist	EB/PRP/NICHD
Other:	George G. Rhoads	Branch Chief	EB/PRP/NICHD

COOPERATING UNITS *(if any)*

Biometry Branch, PRP, NICHD (H.J. Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.25	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)*

This study describes differences in perinatal mortality and age at death in 59 large cities from 1972 through 1981, of rapid change in technology and medical management of high risk pregnancies.

It explores whether high rates of neonatal mortality in certain cities can be explained by shifts in mortality from the late fetal to the neonatal period and compares differences in perinatal experience according to age at death by race and city size. A secondary analysis of data sets provided by the National Center for Health Statistics was done based on 100 percent reporting of perinatal deaths. Review of fetal death rates from 24 weeks gestational age and of neonatal deaths for the periods, 1-7, and 8-27 days is being used to examine potential reporting differences among cities and shifting of neonatal deaths into the latter period. These data have not been available publicly for analysis. The analysis provides an improved standard for comparison of perinatal mortality in differing geographic sites.

PREVENTION RESEARCH PROGRAM (PRP)

NEW BRANCH 1988

ZO1 HD 00343-05 Effect of Westernization on Infants Feeding Patterns Among the Negev Bedouins
Heinz W. Berendes, M.D., M.H.S.

ZO1 HD 01700-01 Study of the Efficacy of IVIG in HIV Infected Children
Heinz W. Berendes, M.D., M.H.S.

Office of the Director, Prevention Research Program

The Prevention Research Program conducts epidemiological and biostatistical investigations in maternal and child health which include determinants of perinatal and infant mortality, risk factors in intrauterine growth retardation and preterm delivery, nutritional aspects of pregnancy, infant feeding practices and their effects on growth and development during infancy as well as specific questions in teratology and also research in pediatric AIDS. The program encompasses case control as well as prospective studies and clinical trials. The research is conducted in this country and also to a more limited extent abroad. The different research projects are conducted by the Epidemiology and Biometry Branch of the Prevention Research Program and by this office.

Despite our limited resources, in keeping with the additional mandate to develop research in the prevention area, several new projects have been initiated with a specific prevention focus of relevance to this Institute and also encouraging prevention research activities in the extramural program of this Institute.

A major effort during the past year was the initiation of a clinical trial of the efficacy of intravenous gamma globulin in the treatment of symptomatic children infected with the human immunodeficiency virus. This project was developed in collaboration with the Pediatric AIDS Coordinator in the Center for Research for Mothers and Children and is testing the hypothesis that intravenous immunoglobulin when administered every 28 days will significantly reduce the proportion of the treatment group who develop at least one invasive or serious bacterial infection or die during the two year treatment period when compared to the control group of HIV infected children who will receive an intravenous albumin placebo every 28 days. The clinical trial is being conducted in 26 hospitals around the country and 130 children have been enrolled as of July 31, 1988.

The congressionally mandated study to determine possible long term effects in children of exposure to a chloride-deficient formula in 1979 has made considerable progress and will be completed later on this year. This most complex and logistically difficult project consists of a population based study of exposed children and controls in counties adjacent to Washington, D.C. and a group of about 30 children from the United States with evidence of hypochloremic metabolic alkalosis while on the chloride-deficient formula.

The analysis of data from the Bedouin Infant Feeding Study in collaboration with investigators from Ben Gurion University in Beer Sheva, Israel has made considerable progress. We were somewhat surprised by the degree of stunting in physical growth

among the Bedouin infants which increases with age. Factors related to stunting include exclusive breastfeeding beyond six months of age but also morbidity, especially gastrointestinal and respiratory diseases in infants. Several manuscripts have been prepared which include perinatal determinants of infant feeding practices at birth, seasonality of Bedouin births, factors associated with low birth weight, infant feeding practices and physical growth during the first year of life and also morbidity during infancy and its relation to infant feeding practices. A workshop is planned in Beer Sheva in April 1989 to present the main findings of this jointly conducted Bedouin Infant Feeding Study to Jewish, Arab, Israeli policy makers, scientists and practitioners in the Negev and to discuss the implications of these findings for undertaking health promotion interventions.

Other international activities include a research project which has finally been approved by the Pakistani Government of a pregnancy outcome study to be conducted in collaboration with the Aga Khan University in Karachi. One component of the study is a survey of maternal mortality and the identification of key risk factors in selected geographical areas of Pakistan in the first two years of the study and a subsequent intervention which would make use of the information learned from the initial survey. Another component of the study deals with risk factors associated with poor birth outcome, especially low birth weight including intrauterine growth retardation and preterm delivery and the effect of these on mortality and morbidity during the first two years of life. This phase of the study will also be conducted in different sites in Pakistan responding to the Ministry of Health of Pakistan. Particular emphasis in this component of the study will be on nutritional factors during pregnancy and the extent to which they may help to explain the high rate of low birth weight, which is estimated to be between 30 to 40% in at least some of the sites under consideration for participation in this project. If the importance of nutritional deprivation can be confirmed during the initial phases of the study, a nutritional intervention during pregnancy will be introduced during the last three years of the project. The aim of the intervention is to increase weight gain substantially to determine to what extent this increase in weight gain would affect a reduction if any in the rate of low birth weight.

As part of the U.S. Polish health agreement, a project has been developed and approved in collaboration with the Children's Hospital in Krakow, Poland to study the reason for the marked increase in the rate of low birth weight in Zakopane, Poland. This phenomenon has been observed and well documented by the principal investigator in Poland and reported and is at present without explanation.

Plans for a workshop on perinatal determinants of child survival in New Delhi, India have progressed substantially. A tentative agenda has been developed in discussion with the Deputy Director of the Indian Council of Medical Research during a visit earlier

this year. The agenda has now essentially been finalized and the U.S. participants have been identified. We have confirmation from the Indian Council of Medical Research that there are accepting the agenda with minor modifications and we plan to conduct this workshop in New Delhi in February 1989. It is hoped that this workshop will result in the identification of one or more interventions to reduce maternal and infant mortality which are feasible in India and which we hope to implement in a collaborative manner.

Several new prevention research activities are worthy of note: A smoking intervention trial is under development by the Epidemiology Branch in collaboration with the American College of Obstetrics and Gynecology which will evaluate different smoking intervention strategies to be conducted in the offices of obstetricians as part of regular prenatal care and to test efficacy and feasibility.

In collaboration with the American Academy of Pediatrics, an educational intervention is under development to train pediatricians more specifically in providing age-specific sex education to their pediatric clientele and their parents as part of regular pediatric care which would also include information to avoid behaviors which increase the risk of AIDS. Once the educational part of the program is developed, it will be implemented in a clinical trials design by randomizing pediatricians in at least two metropolitan areas and by evaluating the impact of this intervention on the pediatricians' practices subsequently.

A workshop is planned focusing on injury prevention in childhood. The particular agenda of the workshop includes the identification based on current knowledge of high priority topics for injury prevention and to discuss the use of clinical trials methodology for their implementation. While in most areas of the medical field evaluation of therapy is now done through double-blind randomized clinical trials, use of such methodology in the area of accident and injury prevention is still rather limited. Yet without the use of this vigorous methodology, it is highly unlikely whether we will ever be able to determine the effectiveness of particular interventions.

With the active participation and the leadership of the Director of this Institute, several meetings and seminars were held on determinants of adolescent pregnancy and on possible interventions with interested staff members of the NICHD and selected speakers from the outside who had done research in this particular field. As part of this effort, discussions have been held with representatives from a nearby metropolitan area who have a particular interest in the prevention of teenage pregnancies about possible collaboration in the future.

Again emphasizing prevention, an international symposium was conducted entitled "Advances in the Prevention of Low Birth

Weight" at the Chatham Bars Inn, Cape Cod, Massachusetts, May 8-11, 1988 in collaboration with the CRMC and the Bureau of Maternal and Child Health and Resources Development. This workshop brought together investigators who had recently completed clinical trials aimed at reducing the rate of low birth weight including intrauterine growth retardation or preterm delivery. The meeting was highly successful because of the excellent presentations by the speakers and the vigorous discussions by the other participants and will be published in proceedings subsequently.

Stimulated by the efforts of the Oxford Epidemiology Unit in the United Kingdom and their development of a register of published trials, we have agreed to a collaborative joint effort to develop a register of currently ongoing trials in perinatal medicine. Reasons for this interest include the current publication bias because of the preferential publication of positive findings from clinical trials, the desire to identify other trials in a given area while planning new ones, and the identification of possible collaborators on planned trials. An advisory panel has been convened, a data form developed and efforts are underway to implement this in North America through the NICHD and in Europe through the efforts of the Oxford Epidemiology Unit. We plan to use the information collected to develop a joint data base which would be available as part of an electronic library presumably through Oxford Press.

Presentations:

Heinz Berendes. The Epidemiology of Perinatal Mortality. Johns Hopkins University, February 1988.

Heinz Berendes. Research on Pediatric AIDS. Society for Pediatric Epidemiologic Research, Vancouver, Canada, June 1988.

Publication:

Forman MR, Berendes HW: Delayed Childbearing: No Evidence for Increased Risk of Low Birthweight and Preterm Delivery. Letter to the Editor. Am J Epid 1988;127:881-3.

Kessel SS, Kleinman JC, Koontz AM, Hogue CJR, Berendes HW. Racial Differences in Pregnancy Outcomes, Clinics in Perinatology. Current Controversies. Richard E. Behrman, Guest Editor (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00343-05 PRP

PERIOD COVERED October 1, 1987 through September 30, 1988

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

Effect of Westernization on Infant Feeding Patterns Among the Negev Bedouins

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

PI: H.W. Berendes, Director, PRP, NICHD

COOPERATING UNITS *(if any)*

Department of International Health, Johns Hopkins University, Baltimore, MD. (M.R. Forman); BB, PRP, NICHD (B. Graubard); Computer Sciences Section, PRP (E. Harley); Ben Gurion University on the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH

Office of the Director, PRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.3	.15	.15

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This is a study of infant feeding practices among Bedouin tribes residing in the Negev, Israel. The objectives are: the evaluation of changes in infant feeding practices during the first year of life and their relationship to physical growth of children and on gastrointestinal and respiratory diseases during the first year of life.

The information obtained covers 5,000 mother-infant pairs. Two samples have been identified, one was identified at birth and a subsample of these births was followed for a period of 5-8 months. Another sample of children was identified at 6 months of age and followed prospectively to 18 months of age.

Several aspects of the data from this project have been analyzed. These include an analysis of the seasonality of births in the Bedouin population showing the preponderance of births in the winter months and a trough in the summer months and possible explanations for the seasonality, determinants of infant feeding practices at birth including socio-demographic characteristics, obstetrical characteristics including complications during pregnancy and around the time of births and conditions in the child during the first two days of life. Other analyses deal with the study of the relationship of infant feeding practices during the first year of life and physical growth during the first year of life. Characteristically this population of Bedouin infants shows an increasing level of stunting during the second half of the first year of life. Part of this is related to the practice of extending exclusive breastfeeding beyond six months of age without any food supplementation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-01700-01 PRP

PERIOD COVERED: October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Study of the Efficacy of IVIG in HIV Infected Children

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

PI: H.W. Berendes, Director, PRP, NICHD

Other: Anne Willoughby, Acting Chief, Pediatric, Adolescent and Maternal AIDS Branch, CRMC, NICHD

COOPERATING UNITS *(if any)*
EB, PRP, NICHD (R. Nugent); BB, PRP, NICHD (G. Reed); OD, CRMC, NICHD (S. Yaffe)

LAB/BRANCH
Office of the Director, PRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.6	PROFESSIONAL: .5	OTHER: .1

CHECK APPROPRIATE BOX(ES)

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

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

This is a placebo controlled randomized clinical trial which will test the hypothesis that intravenous immunoglobulin (IVIG) administered every 28 days, in comparison to an intravenous placebo, will significantly reduce the rate of serious, life threatening bacterial infections and/or deaths in symptomatic children who are infected with the human immunodeficiency virus (HIV).

Eligible HIV infected non-hemophiliac children less than 13 years of age are being assigned to one of two groups on the basis of total T-4 count and clinical staging using the CDC classification system. Group I will contain those patients with more severe clinical disease. Group II will contain patients with less severe clinical illness. Patients within each group are randomly assigned to receive either IVIG or IV albumin placebo. The duration of treatment for each child who is enrolled in the clinical trial will be two years.

Enrollment in the study began around March 1, 1988 and about 28 hospitals have agreed to follow the protocol. As of June 17, 1988 104 children had been recruited into this trial.