National Institute of Child Health and Human Development (NICHD)

NICHD ANNUAL REPORT OF INTRAMURAL RESEARCH

October 1, 1983 through September 30, 1984

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
INTRAMURAL RESEARCH PROGRAM

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR
OCTOBER 1, 1983 - SEPTEMBER 30, 1984

The Intramural Research Program is broadly concerned with the biological and neurobiological, medical and behavioral aspects of normal and abnormal human development. In addition to four major clinical research and training programs in the areas of genetics and endocrinology, a diversity of developmental models are under study in eleven fundamental research Laboratories, drawing upon observations in bacteria, Drosophila, yeasts, viruses, molluscs, frogs, rodents, and subhuman primates. Disciplines employed in these studies include biochemistry, virology, molecular biology, immunology, pharmacology, genetics, cell and neuronal biology, biophysics, mathematical and theoretical biology, reproductive physiology, and developmental psychology.

During the past year, the Program has seen several further major organizational changes. On June 30th, the Pregnancy Research Branch was disestablished consequent to the departure of its Chief, Dr. Gary Hodgen, and several of his colleagues for new positions at the Eastern Virginia Medical Center. The resources of the Pregnancy Research Branch have been employed to establish a new intramural Laboratory, the Cell Biology and Metabolism Branch, and Dr. Richard Klausner of the Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, has been recruited to lead the new Branch. Building 18 is presently being renovated to accommodate these new research interests. The Cell Biology and Metabolism Branch will focus on the developmental aspects of receptor regulation, metal metabolism, organelle structure and function, and the cell biology of gametes. The Branch's laboratory activities will take advantage of the extraordinary opportunities in cell biology occasioned by the recent advent of recombinant DNA, monoclonal antibody, and other new techniques. The Branch's clinical research activities will reflect the Institute's interest in the developmental aspects of metabolism. The human transferrin receptor will provide the major experimental model, and studies will be undertaken to determine how the cell regulates the biosynthesis of this receptor as a function of cellular iron economy and stimuli to cellular proliferation. The gene for this receptor, as well as the genetic regulatory elements, is being cloned in order to examine the molecular basis for transcriptional regulation. This receptor will also serve as a model for elucidating the pathway, mechanisms, and regulation of receptor degradation, inactivation, and sequestration. The Branch will explore the specific biochemical signals which determine the physical routing of the transferrin receptor within the cell.

The clinical studies to be undertaken by the Cell Biology and Metabolism Branch will employ a population of patients with hemochromatosis and other genetic diseases of metal metabolism. Patients (and normal volunteers) will be studied to determine the mechanisms by which cells regulate the distribution of iron throughout the cell, and the molecules responsible for the controlled intracellular traffic of iron. Therapeutic attempts at the level of "gene therapy" are contemplated for this patient population.

In another area of interest, the new Branch will explore the mechanisms by which the normal intracellular architecture is maintained, providing the basis for the function and dynamics of cellular organelles. Focus will be on the microtubule system and the Golgi apparatus, in order to learn how these structures are determined, how molecules restricted to different domains of these organelles are sorted, and whether there are signals that govern the localization and routing of different components of these organelles.

The Laboratory of Comparative Ethology, established in 1983, underwent a major building program during this year, and its extensive outdoor facility for free-ranging primates was opened in June.

A number of new sections were developed in the established Laboratories during this year, in recognition of new and independent research efforts that have emerged in the past several years. These sections and their Heads are:

Section on Comparative Behavioral Genetics, LCE (Dr. Stephen Suomi)
Section on Molecular Biology, HGB (Dr. Michael Zasloff)
Section on Molecular Structure and Protein Chemistry, ERRB (Dr. Hao Chia Chen)
Section on Adrenal Cell Biology, ERRB (Dr. Charles A. Strott)
Section on Metabolic Regulation, ERRB (Dr. K. P. Huang)
Section on the Regulation of Gene Expression, LDP (Dr. Howard J. Eisen)
Section on Drug Biotransformation, LDP (Dr. Ida S. Owens)
Section on Cellular Neurobiology, LNN (Dr. Yoke Peng Loh)
Section on Macromolecular Analysis, LTPB (Dr. Andreas C. Chrambach)
Section on Immunoregulation and Cellular Control, LDMI (Dr. Edgar E. Hanna)
Section on Steroid Hormones, DEB (Dr. D. Lynn Loriaux)
Section on Medical Endocrinology, DEB (Dr. Bruce C. Nisula)
Section on Reproductive Endocrinology, DEB (Dr. Richard J. Sherins)
Section on Developmental Endocrinology, DEB (Dr. Gordon Cutler, Jr.)
Section on Gamete Physiology, CBMB (Dr. Bela J. Gulyas)

Our clinical fellowships in adult, pediatric and gynecologic endocrinology, as well as the fellowship in human genetics, continue to thrive, and in the past year, we have also placed emphasis on recruiting physicians for full-time basic research training without clinical responsibility.

Peer review of intramural research has been strengthened significantly, with rigorous site visits to each Lab at 3-1/2 year intervals. During the past year, visits were made to the Endocrinology and Reproduction Research Branch, the Laboratory of Molecular Genetics, and the Developmental Endocrinology Branch, with detailed critiques prepared as a consequence of these visits. The membership of the Board of Scientific Counselors has been expanded from six to nine, reflecting the increasing diversity of research interests within the Intramural Program. The current Board membership includes:

James W. Lash, Ph.D., Professor of Anatomy, University of Pennsylvania
Aron Moscona, Ph.D., Louis Block Professor of Biological Sciences, University of Chicago
Roger Guillemin, M.D., Chairman, Laboratory for Neuroendocrinology, Salk Institute
John C. Marshall, M.D., Professor of Medicine, University of Michigan
Lewis P. Lipsitt, Ph.D., Professor of Psychology, Brown University

Allen H. Neims, M.D., Ph.D., Professor and Chairman, Department of Pharmacology and Therapeutics, University of Florida

The nominees for the remaining three Board vacancies are:

Story C. Landis, Ph.D., Associate Professor of Neurobiology, Harvard Medical School
Harold Amos, Ph.D., Professor of Bacteriology and Immunology, Harvard Medical School
John Phillips, Jr., M.D., Professor of Human Genetics, Vanderbilt University School of Medicine.

Other developments in the past year include the recruitment of a full-time veterinarian, Dr. John Donovan, who is supervising all aspects of the management of animals in our research. We have continued to see growth in outside sources of support of post-doctoral fellows. This year, the NIH has developed a new Intramural NRSA (National Research Scholarship Award) Program which enables American physicians to obtain research training in the Intramural Program without the need for a formal civil service position. Within the next three years, we shall recruit twelve NRSA trainees. We have also identified a number of bilateral agreements with foreign countries in which the foreign government supports the training of their post-doctoral fellows in our labs, and we are taking full advantage of such bilateral agreements. Good relationships have also been developed with a number of biotechnology companies, and they are endowing fellowships in our research training program as well. Our summer student program was very successful this year, with more than sixty undergraduate and medical students working in our Laboratories. Of this group, more than 50% were women and a third were minority students. The academic credentials of the group as a whole were singularly impressive, and our experience suggests that the decline in the number of talented young people considering careers in biomedical research may be reversing itself.

We have continued to develop new computer-based administrative procedures in the Office of the Scientific Director so as to maximize the efficiency with which our resources are shepherded. These new administrative approaches are ensuring the maximum yield with respect to scientific productivity while the current climate of constrained resources persists. With regard to laboratory space, we are now in the midst of an extensive renovation and building program. In addition to the new primate facilities for the Laboratory of Comparative Ethology at the NIH animal farm in Poolesville, Maryland, as well as the renovation and expansion of Building 18 for our new Cell Biology and Metabolism Branch, we are in the midst of renovating the tenth floor of the Clinical Center which will house the laboratories and offices of four of our Labs and Branches. The Developmental Endocrinology Branch has recently moved into its new corridor within this complex, and two more Labs will have moved by December, 1984. We are also on schedule with respect to the construction of two new floors of laboratory space and additional animal quarters in Building 6. Within established ceilings, our budget base and number of positions were increased significantly during the past year, as was the number of positions allocated for Visiting Fellows from abroad.

Seminars and workshops sponsored by this Program were numerous and popular throughout the year, such that this Institute organized a relatively large

fraction of the NIH's overall intramural seminar and workshop program. During the past year, seven major conferences with participants from throughout the world were hosted by the Intramural Research Program, including:

Mechanisms of Genetic Recombination (Airlie, Va.)
Molecular Biology of Xenopus Development (Airlie, Va.)
Ontogeny of Antibacterial Immunity and Bacterial Vaccines (NIH)
Pertussis Toxin (NIH)
Research on Mastery Motivation in Infancy and Early Childhood (NIH)
Mechanisms and Clinical Aspects of Steroid Hormone Resistance (New York)
Advances in Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency (Toronto)

With regard to the major research interests and scientific results during the past year, particularly notable are the following:

Laboratory of Molecular Genetics--
Igor Dawid, Ph.D.

Investigators in this Laboratory use the tools of molecular and cellular biology to answer questions about gene transmission and recombination, and the regulation of genetic functions during development. The range of model systems under investigation includes bacterial and animal viruses, transformed animal cells, yeast, mouse and Xenopus embryos, and the fruit fly Drosophila melanogaster. Recombinant DNA technology and gene transfer methods are emphasized. The development of novel vectors is being pursued for the introduction and expression of isolated genes in animal cells.

Dawid's section has studied early development of the frog Xenopus laevis by the analysis of a group of gene sequences which are expressed for the first time in the late blastula and gastrula stages of embryogenesis. These sequences were isolated by cDNA cloning and yielded RNA molecules which are absent from the egg but present in the gastrula embryo. DG (differentiating gastrula) RNAs arise first within an hour after the midblastula transition, and show strong developmental regulation. A 17-amino acid peptide has been deduced from the cDNA sequence of one such DG RNA and synthesized chemically; DNA sequencing is being used to locate the 5' end of this DG gene, which will permit study of the gene's control region and the developmental regulation of its expression. In other Xenopus experiments, monoclonal antibodies have been raised against cell surface antigens in embryos in order to study the developmental regulation of their expression. The rationale for these studies lies in the fact that gastrula and neurula development involve cell migration, recognition, and adhesion, and distinct surface molecules undoubtedly play a role in these processes.

Studies by Dawid's group using the fruit fly Drosophila have focused on maternal-effect homeotic genes. Such genes specify the body plan, e.g., the three-dimensional formation of the bithorax complex. To study the fs(1)h homeotic gene, the region of the chromosome carrying it has been cloned by "chromosomal walking." An analysis of RNA transcripts from this region is underway, as is the cloning of cDNAs copied from the RNAs. Studies such as

these promise greatly to improve our understanding of the spatial and temporal integration of the body's members during development.

Okayama's group has continued to develop new vectors for the transfection of expressable full-length cDNA copies of mammalian mRNAs. The system begins with the Okayama/Berg vector, wherein full-length cDNA is synthesized directly onto a plasmid vector which already contains, in the right orientation, an SV40 promoter, splice signals, and a polyadenylation site. That construction is then inserted into a lambda phage vector carrying a selection marker and suitable restriction sites for insertion and release of the insert. The phage is then transferred into mammalian cells which are subjected to selection so as to separate transfectants from unaffected cells. Two clones carrying human HGPRT cDNA have been obtained in this way, demonstrating that rare messages can be cloned by this procedure.

Hinnebusch's group is studying yeast as a eukaryotic organism offering a level of genetic and molecular analysis not possible with higher eukaryotes. The genetic map of yeast has been well characterized, and it is easy to isolate new mutations in this organism. In the past year, this group has been studying the co-regulation of amino acid production in this organism, and finds that both cis- and trans-acting regulatory elements are involved. The minimal cis-acting regulatory element at one gene involved in histidine biosynthesis has been identified, and it has been found that a 14-base pair fragment from this gene's promoter is sufficient to confer the general control regulatory response. Hinnebusch has also found that the hierarchy of control genes involved in amino acid synthesis is subject to both activation and repression, with these influences observed in amino acid starvation or excess. In vitro mutagenesis and in vivo mutant isolation are permitting the identification of other cis- and trans-acting factors involved in the co-regulation of these yeast structural genes.

Levin's group, studying the replication and gene expression of enveloped RNA viruses, has isolated a polymerase mutant which produces a truncated reverse transcriptase. The precise location of the mutation has been identified, leading to a map for the genetic organization of the murine leukemia virus pol gene. In other experiments, this group has further studied the mechanism of reverse transcription and, by computer-aided analysis of the viral RNA sequence, have demonstrated consensus sequences and secondary structure features that may explain "pausing," i.e., the elaboration of partial transcription products during reverse transcription.

Cashel's section is studying the mechanism of coordinate regulation of bacterial metabolism in response to environmental stimuli, focusing on ppGpp as a regulator of the expression of ribosomal RNA. In studies of the promoters responsible for rRNA transcription, this group has shown that these promoters are sensitive not only to ppGpp, but to the surrounding sequences as well. Mutants that affect the basal level of ppGpp are also being developed, and they should help greatly in understanding the multiple regulatory interactions that are mediated by this molecule in the bacterial cell. Cashel's group also has an interest in eukaryotic cells, and is focusing on a cyclic nucleotide-independent protein kinase from a rat adrenal cortical tumor. This enzyme occurs in normal adrenal cells, but is present in 100-fold excess in the tumor tissue. The cDNA homologous to the kinase mRNA is being

cloned, and will be used to determine whether the kinase gene is amplified in the tumor and whether it is associated with an onc gene (and therefore be causily related to tumorigenesis in addition to being a marker).

Crouch and his colleagues are continuing to study RNase H, an enzyme that degrades the RNA component within RNA/DNA hybrids. During the past year this group has shown, for the first time, that RNase H is an essential activity in that the RNase H gene in E. coli is required for normal growth. Mutants in the RNase H gene also interact with certain mutations in DNA, suggesting that a major role for RNase H may be the generation of RNA primers during DNA replication.

Maizel's group is continuing to develop and apply computer-aided methods for the analysis and comparison of the sequences and structures of nucleic acids and proteins, having pioneered the dot matrix method of sequence comparison (which has been used for the analysis and comparison of a variety of important genes in major labs throughout the world). With the installation of a Vax computer, Maizel's group is now folding RNA molecules of several thousand nucleotides, permitting the prediction of secondary structure models with great precision. In the past year, these methods have been used to demonstrate the relatedness of the adenovirus E3 gene product to the major histocompatibility protein family. Additionally, this group has shown that enhancer sequences, although they do not show a consensus sequence, do display a common feature in DNA structure that may be required for enhancer activity.

Weisberg's section is continuing to study the attachment sites that are involved in recombination between phage λ and the E. coli chromosome. In the past year, they have obtained strong evidence for the involvement of sequence complementarity in site-specific recombination: A stretch of seven homologous nucleotides, called the overlap region, will, if mutated in the phage, result in decreased recombination frequencies. However, compensatory mutations in the bacterial overlap region restore complementarity as well as wild-type levels of recombination. Also this year, Weisberg's group has cloned and sequenced the gene which encodes one of the proteins required for site-specific recombination, the integration host factor (IHF). The phage attachment site is also being studied with respect to computer-predicted secondary structure, and it is apparent that such sites may be characterized by specific structural properties. Finally, this group has made progress in analyzing endonuclease I of bacteriophage T7, an enzyme which specifically cleaves branched DNA (Holliday structures) in vitro. These branched DNA structures are believed to be intermediates in genetic recombination, and mutants lacking endonuclease I have a lethal accumulation of Holliday structures.

Westphal's group remains concerned with gene regulation, using viral models. During the past year, they have purified the protein product of the cloned adenovirus E1a gene which appears to promote transcription from several other genes. The purified protein, when injected into mammalian cells, activates the adenovirus E2a gene and complements an E1a deletion mutant virus, allowing expression of the late region of adenovirus. Thus, the bacterially produced E1a protein is fully functional. In a second project, using DNA-mediated gene transfer into fertilized mouse eggs, the group has been

successful in producing mice which have stably integrated the injected DNA in their cells. A Drosophila transposon (the P element) has been employed in these studies, with the result that integration was demonstrable at sites other than the termini of the transposon, suggesting that integration was not the result of specific transposition. Other DNA molecules introduced into the mouse egg employ a eukaryotic promoter fused to a suitable detector gene. These studies are leading to the generation of mouse models of human genetic diseases. For example, a model of galactosemia has been developed by introducing to mouse embryos an E. coli galactokinase gene controlled by the metallothionene promoter.

Laboratory of Developmental Pharmacology--
Daniel W. Nebert, M.D.

Research in this Laboratory has concentrated on attempts to understand drug-induced gene expression at the molecular level. The Laboratory studies mechanisms of drug-induced enzyme regulation, with particular reference to the induction of cytochrome P450-linked drug-metabolizing enzymes by drugs as well as environmental chemicals. There is a particular interest in relationships between the genetics of these enzyme systems and mutagenesis, teratogenesis, and carcinogenesis. The long-range goal is to design molecular biology-based assays to predict the individual risk in humans of drug-induced birth defects and chemically induced malignancies.

This Laboratory has continued to study Phase I and II inducible drug-metabolizing enzymes with the goal of illuminating the genetics of these enzyme systems such that we can expose their role in mutagenesis, teratogenesis, carcinogenesis, and drug toxicity. In the past year, Nebert's group has made great progress in studies on the molecular genetics in rodents of the Phase I enzymes associated with cytochrome P-450 and inducible by polycyclic hydrocarbons. There appear to be at least three P-450 gene families inducible by dioxin (TCDD), phenobarbitol, or steroids. All three gene families are regulated by receptor-mediated drug ligands. The TCDD gene family is controlled by the Ah receptor; the gene family itself is referred to as the Ah (aromatic hydrocarbon) locus. Studies of the TCDD-inducible genes have been carried out in inbred mouse strains and tissue culture lines. This family is composed of two major genes, P_1-450 and P_3-450. During the past year, the two gene products were purified, antibodies to these proteins were raised, and the antibodies were used for polysome immunoadsorption so as to obtain the corresponding mRNAs. By means of the Okayama-Berg vector, full-length cDNA clones were then isolated. A genomic DNA library from mouse liver was also created, and with these reagents, the P_1-450 and P_3-450 genes were isolated and sequenced, including all six introns and more than 1000 base pairs in both the 5'and 3' flanking regions.

The phenobarbital inducible P-450 gene family elaborates an enzyme ($P\text{-}450_{Coh}$) to which an antibody was also raised by Nebert's group. This will permit cloning and sequencing of this gene family as well. Finally, the $P\text{-}450_{PCN}$ protein, which reflects the steroid inducible P-450 gene family, has been similarly used to elicit an antibody; the antibody was used for polysome immunoadsorption, and again, using the Okayama-Berg cloning vector, a full-

length cDNA clone was isolated and sequenced. The study of the protein sequences elaborated by all three P-450 gene families, as deduced from their nucleotide sequences, has led to the conclusion by this group that the TCDD-inducible and phenobarbital-inducible P-450 gene families diverged from a common ancestral gene more than 200 million years ago, and that the P_1-450 and P_3-450 TCDD-inducible genes separated from each other 65 million years ago. Finally, Nebert's group has isolated and sequenced human P_1-450 cDNA and genomic clones. They now intend to use these human DNA probes to assess the human Ah phenotype, the results of which may predict individual human risks for environmentally-induced carcinogenesis, mutagenesis, and drug toxicity.

Eisen's group has concentrated on the cell receptors associated with enzyme induction by TCDD and glucocorticoids. Using somatic cell genetics, they have localized the Ah receptor gene to mouse chromosome 17. Using monoclonal antibodies, they have purified both receptors with final resolution by HPLC. The group is now using these reagents to initiate the cloning of the receptor genes. Eisen's group has also isolated several new clones of a mouse hepatoma cell line which are mutant either in Ah receptor content (i.e., decreased) or which have apparent post-receptor defects. The latter clones may contain the drug receptors, but not be able to mediate enzyme induction because of the post-receptor defects. Preliminary work with certain human cultured cell lines indicates that they too may have TCDD receptor anomalies and be an important new genetic model in this regard. Finally, Eisen's group is employing cloned DNA from the three cytochrome P-450 gene families to study directly the nature of the interaction between these receptors and their cellular DNA binding sites.

Owen's section is studying the regulation of UDP glucuronosyltransferases, which represent a major class of Phase II drug-metabolizing enzymes. After the cytochrome P-450 dependent monooxygenase system converts fat soluble substrates to oxygenated products, the UDPG transferases conjugate these substrates to glucuronic acid, rendering them water-soluble and excretable. As with the Phase I enzyme system, transferase genetics are being worked out in inbred mouse strains and in tissue culture lines. Several mouse and rat liver transferases have been purified and antibodies raised against them. The antibodies have been used for polysome immunoadsorption, and the immuno-enriched mRNAs has been used to yield transferase cDNAs. From this cDNA library, a number of mouse and rat transferase clones have been obtained which share significant homology. Certain of the clones encode constitutive transferase proteins and others are inducible. One of the mouse transferase proteins (51K) undergoes post-translational cleavage and glycosylation, and is the first membrane-bound enzyme found to undergo such a post-translational modification.

Laboratory of Neurochemistry and Neuroimmunology--
Harold Gainer, Ph.D.

This Laboratory utilizes the tools of molecular biology and immunology in a cell biological context to study the biosynthesis, packaging, transport, and secretion of biologically active neural peptides and proteins. Various model

neuronal systems in organisms ranging from molluscs (squid) to vertebrates (e.g., frog, chick, and rat) are used to examine the development and function of defined neural pathways in vivo.

The Laboratory is focused on the development, functional organization, and interactions between the CNS, the endocrine system, and the immune system. The emphasis is on the thirty or more biologically active peptides that characterize these systems, and the roles that they play in intercellular communication.

Gainer's group continues to emphasize the oxytocin and vasopressin magnocellular neurons of the hypothalamus as a model neural development system. (The Laboratory has previously shown, for example, that inappropriately high levels of natural neurohormones [i.e., vasopressin] during fetal development can result in the permanent down-regulation of kidney receptors for the hormone.) These neurons are responsible for the biosynthesis of the vasopressin and oxytocin prohormones, first identified by this Laboratory (the complete amino acid and gene sequences for these hormones have now been elucidated). At the time that the prohormones were identified, this group hypothesized that the initial endopeptidase cleavages, which excise the nascent biologically active peptides from their precursors, occur primarily in secretory vesicles, and that all subsequent processing also occurs within the vesicles. Gainer's group has now demonstrated that the vesicles indeed contain all of the expected processing enzymes suggested by the hypothesis, i.e., prohormone converting endopeptidase, carboxypeptidases, aminopeptidases, and alpha-amidases. Moreover, they have demonstrated specific vesicle membrane proteins, i.e., an ATPase which serves as a proton pump responsible for acidifying the vesicle interior (the converting enzymes have an acid pH optimum), and a cytochrome b561 which donates electrons to yield a reduced ascorbate (which serves as a cofactor for alpha-amidase). Also, during the past year, the group has demonstrated unequivocally, using electron microscopic immunocytochemistry, that the prohormones and converting enzymes are indeed located within vesicles, and are further using these techniques to study the routing of the peptides through the endoplasmic reticulum, Golgi apparatus, and other cell membrane systems. The immunocytochemical methods employ monoclonal antibodies which the group has generated using a new in vitro immunization procedure that takes days rather than months and produces good antibodies against poor immunogens. During the past year, Gainer's group has also used these methods to demonstrate the co-localization of an opioid, dynorphin 1-8, and vasopressin in common secretory vesicles. Further, they showed that this dynorphin is found alone in secretory vesicles in the Brattleboro rat, a mutant which does not synthesize vasopressin. In studies on vasopressin receptors, this group has shown, using a cultured epithelial cell line, that the appearance of these receptors is dependent on the development of an epithelial morphology in culture. Only when the morphology of the cell is appropriate does the vasopressin receptor become coupled to adenyl cyclase, which then triggers the cascade of hormone-dependent signals which eventuate in gene expression.

In other work, Gainer and his colleagues have extended their voltage sensitive dye studies to the mouse pituitary, and found that the action potential is accompanied by a light-scattering effect apparently correlated with hormone secretion. These results may make possible the simultaneous recording

of both the action potential and secretion process with a single oscilloscope sweep. In studies on the squid axon model, it was found that the calcium-activated protease cleavage of the neurofilament protein, as well as the casein-like protein kinase which phosphorylates the neurofilament, both act on a 200K cross-linking component of the neurofilament protein.

Loh's section has continued to study the ACTH/endorphin/MSH family of peptides and its relationship to the oxytocin-vasopressin system. It had been shown that the three peptides are synthesized in the intermediate lobe of the pituitary from a common 32K glycoprotein prohormone (POMC). These intermediate lobe peptides have a specific developmental role in the fetus, as well as a critical role in memory and analgesia later in life. In the past year, the group has found a converting enzyme in the intermediate lobe which cleaves the prohormone at its paired basic residues. The converting enzyme has been characterized as a 68K protein, with an acid pH optimum. As with the vasopressin-oxytocin system, POMC is processed by intravesicular carboxypeptidases and aminopeptidases, as well as the cleavage enzymes. The regulation of synthesis of POMC has been studied using the toad intermediate lobe, and it has been found in this model that dopamine effectively down-regulates the biosynthesis of the prohormone, with the regulation apparently mediated by cyclic AMP. Finally, Loh's group has prepared a cDNA library from toad pituitary, screened the library with a mouse POMC probe, and isolated clones which can be used as probes for studying the regulation of POMC synthesis at the transcriptional and genomic levels. Such reagents will permit an answer to the question of whether the regulation of the converting enzymes and POMC synthesis is coupled during development or later physiologic perturbations such as stress.

Laboratory of Comparative Ethology--
Stephen J. Suomi, Ph.D.

Research in this Laboratory is focused on the development of behavior in humans, primates, and other animal models. The interactions of genetic and environmental factors are explored, using a comparative mammalian approach, so as to determine the origins and evolution of various behavioral phenotypes. Experimental results in animals are correlated with the results of longitudinal studies in human infants and families as well as results obtained by various neuroscience techniques.

Using a core sample of 65 families studied longitudinally in the home environment, Pedersen's section assessed the influence of maternal employment on infant development. This influence was found to be multi-factorial, involving the age and sex of the child, the family's socioeconomic level, the degree of the father's involvement with the child, the satisfaction of the mother with her role, her hours of employment, and the quality of substitute care. Allowing for these variables, there seemed to be a clear difference in response to the employment of the mother related to the child's sex. In general, these results demonstrate that considerable heterogeneity is subsumed under the single concept of "separation." In another longitudinal family study, involving the development of mastery motivation (i.e., the drive to be competent), it was demonstrated that mastery motivation in infancy can

indeed predict later competence, and that infants with Down syndrome display a similar distribution of behavior directed toward mastering various aspects of their environment (although the relative level of mastery behaviors is systematically depressed). The results in both normal and Down syndrome infants indicate that there is a complex interplay between the characteristics of the infant and the characteristics of the parents, mediated by specific areas of competence. Pedersen's group has also been studying psychosocial adjustment in children with endocrine disorders, e.g., precocious puberty and dwarfism. These children do, in fact, show an above normal incidence of adjustment problems, but whether they are related to the child's appearance or to the hormonal disorder per se remains to be established.

In studies on the development of "language" in the squirrel monkey, Symmes' section found that mother monkeys are able to identify their own infants uniquely by the calls they make when isolated from other animals. Using sound spectrographic analysis, the cellular basis for this maternal recognition was explored. Work has also continued on the genetics of the isolation call structure in these animals, documenting the consistent differences in the isolation call between two strains of squirrel monkeys, and the mixture of these vocal types found in hybrid progeny. Two brain pathways have been identified which have differing roles in the expression of this vocalization, the caudal thalamic tegmentum and the rostral cingulate gyrus. Another approach to the study of the brain and behavior in squirrel monkeys involves play. Here too, individual differences in the frequency and roughness of play, and play strategies in general, appear to have a genetic basis; these primate studies should help us to understand the contributions of "nature and nurture" in the origin of "social" behavioral relationships.

The rhesus monkey colony now established at Poolesville includes animals genetically selected for high or low reactivity to novel stimuli and challenges ("up-tight" or "laid-back" monkeys). In a major longitudinal study by Suomi's group, infant monkeys of the two behavioral genotypes are being cross-fostered, with mothers selected for their characteristic style of nurturant or punitive mothering (again, the "laid-back" or "up-tight" genotype). In other studies of these animals, it was found that monkeys who had been identified as "up-tight" in early infancy still react to brief separations at 4-years of age (adolescence) with extreme behavior--agitated, self-directed stereotypy--whereas the infant response had been one of depressive withdrawal. As in earlier studies, the high- and low-reactive adolescents did not differ from one another behaviorally or physiologically in the absence of separation or other stress. Interestingly, when the anti-depressant drug imipramine was administered to these adolescent monkeys, there was a significant reduction in self-directed behavior among the "up-tight" monkeys during separations, but few drug effects were apparent in the behavior of the low-reactive subjects under similar conditions. In another study, rhesus monkey infants were compared with respect to whether they had been reared by their mothers or in a nursery ("kibbutz"). Substantial differences were found in the behavior of these infants as groups initially, but interestingly, these group differences all but disappeared as the infants grew older. On the other hand, the individual differences ascribed to the "up-tight" and "laid-back" genotypes remained highly predictive of individual differences in behavioral and physiologic responses to stress later in life. Finally, the Poolesville colony was studied for a possible relationship be-

tween social dominance and genetics. Groups of peer-reared rhesus monkey infants and juveniles were studied so as not to introduce the variable of the mother's dominance status, and it was found that siblings growing up in different peer groups shared the same relative dominance status, suggesting (as did the squirrel monkey studies) a genetic component in the acquisition and maintenance of social dominance.

Laboratory of Developmental Neurobiology--
Phillip G. Nelson, M.D., Ph.D.

This Lab investigates the neurobiologic mechanisms relevant to development of the nervous system, with emphasis on studies of organotypic cultures at the cellular membrane and molecular levels. The basis for short- and long-term interaction between nerve cells is studied electrophysiologically and biochemically. Combined molecular and morphological methods are used in the analysis of experiential modifications of neuronal function and gene expression. Research activities also focus on the factors critical to the metabolism and function of the pineal gland.

This Laboratory pioneered the development of tissue culture systems derived from the mammalian central nervous system which permit the study of neuronal development and synapse formation in vitro. In the past year, Nelson's group has employed such a system to demonstrate the role of electrical activity in neuronal survival. Using in vitro cultures, they found that neuronal viability is critically dependent upon electrical activity between one and three weeks of development, but this "critical period" applies only to cholinergic neurons and not to GABA-containing neurons. The influence of electrical activity on neuronal development appears to be mediated by cyclic AMP. Moreover, a trophic substance is also necessary for the survival of central cholinergic neurons, and it appears to be vasoactive intestinal peptide (VIP). Interestingly, the VIP content of neuronal cultures reaches a peak at the end of the "critical period." The release of VIP from cultured neurons is blocked by tetrodotoxin (TTX), indicating that the availability of this tropin depends upon ongoing electrical activity. Even in the presence of an electrical blockade, neuronal viability and development can be maintained through the use of exogenous VIP in amounts as low as 10^{-12} M. Furthermore, a marker for cholinergic neurons is choline acetyltransferase (CAT); CAT activity is influenced by the application of VIP only during the critical period. Finally, Nelson's group has data to suggest that the VIP action may be mediated through glial cells, suggesting the importance of glial-neuronal interactions. Taken together, these data offer compelling evidence for an electrical activity-dependent developmental regulatory role for VIP (or a closely related peptide) in the CNS.

Nelson's section has also been analyzing the development of electrical excitability in neuronal cultures, and has found that developmental changes occur in the density of voltage-sensitive sodium and calcium channels. Using voltage-clamp techniques, they found that these ion channels develop within the first week in culture and are distributed on neurites as well as on the cell bodies. These results are consistent with the onset of spontaneous electrical activity, the expression of VIP, and the timing of the critical

period for further neuronal development and viability. The calcium channels are of particular interest because they are involved in the synaptic release of neurotransmitters.

Neurons can also be excited by extra-cellular amino acids, and Nelson's group has employed voltage-clamp techniques to clarify the complexities of post-synaptic responses to excitatory amino acids. The voltage-sensitive conductance mechanism is well suited to the amino acids' role as a neuro-modulator (i.e., a mechanism regulating the availability of neuro-transmitters). The transmitter release mechanism itself has been further studied by this group, who have now established the synaptic bouton as the probable entity underlying release. Most of the bouton elements do not appear to be functional under physiological conditions, i.e., there is a "synaptic reserve." This reserve provides a potentially rich source for alteration of nervous system function, and the ability to switch presynaptic elements on or off could underlie CNS plasticity.

In other work, this group has developed a highly sophisticated computer-based neuronal modelling program in order to analyze elaborate neuronal geometries and complex electrophysiological data. The modelling program is now in wide use throughout the world.

Finally, Nelson's section is increasingly using the tools of molecular genetics to probe gene expression in the developing CNS. In the past year, mRNAs from Xenopus pituitaries were used to construct cDNA libraries in the Pst I site of pBR322 cloned in E. coli. One of these cloned plasmids contains a 435 bp insert homologous to mouse pro-opiomelanocortin (POMC) cDNA. Investigators in the group have also developed libraries of differentiation-specific sequences expressed in a line of mouse neuroblastoma cells, as well as in a neuron-glia hybrid cell line. Several million clones have been inserted in a λ-expression vector, with an initial goal of isolating the gene for choline acetyl transferase. These cloned DNA probes will permit molecular studies of the developmental expression and physiological regulation of critical brain enzymes, neurotransmitters, and neurohormones, and may permit identification of the molecular defect in neurological disorders such as Alzheimer's disease.

Klein's section has continued to work on the pineal's melatonin rhythm generating system. The pineal gland, and the melatonin rhythm generating system, appear to be an excellent model for studies on epigenetic and genetic regulation of intracellular CNS metabolism. This group has now found that the pineal and the retina share several uncommon proteins, including rhodopsin kinase and hydroxyindole-O-methyltransferase. This result probably reflects a common ancestral photoreceptor--although in mammals, light does not act directly on the pineal gland, but through a complex neural circuit, beginning with the retina, proceeding through the hypothalamus and the paraventricular nucleus and the superior cervical ganglia, and terminating in the pineal. This circuit is being dissected by discrete electrical stimulation of various neuroanatomic targets within the circuit, and determining the effect of such stimulation on melatonin production.

Klein's section has also found that the pineal releases norepinephrine, and this neurotransmitter in turn has an important role in regulating melatonin

synthesis. Moreover, norepinphrine acts through both alpha and beta receptors--acting as a transmitter via beta receptors and as a modulator via alpha receptors. These synergistic effects are mediated by cyclic AMP and probably cyclic GMP, but the cyclic AMP and GMP systems, while parallel, are discrete. These results suggest the value of the pineal as a model for detailed studies of neurotransmission and neuromodulation.

Klein's group has also established pineal as well as retinal cDNA libraries, and the gene which encodes rhodopsin kinase is being cloned. These gene probes will permit studies of gene expression during retinal-pineal development, as well as during physiological regulation in the adult. Finally, Klein's group has been successful in transplanting pineal tissue to pinealectomized host animals, and they find that the pineal tissue survives in the brain and elaborates melatonin. Moreover, the transplanted tissue becomes innervated by co-transplanted superior cervical ganglia. If it proves possible to reconstruct the entire melatonin rhythm generating system through transplantation, it may also be possible to engineer these transplanted pineal cells so as to introduce other regulatable neurotransmitters and neuromodulators wherein deficiencies in these peptides are reflected in neurological or mental disease.

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

The Laboratory conducts research into the developmental and molecular biology of "natural" and immunization-induced immunity to bacterial and other antigens. Emphasis has been placed on the study of pathogenic mechanisms, vaccine development, and the immuno-regulatory mechanisms of the young host. Modification of transplantation antigen genes at the DNA level is undertaken in order to determine the structural basis for immunological polymorphism and the function of the gene products, and activation of paternal transplantation antigen genes is studied in developing embryos.

Robbins, Schneerson, and Sekura have continued their work on encapsulated bacteria, e.g., Haemophilus influenzae type b and pneumococci, which remain a serious cause of morbidity and mortality in infants and young children despite effective antibiotics. Effective immunization against these encapsulated bacteria-induced diseases is an important goal, but existing vaccines are poor immunogens in the young and fail to induce a booster ("T cell-dependent") response. Therefore, this group is attempting to increase the immunogenicity of vaccines directed against encapsulated bacteria as well as improve the T cell-dependent antibody response they elicit. They have now succeeded in creating conjugates between the capsular polysaccharides of H. influenzae or pneumococcus and various carrier proteins such as tetanus toxoid or hemocyanin. The polysaccharide and carrier protein are joined by a bivalent spacer molecule (adipic acid dihydrazide). The H. influenzae-tetanus toxoid conjugate has been extremely effective in eliciting protective antibodies in infant rhesus monkeys, with the antibodies directed both toward the polysaccharide and the carrier protein; moreover, a booster response could be demonstrated. The polysaccharide antibodies elicited by the conjugate appear to correlate with clinical protection. As a result of the monkey

trial, this group conducted their next study in normal adult volunteers, using both the H. influenzae-tetanus toxoid and the pneumococcus-tetanus toxoid conjugates. There were no serious adverse reactions, and with both vaccines, the geometric mean increase in antibodies was almost 200-fold, representing the highest levels of polysaccharide antibodies ever observed in humans. In the next clinical trial, Robbins' group will immunize children and infants with H. influenzae and pneumococcus-tetanus toxoid conjugates. They are also employing sonication to produce polysaccharides of various molecular sizes in order to standardize and compare their relative immunogenicities.

Robbins' group is also interested in developing an improved vaccine against typhoid fever, which remains an important cause of morbidity and mortality in underdeveloped nations. The current vaccine, composed of whole bacterial cells, elicits an excess of adverse reactions. However, S. typhi contains a capsular polysaccharide (Vi antigen), and the group has initiated studies in Nepal and India to determine whether the purified Vi polysaccharide elicits protective antibodies. If so, this polysaccharide might serve as the basis for an improved vaccine. In another study, the section has been interested in the age-related acquisition of "natural" antibodies to encapsulated bacteria. Such antibodies, which are protective, have appeared in almost all adults by 20 or 30 years of age without contact with the specific bacteria against which they are protected. For example, Group A meningococcal meningitis is a rare disease in the United States despite the fact that these organisms are rarely isolated. Robbins' group has now shown that the antigens of several E. coli strains, commonly present in the gut, are cross-reactive with Group A meningococcal polysaccharide, explaining the acquisition of "natural" immunity.

Finally, Robbins and his colleagues are devoting much effort to improving the pertussis vaccine. While a vaccine against pertussis is critical to the public health, the current whole cell vaccine is associated with some morbidity and mortality and much litigation. However, pertussis toxin per se appears to be the component of the organism responsible for both the symptoms of pertussis (whooping cough) and the vaccine-induced or disease-acquired immunity. In the past year, this group has greatly improved the yield of pertussis toxin using large scale fermenters. (Yield has been an important problem because B. pertussis is a fastidious organism and releases inhibitors of its own growth during cultivation.) Moreover, the group has been able to improve the purification of the toxin from these large yields. Finally, they have devised a microassay for pertussis toxin antibodies which correlates with vaccine potency, and they have made considerable progress in developing methods for inactivating the toxic activity of the pertussis toxin. Robbins' section thus appears close to developing a new pertussis vaccine with greater specificity and fewer adverse effects than any vaccine currently available.

In basic studies, this group and their collaborators have identified the mechanism of action of pertussis toxin as an enzymatic transfer of ADP ribose to a membrane-bound acceptor protein (similar to the mechanism of cholera toxin). The cell membrane protein ("Ni") appears to be a component of adenylate cyclase and is involved in the regulation of the cyclase. Pertussis toxin-treated cells are no longer responsive to hormones which ordinarily inhibit adenylate cyclase and as a consequence, cAMP accumulates. This mecha-

nism appears to explain the problems with glucose homeostasis and possibly the encephalopathy associated with the toxin.

Ozato's group is interested in the major histocompatibility antigens, in particular the Class I antigens of the mouse. This group has pioneered the structure/function analysis of these complex polymorphic cell surface structures that are critical in the expression and regulation of immune function. Using the H2-L^d antigen as a model, the group has employed oligonucleotide-directed mutagenesis to induce site-specific mutations in the critical areas of the genes which encode various H-2 domains. They have then studied the effect of each mutation upon antigenic expression by transferring the mutagenized genes into H-2 negative mouse L cells. The transfected cells are then characterized through the use of monoclonal antibodies with known reactivities toward the H-2L^d antigen, as well as cytotoxic lymphocytes. This approach has yielded the following results: Mutants with a deletion of the glycosylation site in the first domain had no effect on recognition either by monoclonal antibodies or cytotoxic lymphocytes. On the other hand, replacement of phenylalanine with tyrosine at position 116 in the second domain (the external portion of this H-2 antigen) was associated with a considerable loss of reactivity both to the monoclonal antibodies and the cytotoxic lymphocytes, indicating the importance of the tertiary structure of this external portion of the antigen. Similar studies are now underway with a second Class I antigen, H2-L^k.

Ozato's group is also interested in the embryonic development of the Class I major histocompatibility antigens. This work is of importance because Class I antigens are essential for recognition by the host of foreign antigens that are associated with the cell surface (such as viruses). Moreover, Class I antigens are responsible for most rejection phenomena occurring between incompatible tissues. Among the questions that could be answered were we to have an improved understanding of the development of the Class I antigens during embryogenesis are these: Why does a fetus (histoincompatible by definition since it bears paternal antigens) survive even though the mother should recognize these antigens as foreign? Is the teratogenic effect of early intrauterine infection with some viruses (e.g., rubella) explained by the fact that the fetus can not recognize these viruses except in conjunction with Class I antigens which may not be expressed until later in gestation. In the past year, Ozato's group, using both in vitro and in vivo embryonic tissue, as well as monoclonal antibodies which specifically recognize the Class I antigens, has demonstrated that the onset of Class I antigen expression does not occur before mid-gestation in the mouse. Moreover, they have shown that maternal T-lymphocytes do recognize the paternal contribution of the fetus's H-2 antigens, but maternal antibody directed toward these antigens appears to cross the placenta and may prevent expression of these antigens at the surface of the fetal cells.

Hanna's group has been isolating large populations of rodent lymphoid cells in specific stages of development which express only one of the many immune functions of lymphocytes. Since lymphocytes in vivo are at varied stages of development and as a population represent the full spectrum of differentiated immune functions, Hanna's approach has been to construct hybridomas, one component of which is a lymphocyte at a specific stage of differentiation. Hybridomas containing precursor helper T-lymphoctes or precursor suppressor

T-lymphocytes have now been isolated, cloned, and characterized. These T-cell hybridomas provide a valuable source of differentiated lymphocytes with a single specific regulatory function. Moreover, such reagents are useful for experimental manipulation of these immune functions. For example, in the past year, Hanna's group has shown that streptococcal exotoxin, upon binding to the cloned precursor suppressor lymphocytes, alters the pathway of their development and re-directs this pathway toward a helper function. This effect is associated with a diminution of the expression of the Lyt-2 surface antigen (a marker associated with T-cell suppressors).

Laboratory of Theoretical and Physical Biology--
David Rodbard, M.D.

This Laboratory conducts a wide range of multidisciplinary and theoretical studies, applying mathematical, statistical, and computer-based techniques to the analysis of complex clinical, biological, biochemical, and pharmacological problems. Experimental work in the Laboratory involves the study of ligand-binding receptors, as well as the development of electrophoretic, mass spectroscopic, and other biophysical and physical-chemical techniques.

Rodbard's group, during the past year, has continued to develop computer programs for the analysis of complex ligand-binding systems. In this regard, they have formulated a program which permits a multi-ligand approach, wherein two or more unlabeled ligands are present simultaneously with a labeled ligand. Another new method, the "Kd versus Kd plot," permits the characterization of multi-receptor systems, and identifies distinct classes of binding sites. The programs for ligand-binding analysis have been used to study thrombin receptors on platelets, histamine receptors in the lung, glucocorticoid receptors in human cells which demonstrate steroid resistance, dopamine receptors, and the binding of monoclonal antibodies. Rodbard's group has also improved their programs for the analysis of immunoassays, now reaching a seven parameter extension of the original logistic equation. Programs have also been developed this year for the analysis of complex enzyme-substrate-inhibitor systems, and such a program has been used to characterize an important interaction in cancer chemotherapy, i.e., the competitive interaction of polyglutamate conjugates of methotrexate with thymidylate synthetase. Further, the group has developed a program with immediate clinical application designed to enhance the management of patients with diabetes mellitus receiving intensive insulin therapy. This unique program permits highly refined self-adjustment of insulin dosage and can even be used by the patient at home with a small personal computer.

Other investigators in Rodbard's section have employed direct experimentation, as well as optimizing the computer-based analysis, to demonstrate and characterize the opioid receptors of the brain. Using quantitative ligand-binding studies, they have demonstrated at least 4 types of CNS opioid receptors in the rat (mu_1, mu_2, delta, kappa). These findings have been displayed graphically using the K_d vs. K_d plot. The mu_1 receptor subtype, which plays an important role in analgesia and in the regulation of pituitary function (especially prolactin release) was shown to be selectively inactivated by naloxonazine, an opioid antagonist dimer. These techniques

for characterization of brain receptor systems are widely applicable to other complex receptor systems as well.

Finally, this group designed a series of dimeric analogs of the enkephalins which were then synthesized in the laboratory of Dr. Harry Chen. The analogs bind either to mu or delta receptors in the brain, and demonstrate a dramatic increase in binding affinity relative to the monomer. Studies with the analogs demonstrate that the receptors for enkephalins are themselves arranged as dimers; the dimeric enkephalins bind to two sites within a receptor, but not to two widely separated receptors. Moreover, the binding of two dimers cannot occur simultaneously at any given receptor.

In another area of interest, this section further developed a highly sophisticated computer graphics system which permits three-dimensional reconstruction of anatomical interrelationships, from the ultrastructural to the gross-anatomical levels. With regard to embryogenesis, they have been able to align and digitize histologic sections and reconstruct a three-dimensional image. Such a reconstruction permits the study of teratogenesis as well. At the ultrastrucural level, these investigators have employed their three-dimensional computer graphics method to reconstruct the topography of various intracellular organelles, e.g., the Golgi apparatus. They have also made significant progress on illustrating the three-dimensional interrelationships between neurotransmitters and their receptors in the brain, as well as the brain's microcircuitry.

Chrambach's section has pursued the development of universally applicable, high resolution techniques and strategies for the fractionation of various macromolecules. They have continued to refine the methods of polyacrylamide gel electrophoresis, isoelectric focusing, and chromatography, and applied them to the preparative separation of a number of important proteins which retain their biological activity. In the past year, human growth hormone was prepared from a recombinant DNA source and isolated in high yield in a new scaled-up, single-step electrophoretic procedure. The group's preparative methods have also permitted this year the fractionation of large intracellular organelles (e.g., receptosomes), viruses, and cross-linked, aggregated bacterial immunogens.

Yergey's group has continued to innovate new techniques which permit the mass spectroscopic analysis of biological molecules. Their recent development of the thermospray apparatus has permitted continuous on-line analysis of the effluent from high performance liquid chromatography (HPLC) with great sensitivity, resolution, and specificity. Another new and related method, thermal ionization, has now been applied to the measurement of stable calcium isotopes. Using this method, Yergey's group has studied calcium metabolism in neonates, during normal growth and development, in pregnancy and lactation, in osteoporosis, in disorders of vitamin-D metabolism, and in certain rare congenital disorders of heterotopic calcification (e.g., fibrodysplasia ossificans progressiva). In the latter disease, they have demonstrated that while intestinal calcium absorption is normal, there is virtually no urinary excretion of calcium, and multicompartmental analysis with mathematical modeling demonstrates an extraordinary tissue retention of calcium. Yergey's group has also employed mass spectrometry this year to study acetylcholine metabolism in the brain, acylcarnitine metabolism in various organic

acidurias, and the hepatic uptake and production of glucose in glycogen storage disease.

Human Genetics Branch--
Michael A. Zasloff, M.D., Ph.D.

The Human Genetics Branch's interests range from studies on the etiology, diagnosis, and treatment of genetic and developmental disorders of young people to very basic studies on eukaryotic gene expression utilizing recombinant DNA methodology. There is a broad attempt to apply "genetic engineering" together with tissue transplantation techniques to therapeutic strategies. Current research projects concern genetic disorders of lipid and carbohydrate metabolism, the mucopolysaccharidoses, heritable disorders of bone and connective tissue, lysosomal storage diseases (e.g., cystinosis), temperature-sensitive models of cellular differentiation, the genetics of alcohol-related syndromes, and the structure and function of human tRNA genes.

Zasloff's group has continued to work on the organization and expression of human tRNA genes, with a particular focus on $tRNA^{met}$. This group was the first to study the organization of tRNA genes in higher vertebrates; they have localized the $tRNA^{met}$ gene to a specific chromosome, and demonstrated polymorphisms for this gene. Expression of the gene has been studied both in vitro and in vivo using Xenopus oocytes for studies of gene regulation. These studies have now revealed a transport mechanism in the eukaryotic cell which utilizes nuclear membrane pores to regulate the delivery of tRNA from the nucleus to the cytoplasm. In order for this mechanism to function, the primary transcript from the human tRNA gene must be processed, and Zasloff's section has recently purified the nucleases which carry out the processing. Over the past year, about 30 point mutations have been generated in the cloned human $tRNA^{met}$ gene. Virtually every mutation placed in the wild-type gene generates a species which is less efficiently transported than the wild-type, demonstrating the transport system's extreme specificity. If the mutation leads to disruption of the tertiary structure of the tRNA molecule, the processing step is compromised as well as the transport. The mutations which do not affect processing cluster within the anti-codon loop, and these non-shape related changes are the ones recognized by the transport mechanism. The cell proteins which are known to recognize specific tRNAs are the aminoacyl-tRNA ligase and the initiation factor, EIF-2. Thus, one or both of these proteins may be involved in the nuclear membrane transport system.

Another gene sequence, the transcript of which appears to migrate from the nucleus to the cytoplasm by a specific transport mechanism, is the Alu sequence. About 300,000 copies of this sequence exist in scattered human genomic loci, although no function has been assigned to Alu. In the past year, Zasloff's group has studied the Alu sequence contained within the first intron of the mouse alpha-fetoprotein gene. They have shown that this sequence is transcribed by RNA polymerase III and that the primary transcript is processed by a specific endonuclease to yield a "core" Alu-specific RNA. It is the core Alu RNA which is transported specifically to the cytoplasm wherein it is packaged into a ribonucleoprotein. In the mouse, the processed core Alu RNA is found only in liver, suggesting that the post-transcriptional

pathway is tissue-specific and that the ubiquitous Alu gene sequences may therefore play an important role in differentiation.

Zasloff's section has also been interested in the molecular mechanism of action of thyroid hormone, and to illuminate this mechanism, they have developed new methodology in the past year that permits the identification of low abundance ("rare")species specifically induced by the hormone. In the method, nick-translated cDNA fragments from a recombinant cDNA library are bound to total cellular RNA; binding occurs in proportion to the abundance of complementarity in the total cellular RNA, permitting quantitative analysis of mRNA abundance for each corresponding cloned cDNA. Using this method, the group has now been able to identify those specific messages which vary upon treatment of the rat with high doses of T3.

Mukherjee's group has been studying uteroglobin, a protein elaborated by rabbit endometrial cells, the function of which is uncertain. During the past year, this group found that uteroglobin is a potent inhibitor of platelet aggregation and may thus counteract the hypercoagulable state which characterizes pregnancy. This group has also found that a protein similar to uteroglobin is present in the neonatal human lung. Further to facilitate these uteroglobin studies, the group has transformed an endometrial cell line with SV40; the transformed cells, when stimulated with progesterone, secrete uteroglobin.

Chou's group has been interested in the use of temperature-sensitive cell lines to study cellular differentiation. Using a line of rat fetal liver cells transformed by a temperature-sensitive mutant of SV40, the group has studied various differentiation markers elaborated at the non-permissive temperature (40°C), and observed their disappearance when the transformed phenotype is re-induced at the permissive temperature (33°C). In the past year, the group has shown that at 40°, the differentiated phenotype resembles fetal liver in vivo: The cells synthesize two alpha-fetoprotein variants of 73 and 69K and contain an alpha-fetoprotein mRNA species of 20S. However at 33°C, the level of AFP synthesis is reduced, with only one species of 65K and an AFP mRNA species of 14S (apparently generated by an alternative RNA splicing pathway). This elegant system also appears to be useful for the study of molecules which regulate differentiation and maturation; for example, Chou's section has found, in the past year, that retinoic acid will induce maturation of fetal liver cells in vitro.

In clinical research, the Branch has been interested in a number of metabolic and connective tissue disorders. In preliminary studies on a rare congenital disease involving heterotopic ossification (fibrodysplasia ossificans progressiva), it was found that six of seven children treated with 13-cis retinoic acid experienced an apparent remission of ectopic bone formation. This study will now be expanded with appropriate controls. A new disease was also identified this year, similar to FOP in that it is a disorder of heterotopic ossification, but different in that the lesions have a totally different tissue distribution. In studies on the Hunter syndrome, a heritable lysosomal disorder resulting from a defect in expression of iduronate sulfatase, this enzyme was purified and is now being used to generate antibodies useful in the selection of the relevant mRNA and the corresponding gene. In studies of children with mucopolysaccharidoses, a preliminary observation in

England has been followed up here by skin implantation of human amnion from normal newborns into Branch patients. (Normal amniotic cells can be donated between otherwise histoincompatible individuals; moreover, enzymes elaborated by these transplanted amniotic cells and circulating in the plasma can be recaptured by the host cells even though they are genetically deficient in the enzyme.) About 20 MPS children were treated by amnion transplant over the past year. The circulating serum levels of the lysosomal enzyme involved in MPS did not increase, although there was some clinical improvement and a decrease in urinary mucopolysaccharides in several of the patients.

In other clinical research, Branch investigators determined the transketolase Km for thiamine pyrophosphate (TPP) because of an earlier suggestion that this Km is very high in chronic alcoholics who develop Wernicke-Korsakoff encephalopathy. A high Km would deplete the body of thiamine rapidly in the alcoholic state. Because the high Km might be a genetic variant, it was sought in a population of alcoholic men and their young sons compared with non-alcoholic men and their sons. The Branch investigators confirmed the earlier Korsakoff findings and showed that in general, the sons of alcoholic males have a higher Km than the sons of non-alcoholic men, as is true for the alcoholic men per se. These preliminary data suggest the possibility of a genetic predisposition to thiamine deficiency in the presence of alcohol; such a predisposition might relate to other complications of alcoholism such as the fetal alcohol syndrome.

Gahl and his colleagues continue to investigate the carrier-mediated transport of cystine across leucocyte lysosomal membranes. The group has shown that the lysosomal cystine carrier is saturable, stereospecific for the L-isomer of cystine, capable of exhibiting counter-transport, and deficient in cystinosis--a genetic lysosomal storage disease. In the past year this group has further studied normal cystine transport and its requirements and mechanisms. They have discovered that in another disease, mucolipidosis II, the cultured fibroblasts demonstrate impaired cystine clearance similar to that occurring in cystinosis per se. This tissue culture system may thus provide a good model with which to study defects in lysosomal cystine transport. This group is treating cystinotic children with the cystine-depleting agent cysteamine as part of a national study initiated here. The growth of these patients has been encouraging, and renal deterioration appears to have been delayed. Cysteamine may thus be the first useful therapy for this important genetic disorder. However, because of poor tolerance of cysteamine, other therapies are being sought and in a trial of oral pantethine, cystine-depleting efficacy was demonstrated with improved tolerance.

In studies on disorders of carbohydrate metabolism, Sidbury's group demonstrated the usefulness of raw starch in the oral management of children with Type I glycogen storage disease. This simple therapeutic maneuver appears to stabilize blood glucose levels in patients who had previously been exceptionally labile and difficult to manage.

Finally, Caddell has studied magnesium metabolism in animals and infants with the type of recurrent apnea associated with the "sudden infant death syndrome." It is clear that magnesium deficiency in young rodents does produce apnea, and Caddell has now shown in a large retrospective clinical study that magnesium deficiency is common both in neonates and in infants with recurrent

apnea; in those infants who were given replacement magnesium, the incidence of recurrent apnea was significantly reduced.

Developmental Endocrinology Branch--
D. Lynn Loriaux, M.D., Ph.D.

The endocrine concomitants of normal and abnormal human growth, development, and differentiation are examined in this clinical research Branch. Specific areas of study include the mechanisms underlying the initiation of puberty; the regulatory physiology and biochemistry of the glycoprotein hormones; the roles of sex steroid hormones, growth hormones, and other growth factors in bone growth; and the physiology and biochemistry of hypothalamic releasing hormones. Clinical research on male and female reproductive disorders is also a major interest of this Branch.

The Branch has a strong interest in the endocrine mechanisms which determine the onset of puberty in humans, and has been particularly interested in "experiments of nature" which may illuminate these mechanisms. In this regard, a clinical trial involving patients with central precocious puberty is nearing completion. These children have been treated by Loriaux and his colleagues with an LHRH analog that desensitizes the pituitary response to endogenously secreted LHRH. Over 100 children with this disorder have now been treated with the analogue, with very encouraging results. The secondary sexual characteristics have regressed and the accelerated rate of growth has fallen to normal. Moreover, the psychological maladjustment of these children has tended to improve with treatment. A number of these patients have been treated for as long as 4 years and no adverse reactions have been noted. The LHRH analogue has also been used as a probe to clarify the lesion in two other forms of precocious puberty: The McCune-Albright syndrome and the syndrome of familial male isosexual precocious puberty. Using the analogue, both of these forms of precocious puberty appear to be independent of gonadotropin support and are thus primary gonadal disorders. With this in mind, patients with McCune-Albright syndrome have now been treated with testolactone, an aromatase inhibitor which blocks estrogen formation in the gonad. All patients treated have shown regression of their secondary sexual characteristics and a decreased rate of growth. A number of boys with familial isosexual precocity have been treated with spironolactone, an anti-androgen, and these patients have improved significantly as well.

Cutler's section has been interested in adrenal maturation as an important feature of the pubertal process. In the past year, studies on the mechanism of adrenarche have been carried out in castrated male chimpanzees, which provide a model of human adrenarche. These chimps were hypophysectomized and given replacement therapy with ACTH and thyroid hormone. The plasma glucocorticoids were maintained by this maneuver, but plasma androgens fell significantly, suggesting that a non-ACTH pituitary factor plays an important role in supporting adrenal androgen secretion. Studies were also continued on primary cortisol resistance, an entity characterized in humans by hypercortisolism without Cushing's syndrome, hypertension, and hypokalemic alkalosis. A similar picture was found in New World monkeys which normally have very high free plasma cortisol levels as well as other alterations of

plasma steroid hormones. The human disorder and the normal New World primate physiology might be explained on the basis of receptor-mediated resistance to glucocorticoid action. However, this group found that the abnormality is probably one of binding affinity rather than receptor number. Therefore, studies were undertaken on the physico-chemical nature of these receptors, but it was found that in both models of primary cortisol resistance, the receptors are identical in all respects to those of appropriate controls. A coincidental observation in the New World monkeys was that there appears to have been an evolutionary divergence of thyroid-binding and cortisol-binding plasma proteins, with the binding capacity for thyroid hormone increased and that for cortisol decreased in these animals. This result suggests that the cortisol binding capacity may have decreased as a compensation for the high plasma levels associated with reduced receptor affinity.

Other Branch investigations focused on studies of a synthetic antiglucocorticoid, RU38486, which competes with cortisol for binding to the glucocorticoid receptor. This new drug has been used very effectively to treat the ectopic ACTH syndrome. The diagnosis of this uncommon syndrome has been approached using corticotropin releasing factor (CRF). It was found that patients with Cushing's disease (ACTH secretion from a pituitary microadenoma) respond to CRF with an increase in circulating ACTH, but patients with the ectopic ACTH syndrome fail to respond to this stimulus. It was also found this year that the surgical approach to pituitary microadenomas can be greatly improved by administration of CRF prior to sampling of the inferior petrosal sinus for ACTH. This procedure amplifies the gradients between the sides of the pituitary and makes clear which side contains the adenoma. With this approach, the ultimate surgical success rate has climbed from 50% to virtually 100% in patients with Cushing's disease.

The pediatric endocrinology group has also been interested in the effects of the sex steroids on skeletal growth during puberty. Most of this effect appears to be due to estrogen, and during the past year, the dose-response relationship was determined between exogenous estrogen and bone growth in children with gonadal dysgenesis. Undesirable effects of sex steroids during puberty include the development of hirsutism, acne, and male pattern baldness. Branch investigators found, during the past year, that these systemic effects can be avoided by the topical application of an anti-androgen; the topical drug is effective in treating hirsutism in women and in preventing male pattern baldness in a primate model. A trial to determine whether the anti-androgen can prevent severe acne in adolescents is underway. Branch investigators also had a major therapeutic success in the prenatal treatment of congenital adrenal hyperplasia. A mother who had given birth previously to a child with this disorder of steroid biosynthesis was given dexamethasone early during her next pregnancy, and this prenatal therapy prevented the development of ambiguous genitalia in the second infant. This result is one of the first demonstrations of the value of "fetal therapy."

Studies were continued on the 44 amino acid peptide, GRF (growth hormone releasing factor). It was found that GRF releases the secretion of growth hormone in over 80% of growth hormone deficient children, and induces a growth response indistinguishable from that obtained with exogenous growth hormone *per se*. Since GRF is now readily synthesized and relatively inexpensive, these results are of considerable clinical significance. Branch inves-

tigators also developed a new radioimmunoassay for GRF that permits careful monitoring of patients receiving this factor.

Nisula's section continued their studies on thyroid stimulating hormone. They had previously shown that TSH interacts with two classes of binding sites in human thyroid tissue, having either high or low affinity for the hormone. During the past year, this group employed a specific molecular probe to demonstrate that it is only through the high affinity sites that TSH stimulates adenylate cyclase. In other experiments, Nisula's group continued to explore human choriogonadotropin (hCG) as a molecular congener of TSH useful for structure-function studies. It is this structural feature which appears to account for the thyrotoxicosis of choriocarcinoma. In the past year, the section studied the influence of the carbohydrate moieties of hCG on its thyrotropic activity and found that deglycosylation produced enhanced binding to the TSH receptor, but loss of intrinsic activity. This result supports the concept that the TSH receptor has separate domains for its binding and activation functions, and points to the potential utility of competitive antagonists for the treatment of hyperthyroidism (Graves' disease).

Sherins' group has exploited the opportunity afforded by men with selective gonadotropin deficiency to identify the hormonal requirements for human spermatogenesis. During the past year, they showed that early exposure to FSH plus hCG augments testicular growth and the appearance of sperm in men with complete hypogonadotropism. However, they also found that estradiol overproduction limits sperm production, suggesting that the temporal relationship of FSH and LH in stimulating the testis is critical. They will now use a pulsatile pump for administration of these hormones in order to define the appropriate FSH and LH algorhythms. In contrast to the very successful effect of gonadotropin replacement therapy in hypogonadotropic men, the treatment of men with idiopathic infertility continues to be unsatisfactory. During the past year, Sherins' group found that androgen receptor binding in tissue from these azoospermic men is entirely normal, and treatment of these patients with hCG and Teslac was unrewarding. On the other hand, the group found that sperm from men with idiopathic infertility are deficient in several glycoproteins that appear to be required for normal fertilizing potential, providing a new avenue for fertility research. Finally, these investigators have been interested in the initiation of gametogenesis during puberty. They have now shown that the ovary regulates the frequency of GnRH secretory bursts from the hypothalamus, and thus regulates its own function. On the other hand, prolactin interferes with the ability of the hypothalamus to sense the ovarian signals (estrogen and progesterone) and an excess of prolactin, as occurs with a prolactin-secreting microadenoma, leads to a state of hypogonadism.

Endocrinology and Reproduction Research Branch--
Kevin J. Catt, M.D., Ph.D.

Current research is focused on the mechanisms controlling hormone secretion and action, with particular reference to the structure and function of hypothalamic-pituitary hormones and their receptor-mediated responses in endocrine target cells. Studies in progress include the analysis of receptors

and actions of hypothalamic peptides (GnRH, somatostatin, CRF, GRF), angiotensin II, ACTH, prolactin, and gonadotropins. The characterization and isolation of peptide hormone receptors, clarification of plasma membrane-related second messenger systems, and elucidation of the hormonal control of adrenal steroidogenesis and hepatic glycogen synthesis, are major goals of the Branch research program.

Catt's group remains interested in the characterization, regulation, and activation mechanisms of cell membrane receptors for gonadotropins, angiotensin II, gonadotropin-releasing hormone (GnRH), and corticotropin releasing factor (CRF). In the past year, the major emphasis has been on exploration of the fetal development of gonadotropin receptors, and differences between fetal-neonatal and adult Leydig cells in response to gonadotropic stimulation and treatment with GnRH agonists. The expression of receptors for LH and FSH has been studied in the fetal rat, and a significant correlation has been established between stimulation and the changes in testicular endocrine function that occur during fetal development. When the fetal testis is treated with natural gonadotropins (LH or hCG), expression of the appropriate receptors is stimulated, but in the adult testis, after an initial stimulation there is a loss of receptors and thus a desensitization to the tropin. The adult-type steroidogenic lesions that follow LH stimulation do not occur in the fetal Leydig cell, but such lesions are produced in neonatal rats given supra-physiological GnRH agonists. In the ovarian granulosa cell, as well, GnRH agonists were found to inhibit expression of LH and prolactin receptors and impaired the ability of FSH to activate adenylate cyclase. These inhibitory effects were calcium-dependent, whereas the maintenance of FSH, LH, and prolactin receptors in the ovarian cell was found to be dependent upon the action of cyclic AMP. In the pituitary gland, Catt's group found that GnRH receptors underwent endocytosis after stimulation by agonists, followed by a phase of up-regulation that depended on protein synthesis. With respect to GnRH per se, the early actions of this hormone were found to include increased phospholipid turnover and the release of arachidonic acid. Arachidonate metabolites as well as protein kinase C were shown to have roles in the release of gonadotropins by the pituitary. In other experiments, Catt's group demonstrated GnRH as well as CRF receptors within the brain. In particular, the demonstration of brain CRF receptors by radioautographic topography provides an anatomic basis for the presumed role of CRF in an integrated CNS response to stress.

This section also studied angiotensin II, which mediates the secretion of aldosterone and circulatory homeostasis. They demonstrated that the actions of this hormone in the adrenal zona glomerulosa cell were selectively blocked by calcium channel antagonists, suggesting the close structural relationship between the AII receptor and calcium channels. These data are consistent with the inhibitory effects of calcium-calmodulin antagonists upon AII-stimulated aldosterone production. The central actions of AII within the brain were correlated with the presence of AII receptors in specific brain areas, demonstrated by topographic autoradiography. These AII receptors were localized to those areas of the brain involved in circulatory homeostasis, e.g., the subformical organ which regulates the drinking response to water deprivation.

Dufau's section demonstrated that the activation of adenylate cyclase by LH in the adult Leydig cell was accompanied by guanyl nucleotide binding to membrane components and cAMP-independent phosphorylation of a 42K protein. Guanyl nucleotide-dependent phosphorylation was highly sensitive to calcium. The relationship between membrane phosphorylation and the activation/desensitization phenomenon was demonstrated, and it was confirmed that receptor activation is required before receptor down-regulation and steroidogenic desensitization can occur. These studies on purified Leydig cells were facilitated by development of an elutriation procedure which yields an abundant purified cell population. This group also studied ovarian receptors for LH and prolactin, obtaining a highly purified preparation of these receptors which permitted physico-chemical characterization and a demonstration of their functional relationship to adenylate cyclase.

In in vivo studies, Dufau's group demonstrated that LH is secreted in pulses of high biological activity, and that a significant discordance may occur between immuno- and bio-active LH pulses. These results indicate that a determination of bioactive LH is necessary fully to characterize the physiological patterns of LH secretion during the menstrual cycle.

Strott's laboratory continued to investigate the physiology and regulation of adrenal steroidogenesis, employing the guinea pig as a model for analyzing the differential function of the adrenocortical zones. They demonstrated that the zone reticularis is insensitive to ACTH. However, the zona fasciculata is selectively atrophied following dexamethasone suppression, with loss of ascorbic acid and cholesterol side-chain cleavage activity. These effects do not occur in the zona reticularis. This ACTH insensitivity of the reticularis is not due to a receptor defect, but rather to a deficient cellular response beyond the formation of cAMP. In other studies, this group has raised antibodies against specific adrenal steroid binding proteins; these reagents will permit studies on the mechanism by which cholesterol and pregnenolone are mobilized to and from the inner mitochondrial membrane during stimulation of the cholesterol side-chain cleavage reaction, which is the rate-limiting step in steroidogenesis.

Chen's section continued their work on the analysis and synthesis of peptides and proteins important in reproductive and developmental biology. A major accomplishment was the synthesis of the 41 amino acid ovine CRF molecule, in high yield, 100% purity, and excellent bioactivity . This is the largest peptide with these characteristics yet synthesized. Moreover, the group synthesized a number of fragments of the CRF chain, e.g., 9-41 and 15-41, and employed these reagents for structure-function studies. Interestingly, the 15-41 fragment has agonist activity, but the 9-41 fragment has antagonist activity, suggesting that the steric configuration of the CRF chain around an axis of symmetry determines the nature of receptor interaction. In other work, dimeric GnRH agonists were produced in order to study the role of receptor micro-aggregation and cross-linking in receptor activation. In regard to peptides of interest in developmental biology, the group synthesized a pentadecapeptide segment of a 68K protein encoded by an mRNA which is differentiation-specific to gastrula embryos of Xenopus.

Huang's section continued their studies on the hormonal control of glycogen metabolism, and specifically, the regulation of glycogen synthase activity by

phosphorylase kinases. These model studies are of considerable importance because phosphorylation and dephosphorylation of enzymes which control rate-limiting metabolic steps appears to be the major mechanism by which cellular metabolism is controlled. It is this mechanism which regulates glycogen synthesis. The glycogen system is complex since the synthase is phosphorylated and dephosphorylated at multiple sites by various protein kinases and phosphatases; a determination of how these various enzymes are affected by hormones is the goal of these studies. Huang's group isolated a unique protein kinase during the past year which may be a potential target for the action of glucagon. This kinase not only phosphorylates glycogen metabolizing enzymes, but also muscle contractile proteins such as the microtubules. All of these studies on the mechanisms which regulate the enzyme activity associated with glucose homeostasis were carried out in diabetic as well as normal human and rodent tissues.

Pregnancy Research Branch--
Gary D. Hodgen, Ph.D.

The interests of this Branch have ranged from fertility regulation and the mechanism of fertilization, through toxicologic aspects of ovarian function, to implantation and the endocrine physiology of pregnancy. These studies employ primates and other animal models, and particular emphasis has been given to in vitro fertilization using these animal models as well as surrogate embryo transfer. Other studies involve the medical and surgical correction in utero of fetal metabolic disorders and anomalies, again using animal models.

Gulyas' laboratory has been concerned with the fundamental mechanism of fertilization and with the specific question as to whether the paternal genome is required for normal embryonic development. In a mouse model, this group collected and denuded eggs; using polyethylene glycol and ethanol, two eggs were fused, and the fused eggs, lacking any sperm products, were incubated in vitro initially and then transfered to surrogate mothers. More than a third of the fusion products developed into blastocysts, but in vivo, none of these fused gametes developed beyond the 14 somite stage. Thus, sperm is not required for the pre-implantation stages of development, but normal term embryonic development does not proceed in the absence of sperm. It is not yet clear whether the ultimate dependence upon sperm reflects the necessity for paternal as well as maternal DNA, an extra-genetic factor in sperm, or whether the fused oocytes would develop normally even in the absence of sperm had they not been compromised by the fusion process per se. In other experiments, Gulyas found that aging eggs demonstrate reduced fertilizability apparently because the zona pellucida "hardens" with age, i.e., it becomes less soluble to proteases (such as contained in sperm).

Bercu's group employed the subhuman primate model to study the pulsatile release of pituitary growth hormone and gonadotropin. By careful monitoring of the pulsatile secretion of these peptides, Bercu was able to demonstrate neurosecretory abnormalities that would have been missed by routine sampling for peak plasma levels of the hormones. These primate findings appear to demonstrate why certain short-statured but non-growth hormone deficient chil-

dren do respond to exogenous growth hormone; the gonadotropin results have similar implications.

Mattison's group has emphasized the study of environmental agents which may be toxic to the ovaries, using a mouse model. In one set of experiments, the group found this year that cyclophosphamide (a common chemotherapeutic agent) produces an age-dependent premature ovarian failure; the age-dependence reflects a decreasing number of oocytes with age due to follicular atresia rather than an increasing sensitivity to the alkylating agent. Importantly, this group found that the ovary contains all of the enzymes necessary to metabolize benzopyrene and similar synthetic derivatives to toxic products capable of producing oocyte destruction. Moreover, there appears to be an excellent correlation between the degree of oocyte destruction induced by these toxins and the risk of germ cell mutagenicity and/or carcinogenicity.

Hodgen's group continued to use the subhuman primate model to study fertility as well as the physiology of pregnancy. Since it is known that only hypopituitary women respond predictably to gonadotropin therapy, they administered a potent GnRH antagonist to monkeys, simulating a "medical hypophysectomy," and demonstrated that this maneuver reduced individual variation in the animals' response to therapy with FSH or FSH/LH. In other studies, purified FSH was administered to monkeys through day 12 of the menstrual cycle, resulting in significant ovarian hyperstimulation and the development of multiple bilateral ovarian follicles. In this state, the normal LH surge was prevented, suggesting that when supraphysiological FSH levels persist into the late follicular phase, secretion of an ovarian factor blocks estrogen-induced LH surges ("the gonadotropin surge-inhibiting factor"). Hodgen's group also pursued studies on endometriosis, autografting endometrial tissue to the pelvic peritoneum of monkeys. The incidence of chemical pregnancy (i.e., the appearance of early hormonal markers) was significantly reduced by this maneuver, but when chemical pregnancy did occur, it always eventuated in term intrauterine pregnancy. These results suggest that infertility in monkeys with endometriosis is mediated primarily by failure of follicular rupture, likely due to pelvic adhesions.

Finally, Hodgen's section introduced another variation on the surrogate embryo theme by collecting fertilized monkey embryos from the utero-tubal lumens of donors and transferring these embryos to the utero-tubal lumens of ovariectomized female surrogates. The surrogates were then given sequential estrogen-progesterone replacement therapy, mimicing the natural ovarian menstrual cycle. This maneuver yielded a proliferative, secretory endometrium that fully supported a normal pregnancy. Thus, the combination of surrogate embryo transfer and an exogenous hormone regimen permits pregnancy in primates even in the complete absence of ovarian function.

Office of the Scientific Director

° Section on Viruses and Cellular Biology--
Arthur S. Levine, M.D.

DNA viruses which influence differentiation are used to probe the developmental program of macromolecules that regulate changes in the phenotypes of

normal and transformed animal cells. The DNA viruses are also utilized as models in studies on the mechanism of DNA replication.

This group has begun to identify the phenotypic characteristics of hamster cells transformed by adenovirus 2(Ad2) or SV40 that correlate with their ability to form tumors in syngeneic animals. Through the use of somatic cell hybrids formed between Ad2("non-oncogenic")-and SV40 ("highly oncogenic")-transformed cells, it has been shown that Ad2 is dominant in regulating this phenotype. Hybrids containing both Ad 2 and SV40 genomes have diminished cellular fibronectin, are very sensitive to in vitro lysis by unprimed immune effector cells, and fail to form tumors upon transplantation to adult syngeneic hamsters. Hybrids containing only the SV40 genome have normal amounts of cellular fibronectin, are resistant to lysis in vitro, and form tumors even in allogeneic hosts. This phenomenon, which correlates with the concentration of Ad2-encoded proteins, may reflect specific interactions between viral genes and host cell genes which encode MHC antigens and/or growth factors. Other investigators in the group have found that tumors induced in hamsters by a small t-antigen deletion mutant of SV40 develop more slowly but metastasize more frequently than do tumors induced by wild-type SV40. Finally, SV40 is also being used as a probe to investigate the molecular mechanisms by which DNA-damaging agents induce mutations in mammalian cells. In studies on the replication of UV-damaged SV40 DNA in monkey cells, it was found that when the SV40 replication fork encounters a pyrimidine dimer in either template strand, the fork proceeds beyond the damage, leaving gaps in both daughter strands. Mutation fixation is likely to occur during the gap-filling process. By use of an SV40-derived shuttle factor, it has been possible to induce mutations in the mammalian system, and then to identify and sequence the mutations in a bacterial system, thus characterizing the types of mutations induced by specific agents and correlating them with the mechanism of mutation induction. Because of the mounting evidence that tumorigenesis, metastasis, mutagenesis, and normal events in developmental biology are intimately related, these results should help to illuminate the mechanisms which regulate basic developmental phenomena.

° Section on Growth Factors--
Gordon Guroff, Ph.D.

This section is studying the mechanism of action of growth factors of neurochemical interest, the biosynthesis and degradation of these molecules in neural tissue, and the physiology of nerve growth factor. Other studies concern the evaluation of the effects of somatic growth factors on cellular differentiation and proliferation.

Efforts this year have been concentrated on the intracellular events which follow the binding of nerve growth factor to its membrane receptor and lead to its effect on nuclear events. Using rat pheochromocytoma cells which differentiate in culture in response to nerve growth factor, it was found that two specific phosphorylations, one cytoplasmic and the other nuclear, were altered in these cells as a consequence of exposure to nerve growth factor. Both phosphorylations were demonstrable in a cell-free preparation, and the cytoplasmic activity was identified as a kinase. The phosphorylative changes are followed by changes in the structure of the DNA which are

believed to underlie changes in the transcription of specific neuronal genes. These results expand our understanding of the molecular basis for the role of nerve growth factor in the development and maintenance of the nervous system, as well as its possible role in tumors arising from the neural crest.

Arthur S. Levine, M.D.
Scientific Director

LABORATORY OF MOLECULAR GENETICS

Z01 HD 00066-14 Control Mechanisms in Temperature Bacteriophage λ
Robert A. Weisberg, Ph.D.

Z01 HD 00067-16 Integrative Control of Macromolecular Synthesis
Michael Cashel, M.D., Ph.D.

Z01 HD 00068-13 Factors Influencing Genetic Transcription-Initiation and Termination
Robert J. Crouch, Ph.D.

Z01 HD 00069-12 Molecular Aspects of the Replication of Enveloped Animal RNA Viruses
Judith G. Levin, Ph.D.

Z01 HD 00070-24 Morphogenesis of Animal Viruses During Infection of Mammalian Cells
Jacob V. Maizel, Jr., Ph.D.

Z01 HD 00071-12 Study of Adenovirus Gene Functions
Heiner Westphal, M.D.

Z01-HD 01001-02 Gene Organization and Expression in Drosophila
Igor B. Dawid, Ph.D.

Z01 HD 01002-02 Gene Expression During Embryonic Development of Xenopus Laevis
Igor B. Dawid, Ph.D.

Z01 HD 01003-02 Cloning of cDNAs by Their Expression in Mammalian Cells
Hiroto Okayama, M.D., Ph.D.

Z01 HD 01004-01 Regulation of Amino Acid Biosynthetic Genes in Saccharomyces cerevisiae
Alan G. Hinnebusch, Ph.D.

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Laboratory of Molecular Genetics

During the past year the Laboratory of Molecular Genetics has seen a period of stabilization after the previous turnover of personnel. The Developmental Biology Section has expanded to include three separate research groups. Hiroto Okayama, having arrived in the Laboratory prior to this reporting period, has brought his laboratory to full activity, and Alan Hinnebusch has arrived and initiated a program in molecular genetics of yeast. With these additions the Laboratory of Molecular Genetics conducts research in a broad spectrum of systems from lambda phage to mice. We feel that certain aspects of the molecular and developmental genetics of eukaryotes can be studied particularly well in certain model systems, and we believe that the addition of projects using yeast, Drosophila, Xenopus and mice to the repertoire of experimental organisms used in the Laboratory of Molecular Genetics is an important step in the development of the Laboratory. At the same time the continued strength of research on bacteria and bacteriophage, and on mammalian cells in culture and animal viruses, assures further progress in directions that have been traditional within the Laboratory of Molecular Genetics.

On a more practical level the Laboratory of Molecular Genetics underwent some much-needed renovations of common equipment rooms, and replacement of some equipment that had reached the limit of its useful lifespan. Cell culture and dark room facilities were expanded somewhat to accommodate existing needs. With the projected renovation of two rooms in the near future the Laboratory of Molecular Genetics should have largely achieved a rejuvenation of its physical facilities, providing improved conditions for the execution of its research program.

Description of research program in the Laboratory of Molecular Genetics

The Laboratory is divided into five Sections and one Unit, which together accommodate the programs of nine independent research groups.

Developmental Biology Section

This section includes three research groups headed by Igor Dawid, Hiroto Okayama, and Alan Hinnebusch. Two research projects are carried out in Igor Dawid's group. Early development in the frog Xenopus laevis has been studied by the analysis of a group of genes which are expressed for the first time in the embryo in the late blastula and gastrula. This population of sequences has been isolated by a selective cDNA cloning procedure which yielded a library of copies of RNA molecules which are absent from the egg but are present in the gastrula embryo. These sequences have been named DG RNAs. In the past year several aspects of DG RNA structure and biology have been studied. A set of over 20 DG RNAs has been studied for their developmental accumulation pattern. DG RNAs arise first within an hour after the midblastula transition (MBT) and accumulate thereafter. Different patterns have been found for different RNAs: two examples were found that peak during gastrula and decrease rapidly after this, whereas several other DG RNAs peak at gastrula and neurula, followed by rapid decay during the next day of development.

Only two examples of DG RNAs were seen that continue to accumulate to the tadpole stage. In initial experiments studying spatial distribution of DG RNAs it was found that most of these RNAs appear to accumulate quite evenly in the embryo, but four cases of regional distribution have been observed.

More detailed studies are being carried out with DG 42, an RNA that shows strong developmental regulation. DG 42 cDNA and a homologous cDNA, DG 21, have been sequenced and shown to be 80% homologous, comprising a family of at least two expressed genes. The DG 42 sequence shows a long open reading frame which presumably encodes the gene 42 product. In collaboration with H-C. Chen and J. Morell a 17 amino acid peptide has been deduced from the cDNA sequence and synthesized by chemical methods. This peptide is being used in an attempt to produce antibodies against the parent protein.

Genomic clones encoding DG 42 have been isolated from a library of Xenopus DNA in lambda, and the coding regions have been mapped approximately. At present, DNA sequencing is being used to locate precisely the 5' end of the DG 42 gene, with the aim to identify and further study the control region of this gene which should be involved in the developmental regulation of its expression.

A project aiming to study the developmental regulation of expression of cell surface antigens in Xenopus embryos has been initiated. The rationale behind this work is the fact that gastrula and neurula development involve cell migration, recognition and adhesion, and the presence of distinct surface molecules at these stages may be expected. Polyclonal antibodies against dissociated fixed cells from gastrula and neurula embryos have been generated. These antibodies stain early frog embryos preferentially on their membranes; oocyte surfaces are also well stained, but tadpoles are negative with the exception of staining along the membranes of the gut. Staining of electrophoretically separated proteins (Western blotting) shows a series of bands in samples derived from gastrula and neurula embryos, but very few bands in samples from tadpoles or adult tissues. While preliminary, these experiments allow the conclusion that early embryos carry distinct surface molecules which are absent or rare on cells of later stages. Some of these embryo-specific surface molecules should be amenable to isolation and study. One approach to the analysis of discrete components is the generation of monoclonal antibodies. Several such antibodies have been produced in collaboration with Keiko Ozato, and are being analyzed. The production of additional monoclonals is projected.

The second project in Igor Dawid's group uses Drosophila melanogaster as the experimental organism. The major emphasis during this year was placed on the study of a female effect homeotic gene named fs(1)h. This gene, which is being studied in collaboration with M. Gans and F. Forquignon in Gif, France, is a maternal effect gene that leads, under certain conditions, to bithorax-like transformations of certain segments in the fly. The fs(1)h gene interacts with other homeotic genes on a different chromosome in producing these phenomena, specifically the bithorax complex itself and the gene named trithorax (trx). To allow a molecular study of the fs(1)h gene the region of the chromosome carrying it has been cloned by "chromosomal walking". A translocation from the X to the third chromosome, one insertion and one duplication provided three rearranged fs(1)h alleles that could be mapped onto the DNA. An analysis of transcripts from this region is underway, and so far has yielded two RNA molecules: an abundant 1.6 kb RNA that is most

likely not derived from the fs(1)h gene itself, and a 7 kb RNA that has some properties suggesting that it may be the desired gene product. Cloned cDNAs copied from these RNAs are currently being sought.

The research group headed by Hiroto Okayama is interested in the development and application of techniques for the production of full length cDNA copies of mammalian mRNAs and the reintroduction of such cDNAs into cells under conditions where their information content is expressed. Such methods should allow the selection and characterization of cDNAs and thereby mRNAs on the basis of their functional properties. Procedures for the synthesis of full length cDNAs have been established by Okayama during his earlier stay at Stanford University. More recently, he has developed a vector system which provides all the necessary components for functional expression of the cDNA in the cell. The system starts with the original Okayama/Berg vector, where full length cDNA is synthesized directly onto a plasmid vector which already contains, in the right orientation, a SV40 promoter, splice signals, and a polyadenylation site. The inserted cDNA is thus made in an expressible form. The entire vector with its cDNA is then inserted into a lambda vector which carries the neo gene as a dominant selective marker, and suitable restriction sites for insertion and later release of the insert. The phage is transferred into mammalian cells with the aid of calcium phosphate precipitation; whole phage is more efficient in such transfection than pure DNA. The cells are then subjected to selection with G418, so that only those cells that carry the neo gene will grow. This initial step is critical to select transfectants from the large background of unaffected cells. The transfectants are then analyzed for the presence of the desired cDNA. In control experiments a complete human cDNA library was introduced into $HGPRT^-$ cells, transfectants selected with G418, and subsequently placed under HAT selection. Two clones carrying human HGPRT cDNA were obtained in this way, showing that a rare mRNA can be cloned by this procedure provided that a selective method for its products is available.

The research group headed by Alan Hinnebusch is concerned with molecular mechanisms of gene regulation in yeast. Earlier work had shown that the genes encoding enzymes in histidine biosynthesis are highly suitable for such studies. Histidine and other amino acid production in yeast is co-regulated, and both cis and trans-acting regulatory elements are involved in this pathway. The study of such elements is the subject of this project. The minimal cis-acting regulatory element at one such gene, HIS4, has been identified by the construction of HIS4-CYC1 promoter fusions that place the CYC1 gene under general amino acid control. A synthetic 14 base pair fragment from the HIS4 promoter, containing a single copy of a short nucleotide sequence found repeated upstream from HIS4 and other co-regulated genes, is sufficient to confer the general control regulatory response.

Genetic analysis has revealed a hierarchy of control genes that affect HIS4 and other genes involved in amino acid synthesis in both a positive and negative way. There is evidence to suggest that the element directly interacting with the HIS4 gene is an activator. Molecular analysis of the GCN4 gene, which encodes a trans-acting positive regulator of general control, has shown that GCN4 expression is itself regulated by amino acid starvation and that this regulation is mediated by other general trans-acting factors. Moreover, GCN4 regulation occurs at the translational level and is exerted by sequences found in the 600 nucleotide 5' leader of its mRNA. This control region contains four small open reading frames

of 2-3 codons each. The repression exerted by these sequences appears to be mediated by the GCD1 product, a known repressor of amino acid biosynthetic enzymes. Repression is released during amino acid starvation by the GCN2 and GCN3 products, known activators of amino acid control and antagonists of GCD1. In vitro mutagenesis will now be used to identify more precisely the leader sequences controlling GCN4 translation, and in vivo mutant isolation will be conducted to identify trans-acting factors required for GCN4 translational repression. The GCN4 protein is the best candidate for the transcriptional activator that interacts with the repeated sites of positive control in the co-regulated structural genes. Isolation of the GCN4 protein will be undertaken to test this possibility in vitro.

This work should allow the study of a positive regulator of gene activity in an eukaryotic cell and the analysis of regulation of synthesis of the regulatory molecule itself.

Unit on Viral Gene Regulation

This unit, headed by Judith Levin, studies certain aspects of replication and gene expression of enveloped RNA viruses. Current interest is focused on the process of reverse transcription in an effort to correlate genetic structure with enzymatic function. The genetic defect in a non-conditional pol mutant has now been identified. This mutant produces a truncated reverse transcriptase with reduced levels of enzymatic activity. Using recombinant DNA technology, a molecular clone of the entire viral genome was obtained and the mutation was localized to a 400 base pair region near the middle of the pol gene. Sequence analysis of this region has demonstrated that the mutant genome contains a one-base insertion which brings three TGA codons into phase and results in premature termination of translation at a position consistent with the observed size of the mutant enzyme. Correlation of the precise location of the mutation with the known mutant phenotype has led to a map for the genetic organization of the MuLV pol gene and prediction of a virus-encoded protease at the 5' end of pol. In addition, the active sites for polymerase and RNase H can now be localized to the N-terminal domain of reverse transcriptase.

The mechanism of reverse transcription has been studied further, focusing on partial transcription products that arise during reverse transcription with wild type and particularly with mutant polymerase. These partial products are due to "pause site" which have been studied by computer-aided analysis of the viral RNA sequence, indicating the presence of consensus sequences and secondary structure features that correlate with pause sites. Pausing may thus be caused by certain features in the primary and secondary structure of the viral RNA.

Section on Molecular Regulation

The research group headed by Michael Cashel is engaged primarily in a study of the mechanism of coordinate regulation of cell metabolism in response to environmental stimuli, using E. coli and S. typhimurium as experimental organisms. The activities of guanosine 3',5'-bispyrophosphate (ppGpp) as a regulator of metabolism have been a major focus of this work. The primary and best-known effect of ppGpp in the cell is on the regulation of expression of ribosomal RNA. Two tandem promoters are responsible for rRNA transcription, and Cashel and his colleagues have

shown that the P1 promoter is inhibited by ppGpp and is induced upon reduction in the ppGpp concentration, whereas P2 is on constitutively. P1, studied separately from its surrounding regions, continues to show ppGpp sensitivity, but displays novel properties in its response to coumermycin. P2 also changes its properties when isolated from its surrounding sequences: unlike its behavior in its normal environment a "minimal" P2 is now sensitive to ppGpp. The conclusion is that the rRNA promoters interact with surrounding sequences in establishing the specific regulatory responses that are seen in vivo.

A second project concerned antitermination. rRNA antiterminates, i.e., it reads through otherwise efficient terminators. The ribosomal terminators themselves -- they are two in tandem -- are superefficient and do terminate rRNA transcripts. One possibility was that simply the presence of two terminators in tandem was the cause for their superefficiency. This possibility has been ruled out. It has further been shown that antitermination in the rRNA system is similar though distinct in mechanism to antitermination in bacteriophage lambda.

It is known that ppGpp is also involved in the regulation of expression of genes of amino acid synthesis pathways. Using the Salmonella his operon as a working model because it has been very well characterized genetically, a study was conducted on genes that control ppGpp metabolism. Many mutants have been isolated in the relA and spoT genes, which are loci involved in the inducible synthesis and in the degradation of ppGpp. Mutants in relS are being sought, a gene that is involved in the maintenance of basal levels of ppGpp. These studies should greatly aid in elucidating the genetic control of ppGpp metabolism, which in turn will help understand the multiple regulatory interactions that are mediated by ppGpp in the bacterial cell.

In a second project involving eukaryotic cells, M. Cashel and R. Sharma have focused on a cyclic nucleotide independent protein kinase from a rat adrenal cortical tumor. This enzyme occurs in normal adrenal cells as well, but it is elevated about 100 times in tumor tissue. The kinase phosphorylates only itself and a ribosomal protein, and is specific for serine residues. As an initial attempt to study the mechanism of amplification of kinase concentration and its possible relation to tumorigenesis and regulatory interactions in the adrenal tumor cells, Sharma and Cashel in collaboration with H. Okayama have initiated experiments to clone cDNA homologous to kinase mRNA. A cDNA library has been prepared and is being screened at this time. Further, the presence of kinase has been demonstrated in several tumor lines of different origin, but not in one fast growing but untransformed cultured cell line. The preliminary conclusion is therefore that the kinase may be a marker for transformation, and its amplification might have a deeper connection to tumorigenesis.

The second research group in this section is headed by Robert Crouch. This group is interested in RNA processing and the enzymes involved in RNA metabolism. Studies on both of these aspects have been proceeding, but the major emphasis during the past year has been placed on a detailed analysis of RNaseH. This enzyme degrades the RNA component in RNA/DNA hybrids only. It is distributed ubiquitously in all living forms studied, but until recently there had been no direct evidence that RNaseH was in fact a required activity. Crouch and his colleagues could show by a gene disruption method that the RNaseH gene in E. coli is required for normal

growth. Growth in RNaseH-negative cells is possible when two other enzymes, exo V and exo I, are present in normal amounts. Mutants in the RNaseH gene also interact with certain mutations in dnaA. These results suggest that a major role for RNaseH may be the generation of RNA primers during DNA replication.

Experiments aiming to reconstruct specific rRNA processing steps in vitro are the second direction pursued by R. Crouch. Suitable RNA substrates have been constructed by fusion of a portion of the chicken rRNA gene with the ribosomal promoter from the mouse. The construct can be expressed in vitro in a system from mouse cells that recognizes the rRNA promoter. The RNA produced in this way will be used in the search for an activity capable of in vitro processing.

Section on Molecular Structure

This section is headed by Jacob Maizel and is engaged in two related though distinct research directions. The major aim of the work is the development and application of computer-aided methods for the analysis and comparison of the sequences and structures of nucleic acids and proteins. This laboratory pioneered methods in this field, in particular the dot matrix approach of sequence comparison which has been very useful in the analysis and comparison of a variety of important genes. More recently, the major attention of the section has shifted to the prediction and analysis of secondary structures of RNA and also protein molecules. With the installation of a Vax computer in the Laboratory, much improved computing capabilities have been generated. Together with new and efficient methods it has been possible to fold RNA molecules of several thousand nucleotides and to generate predicted secondary structure models which are in good agreement with conclusions based on enzymatic and evolutionary data.

An important finding is the relatedness in sequence of the adenovirus E3 gene product to the major histocompatibility protein family. This fact is particularly intriguing since earlier work in this laboratory has shown that the E3 protein is a glycoprotein that is localized in the cell membrane.

Searches have also been undertaken to correlate DNA segments of known biological activity with a common structural feature. One example is provided by enhancer sequences, which do not show a consensus sequence but display a common feature in DNA structure that may be required for their activity.

The second major interest of this section concerns the analysis of sequence and biosynthetic activity of certain animal viruses. Rhinovirus, which is related to polio virus, has been analyzed by the cloning of most of the rhinovirus genome and by crosshybridization with the polio virus genome. In spite of their biological similarity the two viral genomes are quite distinct. Suitable cloned fragments are being readied for sequence analysis of the rhinovirus genome.

Section on Microbial Genetics

This section is headed by Robert Weisberg and is conducting research on genetic recombination between virus and host cell genomes. Much of the work employs bacteriophage λ as the experimental system. Weisberg and others have shown previously that recombination between λ and the E. coli chromosome involves specific regions called attachment sites. The phage and bacterial attachment

sites have a stretch of 7 homologous nucleotides, called the overlap region. Mutations in the phage overlap region, which are examples of site affinity (saf) mutations, have been obtained and shown to result in decreased recombination frequencies. However, compensatory mutations in the bacterial overlap region which restore complementarity, also restore wild type levels of recombination. These data provide strong evidence for the involvement of sequence complementarity in site-specific recombination. Other saf mutants which insert a nucleotide into the overlap region, also reduce recombination. It is thought that these changes interfere with the binding of proteins that catalyze recombination to the overlap region.

One of the proteins required for site-specific recombination, the integration host factor (IHF), consists of two subunits. The β subunit is encoded by the E. coli hip gene; this gene has been cloned and sequenced. The protein proved to be related to a set of basic bacterial proteins thought to be DNA binding proteins and having histone-like properties. An overproducing strain has been constructed which will allow more detailed analysis of the properties of the hip gene product.

The study of the phage attachment site has also been pursued in collaboration with Dr. Nussinov at the level of an analysis of computer-predicted secondary structure. A correlation of patterns of twist angle deviation in the DNA of attachment sites has been detected, suggesting that such sites may be characterized by specific structural properties.

A distinct though related project involved the analysis of endonuclease I of bacteriophage T7. This enzyme has been shown to cleave specifically branched DNA structures in vitro. Genetic data with phage T4 have suggested that the analogous enzyme in this phage is involved in recombination; mutants accumulate branched DNA structures, which are believed to be intermediates in genetic recombination. These results form the basis for further genetic and biochemical analyses of the function of this type of enzyme in recombination.

Section on Animal Viruses

This section which is headed by Heiner Westphal, is engaged in two distinct though related research projects. The first project concerns gene regulation during virus infection in mammalian cells. The study concentrates on regulatory events during the early phase of adenovirus infection. The product of the Ela gene is thought to be an activator of several other genes. In collaboration with M. Rosenberg the product of the 13S mRNA of the Ela gene has been generated by expression in E. coli. The purified protein has been injected into mammalian cells to study its function. The injected Ela protein activates the E2a gene, and is able to complement an Ela deletion mutant virus, allowing expression of the late region. Further, the "artificial" Ela product localized in the cell nucleus and is heat stable. Thus, in all properties so far tested the bacterially produced Ela protein is fully functional.

Interactions between different early adenovirus genes have been studied with respect to the AAV helper effect. AAV is a defective virus that requires adenovirus functions for growth. The helper effect was studied by injection of different early adenovirus genes or mRNAs derived from them into AAV-infected cells. The

results show that genes E2a and E4 are sufficient to allow AAV growth when injected at high levels. At lower levels these two genes are not sufficient but require in addition Ela or Elb. It is thought that Ela activates E2a and E4, but the role of Elb in this phenomenon is less clear. These studies continue to illuminate the complex interactions of early adenovirus genes in virus expression.

The second project in this section concerns gene regulation in the mouse. DNA-mediated gene transfer into fertilized mouse eggs is used as the major approach, aiming to introduce and stably integrate genes into mice and to study the regulation of gene activity during development. A variety of technical difficulties in this approach have been overcome, leading to the successful production of several mice carrying the injected DNA in their cells. Several DNA molecules have been injected. The Drosophila P element, a transposon which integrates with high efficiency into the fly genome, has been injected and three mice carrying the element have been obtained. Integration occured at sites other than the termini of the transposon, suggesting that integration was not the result of specific transposition. Other DNA molecules introduced into the mouse egg represent constructs in which an eukaryotic promoter has been fused to a suitable detector gene, usually the bacterial chloramphenicol acetyltransferase (Cat) gene. Such constructs, containing the Rous sarcoma virus LTR and a collagen promoter, have been introduced successfully into mice. When some of these transgenic mice can be shown to express their newly-acquired genetic information, important avenues to the study of certain aspects of gene regulation during development will become accessible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00066-14 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control Mechanisms in Temperature Bacteriophage λ

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

PI:	Robert A. Weisberg	Head	LMG, NICHD
Others:	Eric Flamm	Staff Fellow	LMG, NICHD
	Lazslo Dorgai	Visiting Fellow	LMG, NICHD
	Bernard De Massy	Visiting Fellow	LMG, NICHD
	Janine Robert	Visiting Scientist	LMG, NICHD

COOPERATING UNITS *(if any)* Laboratory of Molecular Biology, NCI (Dr. Max Gottesman); Section on Molecular Structure, LMG, NICHD (Dr. Ruth Nussinov); Max Planck Institut, Berlin, West Germany (Dr. Krysztof Appelt).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Microbial Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3.3	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 long term goal of this project is to study mechanisms of genetic recombination, in particular the mechanism of recombination of virus DNA with the host chromosome (site-specific-recombination). Recombination between bacteriophage λ and its host Escherichia coli is effected by a pair of reciprocal strand exchanges between specialized regions in each DNA called attachment sites. *Int* and IHF proteins specifically promote these exchanges. We have isolated and studied the phenotype of mutants of a central region of the λ attachment site. Certain mutations in this region disrupt homologous pairing between sites, while others disrupt protein interaction with the sites. We have isolated a plasmid that overproduces one of the peptides required for site specific recombination. Analysis of the structure of the attachment sites suggests that *int* protein recognizes a particular pattern of helical twist angle deviations in addition to a specific nucleotide sequence.

We are also characterizing enzymes that promote homologous recombination. We find that endonuclease I of bacteriophage T7 cleaves Holliday structures (branched recombinational intermediates that arise by a reciprocal single-strand exchange). Cleavage occurs by nicking at the branch point.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00067-16 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI:	C. Michael Cashel	Head	LMG, NICHD
Others:	Kenneth E. Rudd	Staff Fellow	LMG, NICHD
	Alan Rauch	Clinical Associate	LMG, NICHD
	E. G. Sarubbi	Visiting Fellow	LMG, NICHD
	Ramesh Sharma	IPA	LMG, NICHD

COOPERATING UNITS *(if any)*
Medical School II, Naples, Italy (Dr. Gianni Chinali); Hadassah Medical School, Jerusalem, Israel (Dr. Gad Glaser); Section on Developmental Biology, Laboratory of Molecular Genetics, NICHD, NIH (Dr. Hiroto Okayama).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.5	3.5	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 overall goal of this project is to understand how a cell coordinates the global expression of its genetic repertoire in response to environmental stimuli. Using bacteria (E. coli and S. typhimurium) as models, we have focused on events regulated by an intracellular hormone-like compound guanosine 3',5'-bispyrophosphate (ppGpp). We have studied both positive and negative regulation of operons regulated by ppGpp (histidine operon and ribosomal RNA operon). As a model for eukaryotic cells, we have initiated studies with a rat adrenal cortical carcinoma cell line that retains partial glucocorticoid hormone responsiveness to adrenal corticotropic hormone (ACTH). Our major findings are:

1. The regions surrounding ribosomal RNA tandem promoters, distinct from the promoters themselves, play significant roles in regulating the differential expression of promoters.

2. Anti-termination of ribosomal RNA operon transcripts has been shown analogous to phage lambda anti-termination both structurally and functionally yet the mechanisms are distinguished by lambda N gene dependence.

3. The dependence of his operon expression on ppGpp has been characterized genetically and has been exploited to yield mutants in ribosome dependent ppGpp synthesis (relA), in ppGpp degradation (spoT), and in more interesting but as yet uncharacterized new loci.

4. Messenger RNA from both normal rat adrenals and an adrenal tumor cell line has been isolated and translated in vitro. The tumor cell line has two unusually abundant ethidium staining RNA regions at about 2 Kb and about 6.5 Kb. A cDNA library has been prepared from the tumor cell line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00068-13 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Influencing Genetic 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:	M. Itaya	Visiting Fellow	LMG, NICHD
	D. Drakeford	Biologist	LMG, NICHD
	C. Chambers	Biologist	LMG, NICHD
	R. Seelke	Microbiologist	LMG, NICHD

COOPERATING UNITS *(if any)*

University of Wurzburg, Biochemistry
Wurzburg, Germany (I. Grummt)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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.)*

RNA plays an important role in cellular regulation -- either by its presence in active form or by its total absence. It has been known for several years that transcription of DNA does not necessarily lead to productive, mature RNA molecules. Cleavage of these RNA molecules often is required for the RNA molecules to mature or to act as an intermediate in other processes (e.g., priming of DNA replication). These cleavage events are a subset of a general maturation pathway known as RNA processing. Work of this Intramural Research Project is concerned with two types of RNA processing, generation of RNA primers for DNA replication and ribosomal RNA processing in higher eukaryotes. We have demonstrated that the requirement for ribonuclease H for cell growth can be supplanted by genes thought to be normally involved in recombination. These results suggest that RNaseH is required for normal DNA replication but a second, poor pathway for DNA replication occurs via recombinagenic activity. Ribosomal RNA processing has been studied by studying an enzyme which we previously described and implicated as being involved in rRNA processing. A small nucleolar RNA (U3) seems to be easily separated from the ribonuclease on DEAE column chromatography. In vitro transcription systems from mouse have been used to synthesize hybrid chick-mouse rRNA. To date, no in vitro processing has been observed in this system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00069-12 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Aspects of the Replication of Enveloped Animal RNA Viruses

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:	Stella Hu	Chemist	LMG, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Human Carcinogenesis, NCI (Brenda Gerwin); Basic Research Program, LBI, NCI - FCRF (Alan Rein); Section on Molecular Structure, LMG, NICHD (Jacob Maizel and Kathleen Currey); NCI - FCRF (Don Court).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Unit on Viral Gene Regulation (Developmental Biology Section)

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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

The goal of this project is to define the molecular mechanisms involved in the replication of enveloped RNA viruses 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 process of reverse transcription in an effort to correlate genetic structure with enzymatic function. The genetic defect in a non-conditional pol mutant has now been identified. This mutant produces a truncated reverse transcriptase with reduced levels of enzymatic activity. Using recombinant DNA technology, a molecular clone of the entire viral genome was obtained and the mutation was localized to a 400 base pair region near the middle of the pol gene. Sequence analysis of this region has demonstrated that the mutant genome contains a one-base insertion which brings three TGA codons into phase and results in premature termination of translation at a position consistent with the observed size of the mutant enzyme. Correlation of the precise location of the mutation with the known mutant phenotype has led to a map for the genetic organization of the MuLV pol gene and prediction of a virus-encoded protease at the 5' end of pol. In addition, the active sites for polymerase and RNase H can now be localized to the N-terminal domain of reverse transcriptase. Studies on "enzyme pausing" in endogenous MuLV reverse transcription have continued. Computer analysis of intermediate bands indicates that the location of pause sites correlates with the presence of a set of C-rich consensus sequences clustered within predicted multibranch loop structures which can be formed by the viral RNA template. Attempts to express the MuLV pol gene in E. coli bacteria are also in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00070-24 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Morphogenesis of Animal Viruses During Infection of Mammalian Cells

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

PI:	Jacob V. Maizel, Jr.	Head	LMG, NICHD
Others:	Charles McLean	Staff Fellow	LMG, NICHD
	Kathleen Currey	Medical Staff Fellow	LMG, NICHD
	John Owens	Chemist	LMG, NICHD
	Devjani Chatterjee	Visiting Fellow	LMG, NICHD
	Ruth Nussinov	Visiting Associate	LMG, NICHD

COOPERATING UNITS *(if any)*
DCBC, NCI (L. Lipkin and B. Shapiro); VB, NCI (G. Vande Woude); LMB, NEI (J. Piatigorsky); LMV, NCI (R. Dhar).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Structure

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
4.8	3.8	1.0

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

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

Techniques of biochemistry, virology, electron microscopy and computer analysis are used to study picornaviruses and adenoviruses. Analyses of proteins and nucleic acids have been developed and implemented. Graphic representations revealing homology, and reverse complementarity are coupled with numerical methods to aid the prediction of secondary structure, splicing, promotors, and recombination in nucleic acid molecules. Programs are developed and installed in a VAX 11/750 system designed for sequence analysis. Structures of up to 2000 bases have been predicted. Methods to assess the significance of predictions use Monte Carlo simulations, evolutionary comparisons and biochemical data. Protein secondary structure is being predicted from amino acid sequences. New sequences are compared with computerized databases to detect relationships with known proteins

Picornaviruses cause diseases typified by polio, colds, hepatitis, and foot-and-mouth disease. c-DNA clones of rhinovirus having approximately 6000 (of 7000) bases have been isolated and mapped by restriction digests. Sequences are being determined on subclones and are being compared with those of poliovirus and other picornaviruses to determine relationships and to predict properties.

Adenoviruses are studied with a goal to understanding early events in replication wherein the cell's metabolism is subverted to viral functions, and late events during which assembly and morphogenesis occurs. Computer analysis of sequences is used to find the structural and functional relationships of intracellular and virion structural nucleic acids and proteins between adenoviruses and their hosts as well as to known proteins from other species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00071-12 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Study of Adenovirus Gene Functions

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

PI:	H. Westphal	Head	LMG, NICHD
Others:	J. Khillan	Visiting Fellow	LMG, NICHD
	P. Overbeek	Staff Fellow	LMG, NICHD
	B. Krippl	Visiting Fellow	LMG, NICHD
	K. Mahon	Staff Fellow	LMG, NICHD
	S. Lai	Chemist	LMG, NICHD
	R. Esherick	Biological Laboratory Technician	LMG, NICHD

COOPERATING UNITS *(if any)* NCI, NIH (B. de Crombrugghe and D. Hamer); Free University, West Berlin (A. Graessmann); NIAID, NIH (A. M. Lewis); NICHD, NIH (K. Ozato); Biocenter, Uppsala, Sweden (U. Pettersson); NEI, NIH (J. Piatigorsky); SKF Laboratories, Philadelphia, Pa. (M. Rosenberg); FCRF, Frederick, MD. (G. vande Woude).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Animal Viruses

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.3	4.3	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Our laboratory investigates mechanisms of gene control in mammalian cells and embryos. One of two major projects deals with regulatory functions of adenovirus. Focal point of these studies is the E1a gene which acts as a transcriptional activator and is involved in malignant transformation. Sequences encoding E1a proteins or certain domains of these proteins have been inserted in prokaryotic expression vectors, and E1a proteins have been produced in E. coli. We have begun to microinject these E1a proteins into mammalian cells and to test their activity. This experimentation will enable us to arrive at a functional anatomy of this important set of eukaryotic regulatory factors. In a second project we analyze patterns of integration and expression of selected gene constructs transferred into mouse embryos. We have begun to insert a number of gene constructs, including the P element transposon or the chloramphenicol transferase gene placed under the control of retroviral promoters or a collagen Ia2 promoter, and we have developed new methods to test tissue specificity and developmental timing of expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01001-02 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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. B. Dawid	Head	LMG, NICHD
Others:	M. E. Digan	Staff Fellow	LMG, NICHD
	S. Haynes	Staff Fellow	LMG, NICHD
	M. Rebbert	Chemist	LMG, NICHD
	B. Mozer	Biologist	LMG, NICHD

COOPERATING UNITS *(if any)*
Centre Genetique Moleculaire, CNRS, Gif-sur-Yvette, France (M. Gans and F. Forquignon); Internat. Inst. Biophys, Naples, Italy (P. P. Di Nocera).

LAB/BRANCH
Laboratory of Molecular Genetics, NICHD

SECTION
Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Previous work from this laboratory has shown that about half the ribosomal RNA (rRNA) genes in Drosophila are interrupted, and that these interrupted genes are inactive, i.e., they are pseudogenes. The molecular basis for the inactivity of these genes is being studied with the aid of a transient expression system using Drosophila cells transfected with rDNA minigenes. Several minigene constructs have been and are being prepared in which the rDNA promoter is placed close to the point of interruption of the rRNA coding sequence by different insertions. Some such constructs initiate transcription at the normal 5' end of rRNA after introduction into cultured cells.

The maternal effect developmental gene fs(1)h is being studied in collaboration with Drs. Gans and Forquignon in Gif. This gene is known to lead to homeotic transformations under certain conditions, and provides an example of a maternal effect gene apparently involved in the specification of body plan. A chromosomal walk across the region containing the fs(1)h gene has been carried out. The gene has been located by mapping three mutations in fs(1)h onto the DNA. The mutations include a reciprocal translocation to the third chromosome, a duplication of several kb of wild-type DNA, and the insertion of a transposable element. These three mutations map within a region of about 5kb. A second gene, lethal(1) myospheroid, has been located within the isolated stretch of DNA. Transcription mapping of the relevant regions is proceeding, and evidence for two transcripts from the region has been obtained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01002-02 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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	LMG, NICHD
Others:	S. Miyatani	Visiting Fellow	LMG, NICHD
	T. Sargent	Staff Fellow	LMG, NICHD
	J. Winkles	Staff Fellow	LMG, NICHD
	M. Jamrich	Visiting Associate	LMG, NICHD
	B. Kay	Staff Fellow	LMG, NICHD
	E. Jonas	Visiting Fellow	LMG, NICHD

COOPERATING UNITS *(if any)* ERRB, NICHD, NIH (H-C. Chen and J. L. Morell)
LDMI, NICHD, NIH (K. Ozato)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.2	5.2	0

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

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

Xenopus laevis is being studied as a vertebrate animal that offers certain advantages for the analysis of molecular events during development. The major approach currently underway is based on the preparation and analysis of an enriched cDNA library which represents those RNA molecules that are present in gastrulae but absent from the egg. Genes differentially expressed in gastrula (DG genes) are being used as a source of molecular markers of early development.

A detailed analysis of accumulation of over 20 DG RNAs during development has been carried out, showing that these RNAs begin to accumulate at a distinct but closely spaced times in late blastula to early gastrula. Most RNAs decrease in concentration during or shortly after neurula. By dissection of neurula embryos the distribution of these DG RNAs has been tested. Most DG RNAs appear evenly distributed throughout the neurula, but four cases have been found which show substantial enrichment in the ventral or the posterior regions of the embryo. In situ hybridization is being used to study the distribution of DG RNAs in further detail.

DG 42 represents a messenger RNA present only in gastrula/neurula stages, and the cDNA clone has been sequenced. A related cDNA clone, DG 21, has the same developmental profile but shares only 80% sequence homology with DG 42. Genomic clones encoding DG 42 and DG 21 have been isolated and mapped, and are currently being subjected to sequence analysis.

Experiments are in progress to express portions of DG cDNAs in bacteria with the aim to produce polypeptides that will be used to produce antibodies against natural DG proteins. Peptides corresponding to parts of the predicted DG 42 protein sequence are being synthesized in collaboration with H-C. Chen and J. Morell, and will also be used for antibody production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01003-02 LMG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cloning of cDNAs by Their Expression in Mammalian Cells

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

PI:	H. Okayama	Visiting Scientist	LMG, NICHD
Others:	M. Kawaichi	Visiting Associate	LMG, NICHD
	C. Chen	Biologist	LMG, NICHD

COOPERATING UNITS *(if any)*

Department of Biochemistry, Stanford University Medical School, Palo Alto, Calif. (Paul Berg).

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.6	1.6	1

CHECK APPROPRIATE BOX(ES)

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

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

As one of the key vectors for cloning cDNAs based on the function they express in mammalian cells, we have developed a lambda phage vector that permits efficient transfection of cultured mammalian cells with a cDNA clone library constructed with the pcD expression vector (Okayama, H., and Berg, P., Mol. Cell. Biol. 3, 280-289). The phage vector contains a bacterial neo gene under the control of the SV40 early region promoter and SV40 RNA processing signals, as a dominant-acting mammalian selectable marker gene, and is capable of accepting a pcD-recombinant with a maximum 9 kb cDNA insert. The recombinant phage particles are directly transfected into cultured cells after co-precipitation with calcium phosphate.

Reconstitution experiments indicate that the vector is able to transduce a phenotype of a particular cDNA into one of 10^6 cells transfected with a library containing one functional clone of the cDNA every 10^5 clones. In confirming this efficiency, transfection of 10^7 hypoxanthine-guanine phosphoribosyl transferase (HPRT)-deficient mouse L cells with a SV40-transformed human fibroblast cDNA library transferred into the phage vector yielded two HPRT-positive transformants that contain the diagnostic human HPRT cDNA sequences and express active human HPRT enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01004-01 LMG

PERIOD COVERED: October 1, 1983 to September 30, 1984

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	Senior Staff Fellow	LMG, NICHD
Others:	Peter Muller	Visiting Fellow	LMG, NICHD
	Alice Ma	Biologist	LMG, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Molecular Genetics

SECTION: Section on Developmental Biology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.8	1	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The goal of this project is to identify the cis and trans-acting elements involved in the coordinate transcriptional regulation of a large number of unlinked genes encoding amino acid biosynthetic enzymes in the yeast Saccharomyces cerevisiae. The minimal cis-acting regulatory element at one such gene, HIS4, has been identified by the construction of HIS4-CYC1 promoter fusions that place the CYC1 gene under general amino acid control. A synthetic 14 base pair fragment from the HIS4 promoter, containing a single copy of a short nucleotide sequence found repeated upstream from HIS4 and other co-regulated genes, is sufficient to confer the general control regulatory response. Molecular analysis of the GCN4 gene, which encodes a trans-acting positive regulator of general control, has shown that GCN4 expression is itself regulated by amino acid starvation and that this regulation is mediated by other general control trans-acting factors. Moveover, GCN4 regulation occurs at the translational level and is exerted by sequences found in the 600 nucleotide 5' leader of its mRNA. The leader control region contains four small open-reading frames of 2-3 codons each. The repression exerted by these sequences appears to be mediated by the GCD1 product, a known repressor of amino acid biosynthetic enzymes. Repression is released during amino acid starvation by the GCN2 and GCN3 products, known activators of amino acid control and antagonists of GCD1. In vitro mutagenesis will now be used to identify more precisely the leader sequences controlling GCN4 translation, and in vivo mutant isolation will be conducted to identify trans-acting factors required for GCN4 translational repression. The GCN4 protein is the best candidate for the transcriptional activator that interacts with the repeated sites of positive control in the co-regulated structural genes. Isolation of the GCN4 protein will be undertaken to test this possibility in vitro.

LABORATORY OF DEVELOPMENTAL PHARMACOLOGY

Z01 HD 00136-16 Pharmacogenetics
Daniel W. Nebert, M.D.

Z01 HD 00137-10 Genetic Regulation of Drug-Conjugating Enzymes
Ida S. Owens, Ph.D.

Z01 HD 00500-06 Receptor Structure and Function
Howard J. Eisen, M.D.

Annual Report of the Laboratory of Developmental Pharmacology
National Institute of Child Health and Human Development
October 1, 1983 through September 30, 1984

SUMMARY

When a human population receives the same dose of any drug, alcoholic beverage, coffee, cigarette smoke, or any other foreign chemical, there will be individual differences in intensity and duration of action of these agents. Both the duration of action and intensity of this response to foreign chemicals depend upon the level of detoxification enzymes in all tissues of the body, most notably the liver. The LABORATORY OF DEVELOPMENTAL PHARMACOLOGY studies the molecular mechanisms of gene expression involving these drug-metabolizing enzymes, a discipline that has been termed pharmacogenetics. Endogenous (constitutive) enzymes that metabolize steroids, fatty acids, prostaglandins, leukotrienes, pheromones, thyroxine and biogenic amines also appear to metabolize the thousands of foreign chemicals that enter our body. Hundreds of drugs are known to stimulate (induce) their own metabolism or the metabolic fate of structurally-related compounds. Steroids, prostaglandins, and small peptide hormones are known to regulate some of these activities. The mechanisms surrounding the induction of these enzymes and expression of these genes are relevant to fundamental molecular genetics, developmental biology, teratogenesis, carcinogenesis, mutagenesis, endocrinology, and drug addiction, tolerance and toxicity. This Laboratory presently comprises three Sections.

A. The Section on Pharmacogenetics and Molecular Teratology, under the direction of Daniel W. Nebert, M.D., is interested in the principle class of Phase I drug-metabolizing enzymes, called "cytochrome P-450." Subsets of this class include at least three P-450 gene families inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; called in the lay press "dioxin"), phenobarbital, and steroids, respectively. Presumably the induction process for all three gene families is governed by receptors. The TCDD-inducible P-450 gene family is controlled by the Ah receptor, and the entire genetic system is referred to as the Ah locus (aromatic hydrocarbon responsiveness). This laboratory has worked principally on the TCDD-inducible P-450 gene family among inbred mouse strains and tissue culture lines. This gene family is composed of two major genes, P_1-450 and P_3-450. The P_1-450 and P_3-450 proteins were purified from 3-methylcholanthrene-treated C57BL/6N mice, and polyclonal antibodies were developed. These antibodies were used for polysome immunoadsorption to purify the corresponding messenger RNAs. By means of the Okayama-Berg plasmid vector, full-length cDNA clones were isolated and sequenced. A genomic-DNA library from C57BL/6N liver was also constructed. The P_1-450 and P_3-450 genes were isolated and sequenced, including all six introns and more than 1000 base pairs in both the 5' and 3' flanking regions. The $P\text{-}450_{Coh}$ protein, responsible for an inbred mouse polymorphism involving coumarin metabolism, was purified and a specific antibody was developed by Lang and coworkers. This antibody will be used to study the mouse phenobarbital-inducible P-450 gene family. The $P\text{-}450_{PCN}$ protein, inducible by steroids such as pregnenolone-16α-carbonitrile, has been purified and a specific antibody was developed by Hardwick, Gonzalez, and Kasper. By means of polysome immunoadsorption and the Okayama-Berg cloning vector, a full-length cDNA clone was isolated in Kasper's laboratory and sequenced in this laboratory. The protein sequences, deduced from the nucleotide sequences, allow us to conclude that the TCDD-inducible and phenobarbital-inducible P-450 gene families diverged from

a common ancestral gene more than 200 million years ago and that the homologous P_1-450 and P_3-450 genes separated from each other at least 65 million years ago. Human P_1-450 cDNA and genomic clones have also been isolated and sequenced. We hope to develop an assay, based on recombinant DNA technology, to assess the human Ah phenotype. Such an assay may predict who is at increased risk for certain types of environmentally-caused birth defects, cancers, and toxicity.

B. The Section on Regulation of Gene Expression, under the supervision of Howard J. Eisen, M.D., compares the mechanism of action of the glucocorticoid receptor and the Ah receptor. Major emphasis is placed on purification of the Ah receptor, development of anti-receptor antibodies, and use of somatic-cell genetics to isolate variants defective in the induction of cytochrome P_1-450. It is hoped that these studies will lead directly to the use of recombinant-DNA methods to clone the gene(s) for the Ah receptor. The glucocorticoid receptor has been purified with a monoclonal anti-receptor antibody for the purposes of peptide mapping and sequencing. During the past year, we have developed a rapid, high-resolution anion-exchange HPLC procedure for assay and partial purification of the Ah receptor. We have isolated and characterized several new variant clones of the mouse hepatoma cell line Hepa-1. These mutants include clones with decreased Ah receptor content ("r^-" phenotype) and clones with apparent "post-receptor" defects. These benzo[a]pyrene-resistant cloned lines will be useful for "rescue" of Ah receptor genes by DNA-transfection experiments. We have shown that certain human cultured cell lines are much less sensitive to TCDD than the mouse hepatoma Hepa-1; these cells are defective in specific TCDD binding and may provide an important new genetic model for study of the Ah receptor. Because there are TCDD-inducible and steroid-inducible gene families, isolation of these receptor proteins will provide appropriate "substrates" for direct analysis of receptor interaction with cloned DNA.

C. The Section on Drug Biotransformation, under the direction of Ida S. Owens, Ph.D., studies the regulation of UDP glucuronosyltransferase(s), one of the major classes of Phase II drug-metabolizing enzymes. These transferases catalyze the conjugation of many potentially toxic exogenous, as well as endogenous, compounds to glucuronic acid. Inbred and heterogeneous stock mice and congenic inbred jaundiced and normal rats have been used to understand the regulation of this interesting gene family. This enzyme system uses fat-soluble substrates converted to oxygenated products by the phase I cytochrome P-450-dependent monooxygenase system. The net result is that the initial highly fat-soluble chemical has been transformed to a highly water-soluble (usually innocuous) metabolite that is more readily excreted by the organism. A transferase protein with a low pI was purified from phenobarbital-treated C57BL/6N mice, and antibodies were developed. The antibody immunoprecipitates at least two transferase proteins from mice, i.e. a $M_r \cong 51{,}000$ form corresponding to the antigen and a $M_r \cong 54{,}000$ form. The 51,000-dalton protein was shown to undergo cleavage and glycosylation unlike the 54,000-dalton form. Immuno-enrichment of mouse mRNA has led to the isolation of a mouse transferase cDNA which encodes a 51,000-dalton constitutive protein and recognizes two messenger RNAs (1900 and 2200 nucleotides, respectively). The antibody to the mouse transferase cross-reacts with the rat and precipitates three rat transferase proteins (49,000 to 52,000 daltons) from control and phenobarbital-treated animals and four transferase proteins (ranging from 51,000 to 57,000 daltons) from 3-methylcholanthrene-treated rat liver microsomes. After immuno-enrichment of rat liver mRNA from nascent polypeptide, three rat transferase clones were isolated from a cDNA library. Two clones (2000 and 2300 base pairs) code for two distinct constitutive transferase proteins of the

same size ($M_r \cong 52,000$) and one clone encodes for a 52,000-dalton phenobarbital-inducible form. Each of the three clones recognizes distinct mRNAs with lengths of about 2300 nucleotides. At least one of the rat transferase proteins undergoes cleavage to lose a peptide of approximately 2,000 daltons, but there is no evidence of glycosylation. Mouse transferase cDNA and genomic clones associated with the Ah locus (aromatic hydrocarbon-inducible), as well as clones of the phenobarbital- and steroid-inducible types, are currently being isolated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00136-16 LDP

PERIOD COVERED
October 1, 1983 to September 30, 1984

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 and Molecular Teratology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	2.25	3.75

CHECK APPROPRIATE BOX(ES)

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

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

The principle class of Phase I drug-metabolizing enzymes is called "cytochrome P-450." Subsets of this class include at least three P-450 gene families inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; called in the lay press "dioxin"), phenobarbital, and steroids, respectively. Presumably the induction process for all three gene families is governed by receptors. The TCDD-inducible P-450 gene family is controlled by the Ah receptor, and the entire genetic system is referred to as the Ah locus (aromatic hydrocarbon responsiveness). This laboratory has worked principally on the TCDD-inducible P-450 gene family among inbred mouse strains and tissue culture lines. This gene family is composed of two major genes, P_1-450 and P_3-450. The P_1-450 and P_3-450 proteins were purified from 3-methylcholanthrene-treated C57BL/6N mice, and polyclonal antibodies were developed. These antibodies were used for polysome immunoadsorption to purify the corresponding messenger RNAs. By means of the Okayama-Berg plasmid vector, full-length cDNA clones were isolated and sequenced. A genomic-DNA library from C57BL/6N liver was also constructed. The P_1-450 and P_3-450 genes were isolated and sequenced, including all six introns and more than 1000 base pairs in both the 5' and 3' flanking regions. The protein sequences, deduced from the nucleotide sequences, allow us to conclude that the TCDD-inducible and phenobarbital-inducible P-450 gene families diverged from a common ancestral gene more than 200 million years ago and that the homologous P_1-450 and P_3-450 genes separated from each other at least 65 million years ago. Human P_1-450 cDNA and genomic clones are also being isolated and sequenced. We hope to develop an assay, based on recombinant DNA technology, to assess the human Ah phenotype. Such an assay may predict who is at increased risk for certain types of environmentally-caused birth defects, cancers, and toxicity.

ATTACHMENT I - Others:

Sanford W. Bigelow	Chemist	LDP	NICHD
Jia-Huan Ding	Guest Researcher	LDP	NICHD
Frank J. Gonzalez	Staff Fellow	LDP	NICHD
Larry L. Heilmann	Jr. Staff Fellow	LDP	NICHD
Wayne E. Jackson	Stay-In-Schooler	LDP	NICHD
Shioko Kimura	Visiting Fellow	LDP	NICHD
Krisit L. Kotz	Junior Fellow	LDP	NICHD
Raul A. Lazarte	Guest Researcher	LDP	NICHD
Hugh A. Privette	Biological Aide Tech.	LDP	NICHD
John A. Robertson	Visiting Fellow	LDP	NICHD
Mary Lynn Sienkiewicz	Biologist (Tech)	LDP	NICHD

ATTACHMENT II - COOPERATING UNITS:

A. C. Collins, Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado 80309

H. J. Eisen, Section on Regulation of Gene Expression, Laboratory of Developmental Pharmacology, NICHD, NIH, Bethesda, Maryland 20205

J. E. Gielen, Laboratoire de Chimie Medicale, Institut de Pathologie, Unite de Biochimie, University of Liege, Belgium

O. Hankinson, Department of Pathology, Laboratory of Biomedical & Environmental Sciences, UCLA, 900 Veteran Avenue, Los Angeles, California 90024

M. E. Harper, Agouron Institute, La Jolla, California 92037

D. E. Harrison, The Jackson Laboratory, Bar Harbor, Maine 04609

H. Kon, Laboratory of Chemical Physics, NIADDKD, NIH, Bethesda, Maryland 20205

R. E. Kouri, Department of Biochemical Oncology, Microbiological Associates, 5221 River Road, Bethesda, Maryland 20016

C. Kozak, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, Maryland 20205

P. Lalley, Oak Ridge National Laboratory, Box Y, Oak Ridge, Tennessee 37830

M. A. Lang, Department of Toxicology, University of Kuopio, SF-70101 Kuopio 10, Finland

A. S. Levine, Office of the Scientific Director, NICHD, NIH, Bethesda, Maryland 20205

D. Lovell, The British Biological Research Association, Woodmansterne Road, Carshalton, Surrey, SM5 4DS, Great Britain

A. M. Malkinson, School of Pharmacy, University of Colorado, Boulder, Colorado 80309

I. S. Owens, Section on Drug Biotransformation, Laboratory of Developmental Pharmacology, NICHD, NIH, Bethesda, Maryland 20205

F. Ruscetti, Building C-327, 5516 Nicholson Lane, Litton Bionetics, Kensington, Maryland 20795

H. Shichi, Institute of Biological Sciences, Oakland University, Rochester, Michigan 48063

E. W. Vogel, Department of Radiation Genetics & Chemical Mutagenesis, State University of Leiden, Wassenaarseweg 72, 2333 Al Leiden, The Netherlands

J. E. Womack, Department of Veterinary Pathology, Texas A & M University, College Station, Texas 77843

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00137-10 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

GENETIC REGULATION OF DRUG-CONJUGATING ENZYMES

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

PI:	I. S. Owens	Head	LDP, NICHD
Others:	P. I. Mackenzie	Visiting Associate	LDP, NICHD
	A. Karkowsky	Medical Staff Fellow	LDP, NICHD

COOPERATING UNITS *(if any)*

D.W. Nebert & coworkers, Section on Pharmacogenetics and Molecular Teratology LDP:NICHD:NIH

LAB/BRANCH

Laboratory of Developmental Pharmacology

SECTION

Section on Drug Biotransformation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

The regulation of the family of UDP glucuronosyltransferase enzymes is being studied by means of DNA, RNA and protein chemistry. The transferase system inducible by phenobarbital-like and polycyclic hydrocarbon-like compounds is essential for detoxification via glucuronidation of many lipophilic foreign and endogenous compounds. Antibodies raised against a purified mouse transferase ($M_r \cong 51,000$) were used to immuno-enrich transferase mRNA from mouse and rat liver. Recombinant cDNA clones (three from rat and one from mouse) were developed in the plasmid pBR322 from transferase mRNA and are undergoing characterization. Messenger RNA complementary to the rat cDNA clone $pUDPGT_R$-2 (2000 bp) is inducible by phenobarbital and specifies a 52,000-dalton protein. Messenger RNAs complementary to the other two rat transferase clones ($pUDPGT_R$-1 and $pUDPGT_R$-3) are constitutive, and each encodes a 52,000-dalton polypeptide. Each of the rat cDNA clones recognizes a 2300-nt mRNA, although each clone is distinct by restriction enzyme analysis. The two contitutive clones share extensive homology; $pUDPGT_R$-2 has less homology to these other two clones. The mouse transferase cDNA clone (1850 bp) recognizes two constitutive mRNAs (1900 and 2200 nt) which generate 51,000-dalton proteins. In the rat a 52,000-dalton transferase, translated from total RNA, undergoes processing by dog pancreatic microsomes, resulting in peptide cleavage of a 2000-dalton fragment and with no evidence of glycosylation. In the mouse a 50,000-dalton transferase translated from total mRNA undergoes processing in the presence of dog pancreatic microsomes to the mature form of 51,000 daltons by peptide cleavage and glycosylation. Further characterization of the types of transferase forms specified by these clones is underway. A human transferase form with a high pI, already purified to more than 90% homogeneity, is undergoing further steps of chromatography to obtain a more highly purified preparation for characterization and antibody production to enable us to isolate and characterize human transferase cDNA clones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00500-06 LDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

RECEPTOR STRUCTURE AND FUNCTION

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

PI:	H. J. Eisen	Head	LDP, NICHD
Others:	C. M. Foster	Medical Staff Fellow	LDP, NICHD
	M. E. Reichman	Expert	LDP, NICHD
	A. K. Jaiswal	Visiting Fellow	LDP, NICHD
	D. W. Towne	Chemist	LDP, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Chemistry, NIADDKD
Laboratory of Biochemistry, NCI

LAB/BRANCH

Laboratory of Developmental Pharmacology

SECTION

Section on Regulation of Gene Expression

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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.)*

Steroid hormones, drugs, and certain environmental contaminants alter gene expression in target cells. This project is focused on identification of the molecular mechanism by which glucocorticoid hormones and polycyclic aromatic compounds induce distinct species of cytochrome P-450 in mammalian liver. Glucocorticoids and polycyclic aromatic compounds bind to distinct intracellular protein "receptors;" the ligand-receptor complexes interact with DNA in the cell nucleus and appear to affect directly the transcription of cytochrome P-450 genes. The major advantage of studying glucocorticoids and polycyclic aromatic compounds is that mutants with defective receptors can be isolated for both systems. During the past year, we have concentrated on the isolation and characterization of mouse hepatoma cell culture mutants that are defective in induction of cytochrome P_1-450 by polycyclic aromatic compounds. With the use of mutants that have markedly decreased Ah receptor levels, we have developed and validated a new, rapid assay for the Ah receptor involving anion-exchange HPLC. We have defined the experimental conditions under which [^{3}H]2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)·Ah receptor complexes bind to DNA; these studies should provide the basis for purification of the Ah receptor and analysis of its interaction with the P_1-450 gene. Human lymphoid cells resistant to glucocorticoid hormones have been analyzed with the use of antibodies to human glucocorticoid receptors. The defective receptor moieties in certain cases can be identified by immunochemical methods. The region affected by mutation can be "mapped" by affinity labeling and limited proteolysis. We have isolated affinity-labeled glucocorticoid receptor with the use of a monoclonal antibody. Although the N-terminus of the receptor is blocked, fragments of the receptor prepared by limited proteolysis may be suitable for determining amino-acid sequence. These data are providing detailed information about the structure of the glucocorticoid receptor.

Z01 HD 00056-09 Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones
Yoke Peng Loh, Ph.D.

Z01 HD 00058-09 Peptides in the Adult and Developing Vertebrate Nervous System
Harold Gainer, Ph.D.

Z01-HD 00705-03 Macromolecules Involved in Neuronal Function and Development
Harold Gainer, Ph.D.

NICHD ANNUAL REPORT

Laboratory of Neurochemistry and Neuroimmunology

October 1, 1983 to September 30, 1984

This laboratory is concerned with the development, functional organization and interactions between three major integrative systems in the body - the central nervous system, endocrine system, and the immunological system. In particular, we are studying the various peptides and proteins that characterize these systems, and the roles that these molecules play in intercellular communication (as hormones, neurotransmitters, neuromodulators, etc.). Our overall approach is cell biological in nature and hence utilizes techniques and concepts from a variety of disciplines, (e.g. physiology, biochemistry, anatomy and immunology).

The activities of the laboratory are divided into two sections.

I. Section on Functional Neurochemistry

There are over thirty biologically active peptides already known to exist in the neurons of the central nervous system. These have been identified, in many cases, as chemical transmitters of information in the nervous system. These known peptides (and presumably those still to be discovered) act as conventional neurotransmitters in a synaptic, paracrine, or autocrine fashion, as well as in neuroendocrine systems. In addition to the well-known hypophysiotrophic regulatory peptide hormones (e.g., CRF, LHRH, TRH, etc.), there are neuropeptides involved in a wide variety of other CNS functions (e.g., pain, blood pressure control, memory (?), etc.) This sections' goal is to study the cell biology of peptidergic neurons in the context of their regulatory functions in the Autonomic Nervous System. In particular, we specifically study the expression of neuropeptides during CNS development and their impact on the development of organismic functions.

We focus our studies on the hypothalamo-neurohypophysial system because the neurons that constitute it (i.e., the oxytocin and vasopressin magnocellular neurons) represent excellent models of peptidergic neurons in the central nervous system. These neurons populate two defined topographic sites in the brain (the paraventricular and supraoptic nuclei), and have a specific axonal pathway and termination site, all of which are accessible to experimental manipulation in vivo by stereotaxic, morphological, and biochemical-pharmacological techniques. Access to all three of the critical neuronal structures, i.e., the cell body, the axon, and the terminal in the hypothalamus, median eminence, posterior pituitary, respectively, permits a cell biological analysis of this system. The cell bodies in the hypothalamus are responsible for the biosynthesis of the vasopressin (AVP) and oxytocin (OT) prohormones. Our laboratory first identified these prohormones, and suggested a molecular structure for these prohormones based on peptide mapping analysis of these molecules synthesized in vivo. Since that time the complete amino acid sequences and the genes for these peptide hormones have been elucidated.

Much of our past year's activity has been devoted to testing the "secretory vesicle hypothesis of precursor processing" which we proposed about seven years ago, based upon our studies in the hypothalamo-neurohypophysial system. This hypothesis states that the initial endopeptidase cleavages which excise the nascent biologically active peptides from their precursors occur primarily in secretory vesicles, and that all subsequent processing events must also occur within the vesicles. A prediction from this hypothesis is that appropriate processing enzymes should be located in secretory vesicles. Dr. Russell's work in this section has provided the technological developments which allowed for the isolation of highly purified secretory vesicles. In collaboration with the Section on Cellular Neurobiology (which has focused on the processing enzymes in intermediate lobe vesicles), we have used these vesicles to demonstrate that they contain all the expected processing enzymes indicated by the hypothesis, i.e., prohormone converting endopeptidase (Lys-Arg-specific), carboxy-peptidase-B-like enzymes, aminopeptidases specific for amino terminal arginine, and α-amidase related enzymes. In addition to these studies, we have studied specific vesicle membrane proteins, i.e., an ATPase which has been shown to be a proton pump responsible for acidifying the vesicle interior, and a cytochrome b561 which serves as an electron translocator. The latter protein appears to donate electrons to semidehydroascorbate generated in the vesicle during α-amidase activity, in order to reduce this molecule to ascorbate (a cofactor for the α-amidase enzyme).

In addition to the above biochemical work, we have been developing a capability in ultrastructural localization of antigens by immunocytochemistry (EM-ICC). The point of this work is to demonstrate unequivocally, by these techniques, the intravesicular location of the above enzymes and peptides (precursors), and to study the routing of these antigens through the membrane systems of the cell (e.g., RER, Golgi, vesicles, etc.). This has involved technological developments (by Dr. Whitnall and Ms. Key) in EM-ICC which allow for good ultrastructure combined with good antigenicity. New fixation procedures, embedding media, and most recently the application of immunogold techniques has allowed us to: 1) show the colocalization of an opioid peptide, dynorphin A (1-8) and vasopressin in common secretory vesicles, 2) locate the dynorphin 1-8 in smaller secretory vesicles in the Brattleboro rat, a mutant which does not contain vasopressin, 3) disprove a hypothesis that axonal SER is used instead of vesicles for hormone transport during dehydration stress, and 4) show that the oxytocin precursor is located in secretory vesicles during fetal development. For these and other studies, we have found that monoclonal antibodies (MABs) are the preferred immunological "reagents". Hence, we have begun to make our own MABs against specific peptides and proteins, using a new "in vitro" immunization procedure. The advantage of this over conventional procedures is that immunization takes 5 days (versus 2 months) and antibodies can more easily be generated against poor immunogens (e.g., endorphin and calmodulin). We are currently making MABs against vasopressin, oxytocin, prodynorphin, secretory vesicle membranes, and calmodulin. We have already made MABs (in collaboration with Dr. Ozato) to neurophysins, and parvalbumin.

The optical studies of nerve terminal activity, begun last year in collaboration with Dr. Salzberg (U.Penn) continues. We have extended the voltage-

sensitive dye work to the mouse pituitary (previously we studied frog pituitary) with similar results, i.e., demonstration of both sodium and calcium components in the action potential. In addition, these new studies on the mouse neural lobe revealed a light-scattering effect correlated with hormone secretion. This effect is now under intensive study, since it might allow for the simultaneous recording of both the action potential and secretion process on a single oscilloscope sweep. In addition we have developed two secretion models: 1) an intact mouse pituitary system stimulated electrically in a perfusion system allowing for RIA measurements of AVP secretion during varied stimulation frequency and pharmacological paradigms, and 2) a neurosecretosome model (equivalent to synaptosome, but from the neural lobe and therefore highly homogeneous and without postsynaptic contaminants) which will be used for basic studies of peptide secretion.

Advances have been made in the vasopressin receptor program. Dr. Lang has demonstrated that the development of functional AVP receptors in the cultured A6 epithelial cell line is dependent primarily on the development of an epithelial morphology in culture. Only when the morphology of the cell is appropriate, does the AVP receptor become coupled to the adenylcyclase. This correlation is intriguing and suggests a common mechanism regulating both phenomena. In addition, a AVP receptor mediated endocytosis in the cells has been demonstrated, and the efficacy of this endocytosis is inversely related to the efficacy of functional activity. Studies of the ligand selectivity of the A6 line AVP receptor showed that it more closely resembles mammalian brain AVP receptors than kidney receptors.

Recent studies on the squid axon model have focused on 1) the Ca^{2+} activated protease's pattern of cleavage of its endogenous substrate, the neurofilament protein, and 2) the casein-like protein kinase in axoplasm which selectively phosphorylates axonal neurofilament protein. In both of the above cases, the action of protease and kinase is on the 200,000 dalton cross-linking (to other cytoskeletal proteins) component of the neurofilament protein, and not on the 60,000 dalton protein constituting the neurofilament core.

II. Section on Cellular Neurobiology

The research goal of this Section is to study brain and pituitary peptides which are involved in intercellular neurocommunication and fetal development. The major focus has been to continue to study the biosynthesis, packaging, post-translational modification and secretion of the ACTH/endorphin/α-MSH family of peptides and its relationship to the oxytocin and vasopressin peptide system. Within the past year, three interrelated projects have been pursued.

The ACTH, α-MSH and endorphin peptides are synthesized in the intermediate lobe of the pituitary from a common, glycoprotein prohormone (pro-opiocortin) of about 32,000 daltons in size. Recently we have assayed for several enzymes involved in the processing of this prohormone. A converting activity which cleaves at the paired basic residues which flank these peptides in the prohormone has been detected in bovine intermediate lobe secretory vesicles. This prohormone converting enzyme (PCE) preferentially cleaves

pro-opiomelanocortin between the Lys and Arg at the Lys-Arg pairs. PCE has been purified to homogeneity and characterized as a 68,000 molecular weight glycoprotein. It has a pH optimum of 4.0, and is functional at the acid intravesicular pH. Purified PCE is inhibited by pepstatin A but not by PMSF, DFP (serine protease inhibitor), or EDTA. Thiol protease inhibitors at high concentration (10^{-3} M) had a partial inhibitory effect. A similar enzyme has also been purified from bovine neural lobe secretory vesicles. Neural lobe PCE cleaved pro-oxytocin and pro-vasopressin, synthesized in the neurohypophysial system, to yield their respective hormones. A carboxypeptidase B-like enzyme and an aminopeptidase which removes the C- and N-terminal basic residues, respectively, from the cleaved peptides were also detected in neural lobe and intermediate lobe secretory vesicles. These enzymes appear to be acid, metalloproteases which are highly stimulated by Co^{++}.

The regulation of synthesis of pro-opiomelanocortin (POMC) has been studied using the toad intermediate lobe as a model system. Organ cultures of the toad neurointermediate lobe shows that dopamine effectively down regulates the biosynthesis of POMC. Furthermore, it was shown that this regulation may be mediated by cyclic AMP.

Finally, we have successfully prepared a c-DNA library from toad pituitaries. We have screened the library with a mouse POMC probe and have isolated a clone with a 430 nucleotide insert. Work is now in progress to sequence this insert, which can ultimately be used as a probe for studying the regulation of POMC synthesis in the toad neurointermediate lobe at the transcriptional and genomic levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00056-09 LNN

PERIOD COVERED

October 1, 1983 to September 30, 1984

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)*

P.I.:	Y. P. Loh	Head	LNN, IRP, NICHD
Others:	David Parish	Visiting Fellow	LNN, IRP, NICHD
	Renu Tuteja	Visiting Fellow	LNN, IRP, NICHD
	Baldwin Wong	Bio. Lab. Tech.	LNN, IRP, NICHD
	Philip Hanna	Bio. Lab. Tech.	LNN, IRP, NICHD
	Brenda Myers	Junior Fellow	LNN, IRP, NICHD

COOPERATING UNITS *(if any)*

Mark Goldman, LC, NIHLB; Bruce Schrier, LDN, NICHD; Vivian Hook, LCB, NIMH

LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The biosynthesis of ACTH, endorphin, α-MSH, vasopressin and oxytocin, was studied, with emphasis on the enzymes involved in the proteolytic processing of the respective prohormones. A prohormone converting enzyme (PCE) which specifically cleaves between the Lys and Arg of Lys-Arg pairs of pro-opiomelanocortin (ACTH/endorphin prohormone) to form the active hormones, has been isolated from bovine pituitary intermediate and neural lobe secretory vesicles. PCE has now been purified to apparent homogeneity and characterized as a 68,000 molecular weight glycoprotein. A carboxypeptidase B-like enzyme and an aminopeptidase which function to remove the basic residues from the C- and N-terminals respectively, from the peptide hormone, following the action of PCE, have been detected in intermediate and neural lobe secretory vesicles. The regulation of biosynthesis of pro-opiomelanocortin in the toad intermediate lobe by dopamine and cyclic AMP was also studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00058-09 LNN

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Peptides in the adult and developing vertebrate nervous system

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

P.I.:	Harold Gainer	Head	LNN, IRP, NICHD
Others:	Mark Whitnall	Staff Fellow	LNN, IRP, NICHD
	Michael Lang	Expert	LNN, IRP, NICHD
	Sharon Key	Biologist	LNN, IRP, NICHD

COOPERATING UNITS *(if any)*
B. Salzburg, Department of Physiology, University of Pennsylvania; M. Brownstein, LCB, NIMH K. Ozato, LMDI, NICHD; M. Castel, Hebrew University, Jerusalem

LAB/BRANCH
Laboratory of Neurochemistry and Neuroimmunology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.5	1.0

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

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

The secretory vesicle hypothesis of peptide precursor processing, originally proposed by our laboratory, continued to gain experimental support. This included findings of prohormone converting enzymes, carboxypeptidase B-like enzymes, aminopeptidases, and peptidyl α-amidases in neurosecretory vesicles. The development of the vasopressinergic and oxytocinergic neurons in the rat hypothalamus has been studied with respect to precursor expression and processing. Oxytocin neurons lag behind vasopressin neurons in the expression of processing mechanisms and neurite outgrowth, but not in peptide precursor synthesis. Optical methods have been used to record action potentials in pituitary nerve terminals, and have also revealed a light scattering phenomenon correlated with secretion. Studies of the kidney vasopressin receptor in vivo shows a permanent down-regulation of receptors after exposure of neonatal rats to excessive hormone. A tissue culture model of vasopressin receptor containing epithelial cells showed that the receptor, coupling proteins and adenylcyclase are expressed early, but that development of a physiologically active receptor/adenylcyclase complex occurs at a later stage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00705-03 LNN

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Macromolecules involved in neuronal function and development

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

P.I:	Harold Gainer	Head	LNN, IRP, NICHD
Others:	James T. Russell	Senior Staff Fellow	LCB, NIMH
	Shirley House	Biologist	LNN, IRP, NICHD
	Seth Wolfe	Bio. Lab. Tech	LNN, IRP, NICHD

COOPERATING UNITS *(if any)*

H.C. Pant, Alcohol & Drug Abuse, NIAA; C. Klee, NCI; R. Pruss, LCB, NIMH; P. Fleming, Georgetown University, D. Njus, Wayne State University

LAB/BRANCH

Laboratory of Neurochemistry and Neuroimmunology

SECTION

Section on Functional Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Two membrane proteins with enzymatic activities have been studied in the bovine neurosecretory vesicle (NSV). These are a Mg^{2+}-dependent ATPase which has been shown to transport protons into the vesicle to acidify the intravesicular space, and a cytochrome b561 which serves to transport electrons into the vesicle. Ascorbic acid has also been measured in the vesicles at a concentration of 20 mM, and the electron transport mechanism appears to necessary to reduce the semidehydroascorbate in the vesicle to ascorbate which acts as a co-factor for the peptidyl-α-amidase in the vesicle (for amidation of the peptide hormones). A neurosecretosome preparation has been developed for the study of secretion-related molecular mechanisms. Monoclonal antibodies are being produced against various calcium-binding proteins (e.g., calmodulin) found in the neurosecretosome. A casein-like protein kinase which phosphorylates neurofilament proteins in the axoplasm of the squid giant axon has been studied.

Z01 HD 00054-10 Structural and Behavioral Analysis of Vocal Communication in Squirrel Monkeys
M. Biben, Ph.D.

Z01 HD 00062-08 Brain Mechanisms of Vocal Production in Squirrel Monkeys
J. D. Newman, Ph.D.

Z01 HD 00702-04 Genetics of Primate Vocal Behavior
J. D. Newman, Ph.D.

Z01 HD 01102-03 Behavioral Correlates of Endocrine Disorders in Children
Robert P. Klein, Ph.D.

Z01 HD 01104-02 An Observational Study of Parent-Infant Interaction in a Family Context
Frank A. Pederson, Ph.D.

Z01 HD 01105-01 A Follow-up Study of Mastery Motivation at 6-1/2 Years
Frank A. Pedersen, Ph.D.

Z01 HD 01106-01 Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity
Stephen J. Suomi, Ph.D.

Z01 HD 01107-01 Adaptation of Laboratory Reared Monkeys to Field Environments
Stephen J. Suomi, Ph.D.

NICHD Annual Report

Laboratory of Comparative Ethology, IRP

October 1, 1983 to September 30, 1984

Laboratory Chief's Summary

The Laboratory of Comparative Ethology (LCE) began official operations as a new NICHD IRP laboratory in October, 1983. The primary focus of LCE research involves the integration of biological and behavioral approaches to study the interaction between genetic and environmental influences on developmental processes in human and nonhuman primates. The LCE evolved out of the old Child and Family Research Branch, now the Section on Child and Family Research (CFRS), with the addition of the Brain, Behavior, and Communication Section (BBCS), formerly the Brain and Behavior Section of the LDN, IRP, NICHD, and the creation of the Comparative Behavioral Genetics Section (CBGS). During the past year a number of independent research projects were carried out within each of the LCE's 3 sections. In addition, efforts were initiated to develop areas of mutual research activity between LCE sections, and formal collaborative arrangements were established and nurtured with colleagues in the National Institute of Mental Health IRP, the National Institute on Alcohol Abuse and Alcoholism IRP, as well as with investigators at other institutions in the U.S. and Europe. Summaries of individual research projects conducted in each of the LCE's 3 sections follow:

1. Section on Child and Family Research

This section, headed by Dr. Pedersen, investigates the influences of early experience in the family on the psychological development of the young child, as well as how some biological functions affect behavioral development. The activities of the section are divided into three units.

A. Unit on Parent-Child Interaction. During the past year major efforts focused on four interrelated projects that derive from a core sample of 65 families studied with time-sampling observations in the home environment when the infants were 3 months old and again at 1 year of age. Additional samples were added to the core sample for special questions or to pursue leads generated from earlier analyses of the core sample.

One major substantive question addressed the effects of varying amounts of maternal work-force participation on the child's early experience in the home environment. Our analyses indicated that the observational context is crucial in discerning behavioral differences associated with maternal employment. Few differences were found in maternal behavior according to the mother's employment status when mother and infant were observed alone, i.e., without the father, but significant differences in parental behavior emerged at both 3 and 12 months when mother, father, and infant were observed together. In an additional sample of 40 mother-infant pairs, more refined observations were carried out before and after mothers returned to employment and compared with observations at similar ages in a group of homemaker mothers. This design allowed examination of possible differences in the way employed mothers relate to their babies prior to as well as after the transition to employment. Measures of frequencies and durations of maternal behavior, however, revealed no differences between the two groups at

either time period. More complex analyses, focusing especially on detailed examination of maternal contingencies to infant cues, are currently being pursued. Finally, an extensive review of the existing maternal employment literature yielded a number of possible mediating variables including age and sex of child, socioeconomic level of the family, father involvement with the child, maternal role satisfaction, hours of employment, and quality of substitute care. This review generated several new hypotheses regarding possible underlying psychological processes which may account for inconsistent effects of maternal employment on children, prompting a new research initiative focusing on the issue of a sex difference in response to maternal employment. This new longitudinal project will examine children previously investigated during infancy, both prior to and after the mother's resumption of employment, when they reach 2 1/2 years of age.

A second area of inquiry addressed basic questions of stability and change in parent and infant behaviors over the first year of life. New information has been generated about how the observational context affects generalizations about behavioral stability. Although our observations not surprisingly showed shifts toward greater autonomy of the child from 3 to 12 months of age, greater consistency over time was found in mother-infant interactions involving daughters than in those with sons during daytime home observations when the father was not present. However, stability of maternal behavior in the evening observational context, which included the father, was comparable for either male or female infants. Fathers, moreover, showed generally similar stability in their behaviors whether interacting with sons or daughters. In addition, mothers in the daytime context showed vastly greater interactional rates with infants than they did in the evening context, largely because of the presence of the father in the evening who shared many behaviors directed toward the infant as well as interacted with the mother. The difference in behavior rates of the 2- versus 3- person context was less obvious at 12 months than at 3 months, however, reflecting the greater autonomy of the infants as they matured.

Another aspect of behavioral stability was examined in a comparison at age 12 months of two small groups of fathers who had contrasting early adaptations to parenthood. At age 3 months the two groups of fathers differed in whether or not they had experienced periods of minor depression, and they were observed to behave distinctively with their infants. Fathers who described periods of "blues" showed lower rates of involvement with their children than did their wives on selected measures, while the reverse pattern held for men who were not depressed at the time. Follow-up analyses of the home observations at age 12 months, however, indicated that these adaptations were apparently transient, as the initial patterns did not persist. The results are consistent with transactional or "self-righting" models of parent-infant interaction, which generally predict lack of long-term persistence of problems except in very extreme cases. These findings were presented at a workshop sponsored jointly by the intramural and extramural programs of NICHD on Men's Transitions to Parenthood. The proceedings of the workshop, involving eleven research papers, will be published as a book.

In a third inquiry involving the core sample, Ainsworth Strange Situation trials were carred out at age 15 months in order to assess mother-infant and father-infant attachment relationships. The previously described 12-month home observations were analyzed as independent variables to determine the antecedents of secure vs. insecure attachments. For the 12-month daytime home observations, measures of pleasurable face-to-face interaction between mother and infant and

ratings of maternal sensitivity to infant cues were both predictive of secure mother-infant attachment relationships, although evening observation of mothers appeared to be less sensitive to individual differences and generally failed to be predictive. Predictors of father-infant attachment also appeared less robust. These results again showed that situational contexts affect the type of results obtained in studies of infants interacting with mothers and fathers.

The study of the mother-infant attachment was also augmented by additional subjects on whom interview data were obtained. From this cohort, as well as the core sample, detailed information concerning the types and frequencies of mother-infant separations that took place during the baby's first year of life was obtained. Seven different types of separation were identified, only two of which have been investigated previously. Great variation in frequencies were found for most types of separations, and different types were found to be associated with distinctive aspects of substitute care. The data indicate that there exists considerable heterogeneity subsumed under the single concept "separation." Future analyses will be directed toward whether or not different kinds of separation experiences affect the development of the mother-infant attachment relationship and whether fathers who have provided extensive care (in the absence of the mother) are distinctive in other aspects of their relationship with the infant.

In a fourth inquiry, the psychological significance of cesarean as opposed to vaginal childbirth continues to be examined. It was previously reported that differences in parent-infant interaction associated with mode of birth had been found in the evening observational context of mother, father, and infant. A new finding, though of a null result, is that the daytime mother-infant interaction appears relatively insensitive to variation due to mode of birth, even though the mother's psychological constructions of cesarean birth are clearly less positive than mother's evaluations of vaginal birth. This finding suggests that, in the daytime at least, the mother-infant adaptational system is relatively robust when functioning in isolation, without the potential support and assistance of the father.

B. Unit on Studies of Mastery Motivation. Several interrelated studies are underway that investigate mastery motivation. The first involves a detailed longitudinal study of the development of mastery motivation in the first year of life for a sample of 75 infants from middle income families. The current focus of this investigation is directed toward elucidating the interrelationships between motivation, competence, and both positive and negative affect. Several series of analyses have revealed that the infant's affective displays are closely intertwined with various aspects of interaction with parents. In addition, important developmental changes occur in the expression of affective displays observed both in the home and laboratory setting. These changes, as well as their relationship to the measures of mastery motivation, indicate the importance of affect as a marker of cognitive-motivational functioning. Furthermore, the patterns of cross-age correlations point to the need to address the issue of developmental stability and change in the context of not only the time in the developmental course that measurements are made but also the sex of the child.

The second mastery study involves follow-up of the above subjects to age 2 1/2 years in order to determine whether mastery motivation in infancy is predictive of later mastery behavior and developmental competence and to investigate the role of early parent-infant interaction in the development of mastery behavior

at 2 1/2 years. Analyses to date suggest that measures of mastery motivation in infancy can indeed predict later competence; moreover, mastery in early childhood has a similar form to that observed in infancy, indicating stability in the structure of this behavior. In addition, preliminary analyses indicate that positive affective expression in infancy may be related to attention and concentration in a structured problem-solving session at 2 1/2 years for girls, but not for boys, suggesting that early personality and social development may predict differentially for the sexes to later behavior in test-taking situations.

A third investigation, in which data collection is still in progress, represents an additional long-term follow-up of the developmental course of mastery motivation in the above subjects at age 6 1/2 years. In addition to establishing longitudinal linkages with earlier measures, the goals of this study include investigations of associations of contemporaneous mastery behavior with achievement motivation, intellectual competence, parent-child interaction, and various child characteristics such as temperament and reaction to stress. An interesting feature of this study is that levels of salivary cortisol will be measured in the children under mild and moderate stress conditions, proving a conceptual link between studies of young humans and nonhuman primates faced with environmental challenges.

In a parallel investigation of mastery motivation and social competence in Down syndrome infants, longitudinal data were collected at 3, 6, and 8 months, and an additional cross-sectional sample was studied at 12 months of age. The findings to date indicate that Down syndrome infants display a similar distribution of behavior directed toward mastering both object-oriented and socially-oriented aspects of their environment when compared to a mental age-matched control sample. However, the relative levels of mastery behaviors are systematically depressed in the Down syndrome sample.

C. Unit on Psychoendocrinology. In a new area of inquiry for the CFRS that promises to shed light on behavioral-biological interrelationships, studies are being carried out in collaboration with the DEB (IRP, NICHD) on children with endocrine disorders, including precocious puberty, Turner's syndrome, growth hormone deficiency, and Prader-Willi syndrome. A first objective was to determine if such children are at risk for problems in psychosocial adjustment. In a sample of children with precocious puberty, we reported that children with precocious puberty do, in fact, show an above-normal incidence of a variety of adjustment problems. A current objective is to ascertain the factor(s) responsible for this finding. Analyses completed to date have focused on the relationship between variations in subject characteristics within the precocious puberty group and results from a psychosocial adjustment measure, the Child Behavior Checklist. Three factors were examined on the basis of a literature review: diagnosis, height, and pubertal stage. There was no evidence of an effect of whether or not precocious puberty was attributable to a CNS disorder. In contrast, greater height relative to age was associated with better psychosocial adjustment, due in part to the fact that while the older children tended to be closer to age-normative height, they also showed more evidence of psychosocial difficulties. Nevertheless, the effect of height remained significant after controlling statistically for age. Possible relationships between pubertal stage and incidence of adjustment problems were examined using Tanner breast stage and Tanner pubic hair stage as indicies of pubertal status. No significant relationship was detected between pubic hair stage and psychosocial adjustment, but more advanced breast

stage was associated with greater psychosocial difficulties. Precise interpretations of these findings are difficult because the maturational indicies are integrative measures and probably reflect the action of more than one hormone. Furthermore, the metabolites of these hormones may influence behavior, and social factors may also be relevant. Thus explication of these results awaits analysis involving the specific hormones, and as those data become available from DEB, these more specific analyses will be carried out. Studies planned for the upcoming year include comparisons of psychosocial adjustment among several groups of endocrine and metabolic disorders, including precocious puberty, growth hormone deficiency, Prader-Willi syndrome, and glycogen storage diseases. Additionally, pre-post treatment comparisons will be carried out for the precocious puberty sample who will be receiving hormone therapy during a circumscribed period at the Clinical Center this coming year.

2. Section on Brain, Behavior and Communication

This section, directed by Dr. Symmes, completed an initial full year of activity at its new laboratory facilities on the grounds of the NIH Animal Center near Poolesville. The primary component of these new facilities, Building T-18, was fully operational throughout this period. While some small problems in the construction remain, the facility has been highly successful, and the opportunities for new types of research have begun to be realized. In addition, a portion of Building 130, on loan from the LBEB (IRP, NIMH), was modified to make it suitable for housing of 2 small social groups of squirrel monkeys and 2 family groups of owl monkeys. Finally, construction of 3 outdoor group cages, 2 for owl monkey families and 1 for a new squirrel monkey group, was completed. These outdoor group cages are currently being utilized in ongoing research projects.

With these new facilities, Drs. Symmes and Biben were able to carry out a study on maternal recognition of individual infant vocal signals that had previously failed to yield clear-cut results when conducted in a different setting. The significant finding in the present study was that mother squirrel monkeys clearly demonstrated by their response to a hidden loudspeaker that they recognized their own infants from a test group of six familiar infants. Tape recordings containing only isolation calls were prepared for each infant and played back with a tape recorder. The study probably succeeded because of the calm, naturalistic test conditions possible at the new laboratory. The results are important because they validate earlier work on the immature isolation call done in the BBCS. Additional studies of affiliative vocalizations of squirrel monkey groups have been continued by Dr. Biben. She is currently carrying out new and more detailed analyses of the temporal spacing of chuck calls in order to identify unique acoustic properties which characterize those calls used in vocal exchanges between adult females.

Dr. Biben has completed data collection in a major longitudinal study of play behavior in a mixed-sex group of 10 yearling squirrel monkeys. Analyses completed to date indicate that individual and sex differences in play strategies are clearly present, with particular youngsters adopting specific strategies that seem maximally beneficial to them. In wrestling play, males avoid partners with whom they are at a disadvantage, or they initiate a milder type of wrestling with them more typical of females. Dominant individuals encourage play by displaying role reversals, which gives subordinate play partners ample opportunities to "win" at play wrestling. These results provide new insights into the role of

play in the behavioral development of healthy, normally socialized animals. Studies on the neuroethology of the isolation call in adult squirrel monkeys have proceeded in collaboration between Dr. Newman and Dr. Paul MacLean, Chief of the LBEB (IRP, NIMH). Two brain pathways have been identified which have differing roles in the expression of this vocalization. We have previously reported that the caudal thalamic tegmentum appears to be involved in normal structural patterning of the isolation call. Studies completed this year, involving a total of eight monkeys, have implicated a second brain area--the rostral cingulate gyrus and surrounding cortex--in controlling the motivational basis for uttering isolation calls.

Dr. Newman has also continued long-term studies on the genetics of isolation call structure. This work is based on earlier, fundamental research in the section on consistent differences in the isolation call between two strains of squirrel monkey (termed Gothic arch and Roman arch, respectively), and the mixture of these vocal types found in hybrid offspring. The hybrid types studied to date generally display vocalizations that are intermediate in their acoustic structure between the Gothic and Roman arch phenotypes. When these hybrids are back-crossed with a pure-strained mate, the offspring's vocal pattern reverts back to the pure-strain phenotype.

Finally, preliminary efforts have been initiated to establish long-term collaborative arrangements with investigators at the Yerkes Regional Primate Research Center in Atlanta, which houses the world's largest collection of captive great apes, the Duke Primate Center in Durham, N.C., which features the world's most successful program of captive breeding of rare prosimian primate species, and with the Caribbean Primate Center in Puerto Rico, which includes a field station containing free-ranging groups of both strains of squirrel monkeys and some clearly identified hybrids. The collaboration will involve audiotaping of selected subjects from each of the above primate populations, with detailed comparative sound spectographic analysis to be carried out at the LCE Poolesville facility. In addition, analysis of human infant and child vocal patterns collected in collaboration with CFRS researchers within the LCE are planned for the near future.

3. Comparative Behavioral Genetics Section

The Comparative Behavioral Genetics Section (CBGS), headed by Dr. Suomi, is a component of the LCE that is new to the IRP, NICHD. The CBGS investigates the processes underlying biological and behavioral development in nonhuman primate subjects by focusing on genetic and environmental factors that either alone or in concert affect the course of an individual's ontogeny over a range of levels of analysis. This approach to developmental study in the CBGS is strengthened considerably through a unique collaborative relationship with the IRP, NIMH. formalized by a memorandum of agreement between the Scientific Directors of NICHD and NIMH.

During the past year a considerable amount of time and effort was directed toward the planning and initial construction of new primate facilities at the NIHAC. Plans for the remodelling of Building 112 have progressed to the stage of opening of bids for construction, while preliminary drawings for a new facility for primate breeding have been completed. Construction of a 5-acre outdoor enclosure containing an open-access shelter was completed in April, 1984, a group of 16

rhesus monkeys was released inside the enclosure in May, 1984, and on June 9, 1984 the enclosure was formally opened in a public ceremony. Detailed observations of the free-ranging monkey group have been ongoing since then. In the meantime, other research projects that will be moved to the Poolesville site when the remodelling and new construction are completed have been actively maintained with IRP support at the University of Wisconsin Primate Laboratory.

A major longitudinal study currently underway at the Primate Laboratory involves the cross-fostering of rhesus monkey infants, genetically selected for high vs. low reactivity to novel stimuli and challenge, with mothers selected for their characteristic style of nurturant vs. punitive mothering. Data collection of the first 6 cross-fostered mother-infant pairs is currently underway, with additional subjects due later in this year's "birth season" at the Wisconsin breeding facility. Temperament, neonatal reflex, behavioral, neurohumoral, and psychophysiological data are being gathered longitudinally on all mother-infant pairs.

Long-term follow-up data representing a comparable range of levels of analysis were obtained from adolescent rhesus monkeys this past year; these 4-year-old subjects had been studied extensively since infancy. The data analyses completed to date indicate that 4-year-old subjects who had exhibited extreme behavioral and physiological reactions to brief separations earlier in life similarly responded to the current 4-day separations with extreme reactions, although the form of prototypical behavioral reaction--agitated, self-directed stereotypy--was quite different from the characteristic depressive withdrawal displayed by the same subjects in response to separations earlier in life. Nevertheless, these high-reactive monkeys continued to exhibit higher levels of adrenocortical activity in response to separations than their behaviorally low-reactive cohorts, paralleling their adrenocortical responses to separations during infancy and childhood. Moreover, as was the case earlier in life, these high and low reactive adolescents did not differ significantly from one another, either behaviorally or physiologically, during periods of group housing. These results indicate that there is remarkable long-term developmental continuity of individual differences in stress reactivity among these subjects, even if the exact form of behavioral expression does undergo substantial change during development. Other physiological data collected from these monkeys during the above study are currently under analysis.

The same subjects were subsequently administered the antidepressant compound imipramine both during periods of brief separation and during periods of stable group housing; a placebo treatment was also administered to each subject in a repeated measures crossover design. Measures of behavior, adrenocortical response, and CSF levels of catecholamine metabolites were obtained from each subject throughout the study. Preliminary results of the behavioral analyses suggest that there are substantial differences in response to imipramine treatment between high and low reactive subjects, with the former displaying reductions in stereotypic and other self-directed behavior during separations, but not during reunions, after 2 weeks of chronic treatment; in contrast, few drug effects are apparent in the behavior of low reactive subjects. The physiological data are currently being assayed.

Another study completed during the past year involved the development of a rhesus monkey neonatal assessment test battery, modelled in large part on the Prechtl and Brazelton neonatal exams for human infants. This test battery was repeatedly

administered to both mother-reared and nursery-reared rhesus monkey infants throughout their first 6 weeks of life. Substantial rearing condition differences were found in these subjects, with mother-reared infants displaying more pronounced grasping reflexes and higher predominant state, while nursery-reared infants tended to show stronger visual and auditory orienting responses. Most of the rearing condition differences, however, all but disappeared as the infants grew older. On the other hand, individual differences in measures of predominant state and muscle tone were highly stable over the period of study, and, more importantly, were highly predictive of individual differences in behavioral and adrenocortical responses to brief separation when the subjects were 6 months of age. A replication study is currently in progress.

A final series of studies conducted at the Wisconsin facility this past year focused on a possible relationship between social dominance status, adrenocortical response to brief separation, and paternity. Groups of peer-reared rhesus monkey infants and juveniles were studied in order to avoid confounds with mothers' dominance status. It was found that low-ranking peer-group members tended to display high adrenocortical responses to brief separations, but the reverse was not true for mid and high ranking subjects. On the other hand, paternal half-siblings growing up in different peer groups tended to share relative dominance status (and, if they were low-ranking, adrenocortical response to separation as well). This possible genetic component in the acquisition and maintenance of social dominance in peer-reared groups of monkeys is potentially of considerable importance, and we are following up the original study with additional research utilizing other groups of monkeys.

Finally, the opening of the new outdoor enclosure at the NIHAC in Poolesville permitted the continued longitudinal study of a free-ranging group of laboratory born and reared 10-year-old adults and their progeny. All members of the group adapted to the move from Wisconsin to Maryland in highly predictable fashion, and the group now seems well adjusted to its new physical environment. Long-term observations of this group will be continued within the new enclosure. In addition, the vocal repertoire of each group member is now being sampled, and following sound spectographic analysis (in collaboration with the BBCS), play-back studies utilizing prerecorded auditory stimuli will be carried out within the group. Preliminary analyses of the sound spectographs from these monkeys reveal strong similarities to published sound spectographs obtained from rhesus monkey groups living for many generations in natural habitats, despite the fact that none of the present subjects have ever been exposed to any monkeys born and reared in the wild. These preliminary findings suggest that at least the basic components of rhesus monkey vocal repertoires are preserved in their genetic heritage, even after many generations of laboratory housing and isolation from feral-born conspecifics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00054-10 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	M. Biben	Senior Staff Fellow	LCE, NICHD
	D. Symmes	Head	LCE, NIDHD
Other:	J. D. Newman	Research Psychologist	LCE, NICHD
	N. Masataka	Visiting Fellow	LCE, NICHD
	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Brain, Behavior, and Communication Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.4	2.0	0.4

CHECK APPROPRIATE BOX(ES)

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

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

This project investigates the use of vocal signals by squirrel monkeys in specified contexts as a model language. Three main areas of research were pursued during the reporting period. 1) Affiliative vocalizations continued to be a major focus, with new findings being reported on the temporal patterning of vocal exchanges between individual females sharing affiliative relationships. 2) A major study of play behavior in a group of 10 yearling monkeys was completed, with these results: Youngsters show both sex and individual differences in the frequency and roughness of their play. Females and subordinate males avoid partners which would overpower them in wrestling play, or they initiate play of a milder type with these partners. The tendency of dominant individuals to take on a subordinate role in play (role reversal) encourages play between youngsters of differing abilities or size. These strategies foster play within a group and enable immature monkeys to maximize play experiences which promote normal social and behavioral development. Vocal correlates of play are still being analyzed. 3) Information content in contact and alarm calls has been studied utilizing playback methodology. This work involves presenting tape recorded sounds to multi-age, multi-sex groups of monkeys, and has been made possible by construction of new outdoor habitats. A significant finding demonstrates that mothers can recognize their own infants on the basis of a particular class of contact call - the isolation call. Early results with alarm calls suggest some individuality there as well and raises the possibility that listeners may respond differentially to alarm calls from near relatives.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00062-08 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Brain Mechanisms of Vocal Production in Squirrel Monkeys

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

P.I.:	J. D. Newman	Research Psychologist	LCE, NICHD
Co-Investigator:	P. D. MacLean	Head	LBEB, NIMH
Other:	D. Bernhards	Bio. Lab Tech	LCE, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Brain Evolution and Behavior, NIMH

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Brain, Behavior, and Communication Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.5	0.3

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 study has identified two neural pathways that play a major role in the expression of the isolation call, a stereotyped vocalization elicited by brief separation from conspecifics. One pathway, encompassing the caudal thalamic tegmentum and adjacent core gray matter, is involved with the normal structural patterning of this call. This pathway plays no significant role in the motivation to produce isolation calls or in the normal structural patterning of other vocalizations. A second pathway involves the rostral limbic cortex (cingulate and subcallosal gyrus) and adjacent frontal neocortex. This neural tissue is related to the tendency to produce isolation calls. Bilateral lesions result in a failure to vocalize when visually and acoustically isolated from conspecifics, although some tendency to vocally respond upon hearing squirrel monkey sounds is retained. Other studies have shown this same cortical area to be rich in opiate receptors. Therefore, our finding that systemic administration of an exogenous opiate, morphine, blocks the tendency to produce isolation calls suggests a role for endogenous opiates acting on neurons within this rostral midline cortex in regulating production of this vocalization. Parts of this same brain region also influence the tendency to express vocalizations associated with the mirror-directed genital display, while leaving non-vocal display components relatively unaffected. Vocalizations by adult males in this context often closely resemble infantile versions of the isolation call in their combined noisy and tonal structure and variably placed frequency modulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00702-04 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

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	Research Psychologist	LCE, NICHD
Other:	D. Bernhards	Bio. Lab Tech	LCE, NICHD

COOPERATING UNITS *(if any)*
Duke University Primate Center

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Brain, Behavior, and Communication Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

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

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

The typical vocalization of many primates when separated from conspecifics is a tonal sound of relatively long duration and little abrupt frequency change or noise. These structural features characterize the isolation calls of primates as phylogenetically diverse as lemurs, macaques, and human infants, suggesting that the same genetic program determining isolation call structure may be widespread across the primate order. This same basic structure is also found in the isolation calls of squirrel monkeys (Saimiri). Analysis of the squirrel monkey isolation call has revealed a subtle but consistent difference in structural details of isolation calls from two physically distinct squirrel monkey types or species, the Gothic-arch and Roman-arch types. Studies leading up to the present project showed that these structural differences are present in newborn infants and persist regardles of subsequent experience, suggesting a major role for genetic determination of the isolation call's species-specific attributes. In the present project, adults of both Saimiri types are cross-bred and the isolation calls of the hybrid offspring analyzed for resemblance to parental phenotype. Roman-arch adults from Peru bred with Gothic-arch adults from Peru or Colombia produce offspring whose isolation calls resemble the Roman-arch parent. In the majority of cases the degree of resemblance is only approximate; discriminant analysis indicates that measures of hybrid isolation call structure typically produce discriminant scores intermediate to those of the parents, or to isolation calls of age-matched pure-bred offspring reared under the same conditions. In related crossbreeding studies, Roman-arch males from Bolivia were mated with females from Guyana, representing the most geographically and phenotypically disparate South American populations of Saimiri. Hybrid offspring from these pairings produce isolation calls closely resembling the Gothic-arch (maternal) phenotype, while their physical appearance more nearly resmbles that of the Roman-arch parent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01102-03 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Behavioral Correlates of Endocrine Disorders in Children

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

PI:	R. P. Klein	Senior Research Investigator	LCE, NICHD
Other:	W. A. Sonis	Medical Staff Fellow	LCE, NICHD
	C. Rahn	Research Psychologist	LCE, NICHD
	N. F. Gist	Research Psychologist	LCE, NICHD
	M. Fivel	Research Pscyhologist	LCE, NICHD

COOPERATING UNITS *(if any)*
Developmental Endocrinology Branch, NICHD; Laboratory of Developmental Psychology, NIMH; Child Studies Center, University of Maryland; Division of Endocrinology, Children's Hospital Medical Center

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Child and Family Research Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)
- [x] (a) Human subjects
  - [x] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

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

This project encompasses a series of studies examining the behavioral correlates of endocrine disorders, including children with precocious puberty, Turner's syndrome, and growth hormone deficiency. A first objective was to determine whether these children are at risk for problems in psychosocial adjustment. In a sample of children with precocious puberty, we reported that these children do, in fact, show an above-normal incidence of a variety of adjustment problems. A current objective is to ascertain the factor(s) responsible for this finding. Analyses completed to date have focused on the relationship between variations in subject characteristics within the precocious puberty group and measures of psychosocial adjustment based on the Child Behavior Checklist. Three factors were examined on the basis of a literature review: diagnosis, height, and pubertal stage. There was no evidence of an effect of whether or not precocious puberty was attributable to a CNS disorder. In contrast, greater height relative to age was associated with better psychosocial adjustment, due in part to the fact that while the older children tended to be closer to age-normative heights, they also showed more evidence of psychosocial difficulties. Nevertheless, the effect of height remained significant after controlling statistically for age. Possible relationships between pubertal stage and incidence of adjustment problems was examined using Tanner breast stage and Tanner pubic hair stage as indicies of pubertal status. No significant relationship was detected between pubic hair stage and psychosocial adjustment, but more advanced breast stage was associated with greater psychosocial difficulties. Additional analyses are planned to clarify this finding. Future studies will include pre-post treatment comparisons of adjustment for the precocious puberty sample.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01104-02 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
An Observational Study of Parent-Infant Interaction in a Family Context

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

PI:	F. A. Pedersen	Head	LCE, NICHD
Other:	M. J. Zaslow	Staff Fellow	LCE, NICHD
	J. D. Demetre	Visiting Fellow	LCE, NICHD
	R. L. Cain	Research Psychologist	LCE, NICHD
	J. D. Suwalsky	Research Psychologist	LCE, NICHD
	B. A. Rabinovich	Research Assistant	University of Maryland

COOPERATING UNITS *(if any)*
Parent and Child, Childbirth Education Associates, University of Maryland

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Child and Family Research Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

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

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

This project encompasses four areas of investigation based on a core sample of 65 middle-class families, each with a first-born infant, as well as additional participants to address specific questions. Procedures include observations of mother-infant and mother-father-infant interaction, interviews, assessments of child temperament, and structured laboratory assessments of the parent-infant attachment relationship. The first area of inquiry concerns the effects of maternal workforce participation on the child's early experience in the home environment. Included are analyses of parent-infant interaction in families with employed or homemaker mothers, more detailed observations of mother-infant interaction in the home both prior to and after mothers resume employment, and laboratory observations of toddlers and either employed or homemaker mothers. A second area concerns longitudinal stability and change of parent-infant interaction rates for the sample as a whole and as a function of various contrasting circumstances such as characteristics of the child or parent as well as levels of social engagement of the parents. The third area concerns the parent-infant attachment relationship, its antecedents including quality of parent-infant interaction and the child's separation experiences, and the developmental consequences of contrasting qualities of attachment. A unique feature of this project is that the attachment assessment and the child's separation history are procedures common to several different samples, allowing coordination and replication of several research questions. The final area is a comparison of parent-infant interaction in families that experienced either cesarean or vaginal childbirth, as well as a substudy of families in which cesarean birth occured when the expectant father was present with the mother or not permitted to be with her.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01105-01 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Follow-up Study of Mastery Motivation at 6 1/2 Years

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

P.I.:	F. A. Pedersen	Head	LCE, NICHD
Others:	P. M. Vietze	Head	MRRC, NICHD
	R. H. MacTurk	Research Associate	University of Maryland
	M. E. McCarthy	Research Associate	University of Maryland
	F. T. Hunter	Research Psychologist	LCE, NICHD
	L. Martini	Research Assistant	LCE, NICHD
	J. Demetre	Visiting Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Institute for Child Study, University of Maryland

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Child and Family Research Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.95	1.10	.85

CHECK APPROPRIATE BOX(ES)

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

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

Several interrelated studies are underway that investigate the development of mastery motivation. Three of the projects employed the same sample of 75 children to study the interrelationships between cognition, motivation, and the environment at 6, 12, 30 months, and 6 1/2 years of age, respectively. At each age point, methods were developed that were developmentally appropriate yet conceptually similar to the data collected at the earliest age. Two separate studies were conducted to investigate the expression of mastery motivation in Down syndrome infants and to elucidate the cognitive mechanisms underlying the processing of manual actions in infancy. This last study, as well as the 6 1/2 year followup study, are currently in the data collection phase. The current focus of the 6, 12, and 30 month studies is directed toward elucidating the relationship between mastery motivation, competence, and positive and negative affect. The results indicate that affective displays are closely intertwined with aspects of parent-child interaction. Developmental changes in the expression of affect indicate that affect is a salient marker of cognitive-motivational functioning into the third year of life. With regard to mastery motivation in Down syndrome infants, the findings to date indcate that these infants display a similar distribution of behavior directed toward mastering both object-oriented and socially-oriented aspects of their environment when compared to a mental age-matched control sample. However, the relative levels of mastery behaviors are systematically depressed in the Down syndrome sample.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01106-01 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	M. Linoilla	Clinical Director	IRP, NIAAA
	T. R. Insel	Scientist	CNB, IRP, NIMH
	C. J. Eisele	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

Primate Laboratory, University of Wisconsin-Madison
CNB, IRP, NIMH; IRP, NIAAA

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Comparative Behavioral Genetics Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	.75	.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.)*

This project examines behavioral and physiological development in rhesus monkeys, focusing on individual differences in temperament reactivity and their developmental consequences in different rearing environments. Research this past year focused on 4 studies. In the first study, adolescent monkeys with previous separation experience as infants and juveniles were removed from their social groups for four 4-day periods, and behavioral and physiological measures were obtained prior to, during, and after each separation. Analyses of behavioral and neurohormonal data revealed that (a) individual differences in stress reactivity remained quite stable from infancy through adolescence even in the face of major developmental changes in behavior and physiology, (b) these differences were masked during periods of stable group living, and (c) although behavioral reactions to separation changed dramatically as monkeys entered puberty, physiological patterns of arousal remained the same. A second study using these same monkeys examined the effects of the antidepressant imipramine prior to, during, and following subsequent brief separations. Preliminary results showed that the effects of imipramine were different in monkeys who displayed extreme reactions to previous separations than in monkeys whose reactions to previous separations were mild. The third study developed a neonatal assessment system designed to identify individual differences in reflex and temperament development in rhesus monkey neonates and to use the assessments to predict subsequent individual differences in stress reactivity. Results to date indicate that the assessment system is sensitive to early rearing condition differences among subjects, that temperament and muscle tone measures can identify neonates at risk for high stress reactivity later in life, while certain orienting measures seem to predict subsequent hyperactivity. The fourth study, initiated this past year, uses these neonatal measures to identify potential high and low reactive infants, who are then cross-fostered with multiparous females who differ dramatically in their style of mothering.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01107-01 LCE

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	J. W. Newman	Scientist	LCE, NICHD
	P. O'Neill	Research Psychologist	LCE, NICHD
	D. Barber	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

Primate Laboratory, University of Wisconsin-Madison

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Comparative Behavioral Genetics Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.415	.25	1.155

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 continues the longitudinal study of a group of 10-year-old laboratory born and reared rhesus monkeys and their progeny who now live in a 5-acre enclosure at the NIHAC near Poolesville. Although the core group of adults had been reared without mothers and early in life exhibited predictable behavioral disturbances, their behavior in the new outdoor environment, along with that of their progeny, appears to be indistinguishable from that reported for monkeys living in natural habitats on the Indian subcontinent. Furthermore, measures of individual differences in behavior and social status obtained earlier within a laboratory setting appear to generalize to these monkeys' new outdoor environment. A recent addition to this longitudinal project involves the recording and detailed sound spectographic analysis of these subjects' vocal repertoires, in order to compare them with previously published sonographs obtained from monkeys living in feral environments and with those obtained from monkeys growing up in more physically and socially restricted laboratory environments.

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

Z01 HD 00047-15 Biochemical & Morphologic Studies of Neuronal and Other Cell Types
Douglas E. Brenneman, Ph.D.

Z01 HD 00048-10 Studies of Transcriptional Level Control of Neurobiologic & Development Phenomena
Bruce K. Schrier, M.D., Ph.D.

Z01 HD 00064-08 Neurobiologic Studies of Neurons and Glia in Cell Culture
Phillip G. Nelson,M.D., Ph.D.

Z01 HD 00094-14 Pineal Regulation: Environmental and Physiological Factors
David C. Klein, Ph.D.

Z01 HD 00095-14 Pineal Regulation: Transsynaptic and Intracellular Mechanisms
David C. Klein, Ph.D.

Z01 HD 00703-02 Effect of Long Chain Fatty Acids on Developing Neurons in Cell Culture
(Inactive)

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

NICHD ANNUAL REPORT

Laboratory of Developmental Neurobiology, IRP

October 1, 1983 to September 30, 1984

Work of the Laboratory of Developmental Neurobiology over the past several years has resulted in the establishment of several tissue culture systems derived from the mammalian central nervous system which are in use in many laboratories around the world. We are now focusing on questions of the regulation of neuronal development of synaptic formation and the mechanisms involved in central synaptic action, using preparations most appropriate for particular questions. A broad spectrum of techniques are brought to bear on these questions including electrophysiological and biophysical, biochemical and molecular genetic approaches, and light and electron microscopic methods.

The pineal gland serves as a model system for studying biological rhythms and nervous system control of gene expression. Relationships between retinal and pineal function constitute an exciting new area of study.

Dr. David Symmes' Section on Brain and Behavior within the LDN was transferred to the newly constituted Laboratory of Comparative Ethology during FY 1984.

1. Section on Neurobiology

Major progress has been made in establishing the mechanisms involved in the control of neuronal survival by electrical activity. This regulation exhibits a temporal "critical period" with maximum vulnerability to blockade of electrical activity at 1-3 weeks in vitro. Cholinergic neurons are affected but gamma-aminobutyric acid (GABA) containing neurons are not. A number of observations indicate that a cyclic AMP mediated step is involved in coupling electrical activity to neuronal development:

1) Blockade of electrical activity results in a decrease in cAMP levels in the cultures.
2) Conditioned medium which reverses the effects of electrical blockade raises cAMP levels to normal.
3) Treatment of cultures with 8-Bromo cAMP can itself reverse the deleterious effects of electrical blockade.

Novel and important evidence has been obtained that supports the hypothesis that vasoactive intestinal peptide (VIP) or a VIP-like peptide is an activity-dependent trophic substance for central, in particular cholinergic, neurons.

1) Vasoactive intestinal peptide-containing neurons were shown to exist in dissociated spinal cord cultures. Immunofluorescent studies demonstrated that 3-5% of the total neurons had VIP-like immunoreactivity. The VIP content of the culture increased during development, reaching a maximum at 21 days in vitro.

2. Spontaneous release of VIP-like immunoreactivity was shown to occur in culture. This release was blocked by tetrodotoxin (TTX). Thus, the availability of this peptide appeared to be dependent on ongoing electrical activity. Studies of the spontaneous release of Met-enkephalin did not show the same sensitivity to TTX.

3. Neuron survival in electrically blocked cultures was increased after treatment with exogenous VIP. Maximum increase in survival was observed at 10^{-10} M VIP. Significant increases were demonstrated at levels as low as 10^{-12} M. The potency of this peptide suggests physiological relevancy. The activity of choline acetyltransferase (CAT) was also shown to be increased at the concentrations indicated above. The stimulation of this cholinergic marker enzyme was shown to be dependent on the age of the cultures. Application of VIP before or after the critical period of vulnerability to TTX-mediated cell death resulted in no change in CAT activity.

These results represent the most compelling evidence available for an activity-dependent developmental regulatory role for a peptide in the central nervous system. There is reason to hypothesize that this regulation may be mediated by an action of VIP on glial cells. If so, this would allow an approach to a large and important area of neurobiological research (glial-neuron interactions).

It is of substantial importance to obtain cellular markers identifying cholinergic neurons. Attempts to stain cholinergic neurons in culture using anti-CAT antibodies have been unsuccessful. An alternative approach has been histochemical staining of intracellular AChE; somewhat less than 10% of neurons in ventral horn cultures and less than 1% of neurons in dorsal horn cultures are stained by this method.

The sensitivity of neuronal development to blockade of electrical activity in neuronal cultures has been studied in parallel with analysis of the development of electrical excitability in these preparations. Biochemical measures (^{3}H-saxitonin binding) suggest that developmental changes occur in the density of voltage sensitive Na^+ channels. We have found that the onset of spontaneous electrical activity (day 6-7 in culture) corresponds very closely to the beginning of the period when cell death is produced by tetrodotoxin blockade of electrical activity. The ionic mechanism for action potentials and the cellular localization of action current generation have been analyzed with a novel combination of voltage-clamp techniques with local application of sodium-containing solutions. In contrast to some other preparations, central neurons exhibit very early expression of Na^+ channels and these channels are distributed on neurites, as well as on the cell body as neurites develop.

Both biochemical and electrophysiological studies of the development of the voltage-dependent Ca^{++} conductance mechanism have been undertaken. Calcium channels are of special interest in that they are involved in the process of synaptic release of neurotransmitters. Nitrendipine, a 1,4 dihydropyridine, is believed to bind to the voltage-sensitive calcium channel, and kinetic studies indicate that two classes of binding sites are present throughout development. Dissociation constants (Kd's) increased for both affinity sites during develop-

ment. The Bmax increased two-fold for the high affinity and almost six-fold for the low affinity receptor from day 3 to day 21. In young cultures, tetrodotoxin (TTX) (1 μM) displaced ^{3}H-nitrendipine binding from both the high and low affinity binding sites. In one month old cultures, TTX had no effect on ^{3}H-nitrendipine binding. Interactions between TTX and nitrendipine in young cultures suggest ligand binding site similarities for the sodium and calcium channels during development.

Initial electrophysiologic study of nitrendipine action utilized the calcium spike as an estimate of calcium channel activity. Recordings were made in 3-4 week cultured dorsal root ganglion cells. At low concentrations (5 μM) of nitrendipine, the duration of the calcium spike was prolonged. At high concentrations (100 μM) of the drug, the calcium spike was blocked with secondary enhancement as the drug diffused away from the cell. Additional studies, e.g. voltage clamp, are necessary to assure that these effects were due to the drug's action on the voltage-sensitive calcium channel.

Voltage clamp techniques have been used to clarify the complexities of postsynaptic responses to excitatory amino acids. These acidic amino acids displayed three types of behavior based on the voltage sensitivity of the current flow through the agonist-activated channels. N-methyl-D-aspartate (NMDA) and L-aspartate were highly voltage-sensitive; i.e. the current-voltage relationship for these agonists showed a negative slope conductance at membrane potentials negative to -30 mV, such that there was little current flow through channels activated by these agonists near the normal resting potential (approximately -60 to -70 mV). The current-voltage relationships for kainate and quisqualate were voltage-insensitive, and thus continued to increase in amplitude as the membrane was hyperpolarized. The reversal potential for both types of responses was near 0 mV, suggesting that Na^{+}, K^{+}, and Cs^{+} pass through the channels. The reversal potential was similar to that found for excitatory synaptic potentials in this preparation.

The basis of the voltage sensitive conductance mechanism was found to be due to a voltage-dependent block of NMDA-activated channels by physiological levels of Mg^{++} (0.5 - 1.0 mM). Such a mechansim is similar to the "fast open channel block" of ACh channels at the neuromuscular junction by a number of charged molecules. The chord conductance increased e-fold per 22 mV depolarization. Using this value with a single site channel block model, Mg^{++} appears to enter the membrane electrical field from the outside and block the channel approximately halfway through the membrane.

The properties of the voltage-sensitive conductance mechanism are well suited to a role as a neuromodulator. Synaptic depolarization of the neuron could be "boosted" by activation of NMDA receptors either by agonists released synaptically or by levels of aspartate or glutamate present in extracellular fluid. In addition, changes in extracellular Mg^{++} may also play a role in modulating the behavior of NMDA-activated channels.

The third category of agonists showed a voltage-sensitivity intermediate between the highly voltage-sensitive and the voltage-insensitive agonists. This intermediate group included L-glutamate and D-homocysteate. However, in the presence of the selective NMDA antagonist, 2-amino-5-phosphonovalerate (2-APV), the L-glu-

tamate response was converted to a voltage-insensitive response suggesting that L-glutamate acts as a "mixed" agonist on both the voltage-sensitive and voltage-insensitive conductance mechanism. The behavior of L-glutamate as a mixed agonist suggests that the behavior of glutaminergic synapses may be quite different depending on the postsynaptic receptor type that is activated by the presynaptic release of glutamate.

We have used kinetic and pharmacological data to characterize the excitatory synaptic responses of cultured neurons. These properties of synaptically mediated excitatory responses appear incompatible with those responses produced by activation of NMDA receptor, but may fit well with those of "non-NMDA" activated receptors.

Electrophysiological and morphological studies of the transmitter release mechanism have established the synaptic bouton as the probable morphological entity underlying release. Work from our and other laboratories indicates that some, and in some instances most, of these release elements are not functional under physiological circumstances. The 'synaptic reserve' such non-functional boutons represent, is a substantial potential source for alteration of nervous system function. The mechanisms involved in the plasticity that switching presynaptic elements on and off would represent is an important area for future research.

Tetanus toxin is a molecule with enormous potency and specificity as a neurobiological tool. It is capable of blocking all measurable transmitter release in extremely low doses without affecting any other electrophysiological parameters. Such electrically quiet cultures have been studied for up to six weeks after toxin exposure. It was shown by radioautography that the toxin remains associated with neurons and that there are no obvious alterations in synaptic ultrastructure that correlate with electrical quiescence. Both light and heavy chains of the toxin molecule have a half life of approximately 5-6 days. With fluorescent staining techniques, it was demonstrated that the toxin is internalized in neurons. Initial ultrastructural studies using tetanus toxin-colloidal gold complexes indicate that toxin enters neuronal cell bodies, apparently via receptor-mediated endocytosis, and is seen within synaptic vesicles, possibly related to the mechanism for synaptic vesicle membrane recycling. The relevance of this internalized toxin to its electrophysiological effects remains to be determined.

In order to understand the functional significance of elaborate neuronal geometries and to interpret rigorously a variety of electrophysiological data, computer modelling techniques have been developed. Frequency domain analysis has been combined with morphological reconstruction and compartmental modelling to describe the electrical structure of single ventral-horn and dorsal-root ganglion neurons. Detailed compartmental modelling efforts have demonstrated that the membrane properties of these neurons are not uniform; rather, the somal membrane resistivity is up to ten times greater than the membrane resistivity of the dendritic tips.

This non-uniform membrane resistivity gradient can be shown to have significant effects on the relative efficacy of synaptic inputs throughout the neuron. In addition, these methodologies are being used to analyze the development of electrical structure. Once the source of the non-uniform distribution of mem-

brane resistivity has been determined, neurons can be studied from very early ages (1 day in culture) to follow development of both the expression of specific ionic conductances, and the distribution of these conductances within the structure of the neuron.

We have developed a generalized neuronal modelling program ("NEUROS") to aid in the analysis of these experiments. There has been general widespread interest in this approach, and particularly in the program. The program is freely available to any interested scientist in the U.S., and is now being used in laboratories at Yale Medical School, Washington University Medical School, and the University of California, Berkeley.

The techniques developed in studies of synaptic transmission have been very useful in analyzing the mechanism of action of a number of neuroactive compounds. We had shown earlier that transmitter output from sensory neurons was diminished by opiates preferring μ receptors. Such receptors are rare in spinal cord cells but functional delta and kappa receptors are present in a relatively high proportion of spinal cord neurons. Combined physiological and autoradiographic studies of the localization and regulation (see below) of these receptors are in progress. We have shown that functional cholinergic receptors in hippocampal cultures are primarily localized to presynaptic structures. Abundant muscarinic cholinergic receptors can be demonstrated on spinal cord cells and further physiologic studies of cholinergic function in this preparation are planned. With respect to both opiate peptides and acetylcholine, questions pertaining to physiologic mechanism of action need to be addressed and the regulation of the synthesis and organization of an appropriate receptor system have substantial neurobiologic importance.

We have been studying the ontogeny of receptors in neural tissue using neuropeptide receptors as a model. In particular, we have investigated the role of neuropeptides in the development of their receptors. Our experiments, performed both in vivo and in neuronal cell culture, indicate that the neuropeptide transmitters regulate, during development, the number of their receptors that will be expressed by the adult.

The in vivo experiments demonstrate that several types of peptides, when administered to neonatal rats, permanently alter the number of their receptors in various target tissues. The altered receptor number has physiological consequences for the adult animal, rendering it hyper- or hyposensitive to its endogenously released peptides. The tissue culture experiments demonstrate that the altered receptor number is due to a change in the number of receptors per neuron, rather than a change in the number of cells having receptors.

These studies indicate a mechanism by which neurotransmitter receptors are regulated during development, and provide additional evidence that presynaptic signals are important in determining certain phenotypic characterisitcs of the developing neuron.

Messenger RNAs from the anterior and neurointermediate lobes of pituitaries of Xenopus laevis were used to construct dDNA libraries in the Pst I site of pBR322 cloned in strain MC 1061 of E. coli K12. One of these cloned plasmids contains a 435 bp insert which hybridizes to a probe of mouse pro-opiomelanocortin (POMC)

cDNA. This probe also hybridizes on southern blots with portions of each of 3 different clones of Xenopus genomic DNA in phage λ. We are presently sequencing the 435 bp insert and attempting to identify POMC-containing restriction fragments from the λ clones for future subcloning and sequencing. We have also developed libraries of 400,000 - 600,000 clones of cDNAs for sequences expressed in differentiated cells of NS20Y mouse neuroblastoma cells and the neuron-glia hybrid cell line NG108cc15. We are presently selecting probes of differentiation-specific sequences from each of these cell lines by cascade hybridizations with heterologous (undifferentiated cells of the same cell line) and homologous mRNAs. A library of several million clones of high molecular weight cDNAs from NG108cc15 cells has been prepared in the expression vector λgt11. Portions of this library are being screened with monoclonal antibodies which have been prepared against the enzyme choline acetyltransferase (CAT) from rat brain.

Central cholinergic systems, and CAT in particular, are hypothesized to play a central role in Alzheimer's disease and Down's Syndrome. This Laboratory has extensive experience with physiological regulation of the enzyme, and the molecular genetic approach to analysis of cholinergic development has a high priority for adding impetus to those other components of the laboratory program.

2. Section on Neuroendocrinology

The Section on Neuroendocrinology conducts pioneering studies on the pineal gland. This group of investigators, which has contributed heavily to our knowledge of how melatonin production is regulated, continues to make exicting advances in several areas. A notable growing interest in this program is the relationship of the retina and the pineal gland. Both tissues share uncommon proteins, including rhodopsin kinase and hydroxyindole-O-methyltransferase, which probably reflects the "third eye" function of the pineal complex in lower vertebrates and a common ancestoral photoreceptor having the capacity to make melatonin. In mammals, however, light does not act directly on the pineal gland, but rather through a complex neural circuit. Together with the pineal gland this comprises the melatonin rhythm generating system.

This group and their collaborators have provided evidence that this neural circuit is: retina->retinohypothalamic tract->suprachiasmatic nucleus->paraventricular nucleus->intermediolateral cell column->superior cervical ganglia->nervi conaril->pineal gland. This problem is being extended with the goal of determining where synaptic connections are made and which transmitters are involved. The approach that has been initiated with workers at the Department of Physiology, University of Pennsylvania, is to electrically stimulate targets in the circuit and study the effect of stimulation on melatonin production. The latter is determined by measuring urinary 6-hydroxymelatonin. Initial experiments have shown that electrical stimulation of the paraventricular nucleus during the day does not stimulate melatonin production. The possibility that this stimulation will block neural stimulation of the pineal gland at night is now being studied.

Neural stimulation of nerves in the pineal gland results in the release of norepinephrine, and a continued interest of this group has been to describe how norepinephrine acts on the pineal gland. In this regard, the Section on Neuroendocrinology also views the pineal gland as an excellent model to use in studying neurotransmitter regulation of intracellular metabolism.

Working at the level of the extracellular surface, investigators in the Section have described alpha 1 adrenoceptors on both rat and sheep pinealocytes. In addition they have found that denervation causes the number of these receptors to increase. This work is important as part of a body of evidence which now indicates that a generally accepted idea is wrong. It had been thought that the pineal gland is a purely beta 1-adrenergic system, and that melatonin was regulated exclusively by beta adrenoceptors. This group has presented clear evidence that norepinephrine acts through both alpha and beta adrenoceptors. Alpha adrenergic agonists potentiate the effects of beta adrenergic agonists.

The mechanism of alpha and beta adrenergic synergism is being investigated by studying cyclic nucleotides in intact pinealocytes, and the enzymes involved in the synthesis and degradation of cyclic AMP and cyclic GMP. It is thought that cyclic AMP mediates all the effects of norepinephrine on melatonin production, and that the synergisic actions of alpha and beta adrenergic agonists on melatonin production are mediated via cyclic AMP. The function of cyclic GMP, which is unknown, is being studied.

Detailed studies using dispersed adult pineal cells have now demonstrated unequivocally that beta adrenergic activation is required for the full 100- to 300-fold stimulation of cyclic AMP or cyclic GMP by norepinephrine. Alpha adrenergic stimulation potentiates the effect of beta adrenergic stimulation of cyclic nucleotides.

Recent findings suggest that adenylate cyclase activation by beta adrenergic agonists is enhanced by alpha adrenergic agonists. The former appears to act through a stimulatory guanine nucleotide binding protein to activate the enzyme. Alpha adrenergic agonists, acting through a phosphatidyl inositol->diacylglyerol ->calcium dependent protein kinase mechanism appears to increase the efficiency and effectiveness of beta-adrenergic activation. The mechanisms underlying the 100-fold adrenergic stimulaton of cyclic GMP is less clear, but might involve a similar synergistic action focused on guanylate cyclase.

It should be noted that although cyclic AMP and cyclic GMP are both regulated by a synergistic alpha- and beta-adrenergic mechanism, a number of striking differences in their regulation are apparent. Most importantly, the Section has discovered that when cyclic AMP responsiveness exhibits two-fold denervation supersensitivity, that cyclic GMP responsiveness exhibits nearly complete denervation desensitization; a process they call see-saw signal processing. Recent work indicates other differences: cyclic AMP regulation but not cyclic GMP regulation appears to involve calcium-dependent protein kinase; cyclic GMP regulation but not cyclic AMP regulation is strongly dependent upon extracellular calcium; full stimulation of cyclic AMP is more dependent upon beta-adrenoceptors, whereas full stimulation of cyclic GMP is more dependent upon alpha-adrenergic mechanisms. These observations are important because they are beginning to describe two parallel systems for the regulation of cyclic AMP and cyclic GMP, by norepinephrine. This should provide important clues to neurobiologists about how transmitters and neuromodulators interact, especially in view of the marked increase in examples of neuromodulators, compounds which modulate the response of systems to transmitters. In the case of the pineal gland, norepinephrine acting as a beta 1-adrenergic agonist is the transmitter and is also the modulator, acting via alpha 1-adrenoceptors. Using selective alpha- and beta-adrenergic

drugs, it has been possible to use this system to study the molecular mechanism involved in neuromodulation of neurotransmission.

Cyclic AMP functions in the pineal gland to control a number of processes, including taurine release, biopterin, membrane polarization, and N-acetyltransferase activity. The latter enzyme was found by this group to regulate large changes in melatonin production. This line of investigation has been extended in several directions. Aromatic amine N-acetyltransferase activity in the pineal gland has been found to reside in two distinct enzymes, which have been chromatographically separated. One form is regulated by an adrenergic-cyclic AMP mechanism and is specific for arylalkylamines, including serotonin, and the other specific for arylamines. This situation is unusual, because in most tissues studied, one enzyme with broad specificity appears to act on both classes of substrates. Efforts have continued towards developing highly specific antiserum against purified N-acetyltransferase, which will be useful in studies of this enzyme. Purification procedures for this enzyme have been developed. The enzyme can be purified 200- to 400-fold in a preparation which contains several proteins. Two of these are thought to be N-acetyltransferase.

The study of N-acetyltransferase has been undertaken in sheep because of practical and scientific considerations; this effort has revealed a number of unexpected and intriguing observations. Based on studies in the rat it has been proposed by workers in the Section that daily rhythms in melatonin production in general are regulated by large changes in N-acetyltransferase activity. However, it has been possible in sheep to observe large changes in circulating and pineal melatonin, which are not accompanied by large changes in N-acetyltransferase activity. This puzzle has not been explained yet. However, it has been found that the precursor of melatonin, N-acetylserotonin exhibits marked changes in sheep, which are similar to those in the rat. Thus it appears that in both species melatonin production is regulated by mass action. However, it seems possible that in sheep, N-acetylation is regulated primarily by an activation mechanism and that activation of N-acetyltransferase in sheep is not reflected in broken cell enzyme assays of the sheep gland. The study of sheep is important because it appears that like sheep, a number of species have an apparent small N-acetyltransferase rhythm as measured in broken cell preparations.

The study of N-acetyltransferase, hydroxyindole-O-methyltransferase and other pineal gene products is now being extended by efforts to establish a pineal cDNA library and to eventually clone the genes of interest. This work is being initiated by an attempt to use retinal cDNA libraries. This approach seems a good one because initial studies have provided evidence that bovine rhodopsin cDNA probes hybridize with pineal mRNA. This preliminary indication is now being evaluated with more extensive investigations. Another pineal-retinal gene product that is being pursued is rhodopsin kinase, which phosphorylates photolyzed rhodopsin. This group, working with NEI collaborators, have discovered this enzyme in the pineal gland, and have provided several lines of evidence to indicate that the enzyme in both tissues is very similar, if not identical. Attempts are now being made to determine if purified preparations of this enzyme act to phosphorylate receptors other than rhodopsin, including alpha and beta adrenergic receptors. This work might answer the question of the function of pineal rhodopsin, as well as helping to explain the evolution of receptors in general and the pineal gland specifically.

An important new effort by this group and collaborators in the NINCDS and NIMH is the transplantation of pineal glands to pinealectomized host animals. This provides a highly useful model to study survival and function, because the transplanted pineal tissue will be the only source of melatonin, and production can be monitored by measuring urinary 6-hydroxymelatonin. Initial results indicate that pineal tissue survives in the brain and continues to make melatonin. In addition, the transplanted tissue becomes innervated by cotransplanted superior cervical ganglia. A long-term goal of these studies is to determine if most of the melatonin rhythm generating system can be reconstructed through transplantation procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00047-15 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical and Morphologic Studies of Neuronal and Other Cell Types

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

PI:	D. E. Brenneman	Staff Fellow	LDN, IRP, NICHD
	G. Handelmann	PRAT Fellow	LDN, IRP, NICHD; NIGMS
Others:	G. Westbrook	Staff Fellow	LDN, IRP, NICHD
	M. Litzinger	Medical Officer	LDN, IRP, NICHD
	S. Fitzgerald	Biologist,	LDN, IRP, NICHD
	D. Warren	Bio. Lab. Tech.	LDN, IRP, NICHD

COOPERATING UNITS *(if any)*
W.H. Habig, DBP, BB; R.E. Siegel, LCB, NIMH; L.E. Eiden, LCB, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.5	1.7	1.8

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

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

Cell cultures prepared from fetal mammalian central nervous system were used to study the regulation of neuron development. The mechanisms which mediate the regulatory role of electrical activity were investigated. The onset of a critical period for neuron death after electrical blockade was found to correspond to the following developmental events: 1) the onset of spontaneous action potentials in spinal cord neurons, 2) a major increase in basal choline acetyltransferase activity, 3) a significant increase in sodium channel density, as measured by ^{3}H-saxitoxin binding, 4) an increase in the organization of neuronal aggregates and an increase in the size of neuritic cables, 5) the beginning of the period of naturally occurring neuron death, as determined by cell counts.

A trophic substance, obtained from spinal cord cultures before or after the critical period, was shown to increase neuronal survival during electrical blockade. The release of this substance was shown to be inhibited during impulse blockade. Addition of cyclic AMP-stimulating agents was also found to increase neuron survival during electrical blockade. Vasoactive intestinal peptide (VIP) was shown to exhibit many of the properties of a activity-dependent neuron survival factor: 1) VIP-like immunoreactivity was demonstrated to be present in 3-5% of the spinal cord neurons in culture, 2) the survival-promoting action of VIP was evident at physiologically relevant concentrations, 3) the spontaneous release of VIP was shown to be inhibited by electrical blockade.

We have investigated the possibility that the peptide neurohormones and neurotransmitters play a role in the development of their target organs. The experiments demonstrate that early exposure to the neuropeptides permanently affects the expression of neuropeptide receptors, and that this alteration in receptors is of physiological importance to the mature animal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00048-10 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Studies of transcriptional level control of neurobiologic & development phenomena

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

P.I.:	B.K. Schrier	Medical Officer	LDN, IRP, NICHD
Others:	W. Strauss	Senior Investigator	LDN, IRP, NICHD
	F.M. Neal	Bio. Lab. Tech.	LDN, IRP, NICHD

COOPERATING UNITS *(if any)*

M. Giovanni, NHLBI, B. Raj-Amaladoss, NHLBI; Y. Peng Loh, LNN, NICHD; D. Hilt, NHLBI

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.3	2.1	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)*

Messenger RNAs from the anterior and neurointermediate lobes of pituitaries of Xenopus laevis were used to construct cDNA libraries in the Pst I site of pBR322 cloned in strain MC 1061 of E. coli K12. One of these cloned plasmids contains a 435 bp insert which hybridizes to a probe of mouse pro-opiomelanocortin (POMC) cDNA. This probe also hybridizes on southern blots with portions of each of 3 different clones of Xenopus genomic DNA in phage λ. We are presently sequencing the 435 bp insert and attempting to identify POMC-containing restriction fragments from the λ clones for future subcloning and sequencing. We have also developed libraries of 400,000 - 600,000 clones of cDNAs for sequences expressed in differentiated cells of NS20Y mouse neuroblastoma cells and the neuron-glia hybrid cell line NG108cc15. We are presently selecting probes of differentiation-specific sequences from each of these cell lines by cascade hybridizations with heterologous (undifferentiated cells of the same cell line) and homologous mRNAs. A library of several million clones of high molecular weight cDNAs from NG108cc15 cells has been prepared in the expression vector λgt11. Portions of this library are being screened with monoclonal antibodies which have been prepared against the enzyme choline acetyltransferase from rat brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00064-08 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

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)*

P.I.:	P.G. Nelson	Head	LDN, IRP, NICHD
Others:	E.A. Neale	Physiologist	LDN, IRP, NICHD
	G. Westbrook	Staff Fellow	LDN, IRP, NICHD
	P. Guthrie	Staff Fellow	LDN, IRP, NICHD
	M. Litzinger	Med. Staff Fellow	LDN, IRP, NICHD
	R. Pun, M. Jia	Visiting Fellows	LDN, IRP, NICHD
	P. Sonderegger	Visiting Fellow	LDN, IRP, NICHD

COOPERATING UNITS *(if any)*

P.F. Lemkin, NCI; M.C. Fishman, Mass. Gen. Hospital & Harvard Med. School; H.C. Bauer, Molekular Biologisches Institute, Austria

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.8	4.3	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)*

Physiological studies have shown that substantial numbers of anatomically defined transmitter release elements may be non-functional. Such inactivated synapses represent a potential basis for modifiability or plasticity in neural systems. Excitatory amino acids elicit three distinct types of membrane conductance changes: a highly voltage sensitive response, a voltage independent conductance change, and an intermediate type of response. Glutamate elicits this latter response which can be shown to be due to joint activation of receptors responsible for the other two types of responses. Development of Na^+ dependent mechanisms underlying electrical excitability has been followed both biochemically with apropriate ligand binding assays and electrophysiologically. Na^+ channels are present at very early times in vitro and increase in number and in their distribution over the neuronal surface with little change in kinetic properties. Opiate peptide receptor activation reduces excitatory transmitter release. Delta and kappa type receptors mediate this effect on spinal cord cells, while μ receptors are essentially restricted to sensory neurons. Dorsal root ganglion (DRG) and ventral spinal cord (VSC) neurons were grown in a compartmented cell culture chamber such that their axonal protein composition could be compared after metabolic labelling and SDS-polyacrylamide gel electrophoresis. Distinct quantitative differences can be demonstrated with this system in a small number of axonal proteins when axons are co-cultured with neuroglial cells from the central or the peripheral nervous system. Two proteins were changed specifically by co-culture with peripheral, but not central neuroglial cells, whereas 5 proteins were specifically changed under the influence of central, but not peripheral, glial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00094-14 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	D. Sugden	Visiting Associate	LDN, IRP, NICHD
	P. Voisin	Visiting Fellow	LDN, IRP, NICHD

COOPERATING UNITS *(if any)*
D. Jacobowitz, Section on Histopharmacology, NIMH; S. Markey, Section on Analytical Chemistry, NIMH; J. Pierce, Section on Animal Surgery, NIHLB; M.A.A. Namboodiri, Georgetown Univ.; R. Janowsky, U. of Penn.; M. Brightman, NINCDS.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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.)*

This project studies the environmental and physiological regulation of the pineal gland. Major new discoveries within the last year were: (1) A reciprocal relationship exists between oxidation and N-acetylation products of serotonin in the pineal gland. At night the former are depressed and the latter are increased; the opposite is true during the day. Serotonin concentrations follow the pattern of oxidation products, and the major shifts appear to be regulated by serotonin N-acetyltransferase activity. This underlies the potential importance of N-acetylation of serotonin in regulating serotonin concentrations throughout the brain. (2) Melatonin production can be markedly increased by loading sheep with hydroxytryptophan. This observation raises the possibility that a pineal function test, based on the response of subjects to hydroxytryptophan, might be developed and used clinically. (3) A potent inhibitor of the nocturnal increase in melatonin was discovered: the alpha1-adrenoceptor blocker prazosin. This points to the use of alpha adrenergic drugs to study melatonin production in humans, which has received little attention. (4) Pineal glands were successfully transplanted to the fourth ventricle. They continue to secrete melatonin, albeit at a reduced rate. Innervation takes place when superior cervical ganglia are cotransplanted; vascularization comes from surrounding brain. Using this approach it may be possible to learn how to construct an entire neural circuit, in this case the melatonin rhythm generating system. In addition, it should be possible to transplant genetically engineered pineal cells which produce novel compounds, including psychoactive chemicals, regulated by the adrenergic system which controls melatonin production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00095-14 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

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	Physiologist	LDN, IRP, NICHD
Others:	P. Voisin	Visiting Fellow	LDN, IRP, NICHD
	D. Sugden	Visiting Assoc.	LDN, IRP, NICHD
	J. Vanecek	Guest Researcher	LDN, IRP, NICHD

COOPERATING UNITS *(if any)*
K. Kirk, NIAAMD; W. Anderson, NCI; S. Beckner, NIAAMD; J. Pierce, OD, NHLBI; M.A.A. Namboodiri, Georgetown Univ.; D.Goldman, C. Merrill, D. Jacobowitz, NIMH.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	1.4	0.3

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

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

This project focuses on the mechanisms involved in the neural control of metabolism and gene expression and uses the pineal gland as a neural model to study these questions. Major new discoveries in the project are: (1) Both cyclic AMP and cyclic GMP are regulated by the synergisitc action of two receptors for norepinephrine, an alpha1-adrenoceptor and a beta1-adrenoceptor. This discovery is of profound importance, because it is an exception to the general belief that these receptors always oppose each other. (2) Alpha1-adrenoceptors appear to regulate cyclic AMP through a fatty acid second messenger cascade system, involving phosphatidylinositol turnover, production of diacylglycerol, stimulation of protein kinase c, and sensitization of adenylate cyclase to beta1-adrenoceptor stimulation. (3) It was found that tonic neural stimulation of the pineal gland is required for the stimulation of one enzyme, hydroxyindole-O-methyltransferase, whereas the activity of another enzyme involved in melatonin synthesis, N-acetyltransferase, responds immediately to neural stimulation. This is important because it provides investigators with a model to study how neural signals control both rapid and gradual changes in the activities of specific enzymes. (4) N-acetyltransferase in the pineal gland, in sharp contrast to other tissues, is actually two enzymes, arylakylamine N-acetyltransferase and arylamine N-acetyltransferase. Only the former is neurally regulated and can acetylate serotonin. These two enzymes, which may represent the product of either one or two genes, were characterized. (5) It has been possible to purify hydroxyindole-O-methyltransferase rapidly, using a novel affinity column. This will make future studies more efficient for workers in this area.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00703-02 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Effect of long chain fatty acids on developing neurons in cell culture

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

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 00704-03 LDN

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters 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)*

PI:	E.A. Neale	Physiologist	LDN, IRP, NICHD
Others:	P.G. Nelson	Chief	LDN, IRP, NICHD
	D. Brenneman	Staff Fellow	LDN, IRP, NICHD
	S. Fitzgerald	Biologist	LDN, IRP, NICHD
	L. Bowers	Bio. Lab. Tech.	LDN, IRP, NICHD

COOPERATING UNITS *(if any)*
W.H. Habig, DBP, BB; G.K. Bergey, Univ. of MD Medical School;
J.G. Kenimer, DBP, BB; D.R. Critchley, Univ. of Leicester, England

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	0.5	0.5

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

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

Neurons in cell cultures directly exposed to tetanus toxin undergo a period of increased excitation followed by a loss of spontaneous and evoked synaptic activity. The time to onset of the convulsant action of the toxin is dependent on toxin concentration. Morphologic techniques are being used in an attempt to correlate toxin localization with the characteristic stages of toxin action. Radioisotope studies indicate that the toxin is degraded in cultures with a half-life of approximately six days. Light microscope radioautography demonstrates that the toxin is preferentially associated with neurons and its neuronal distribution does not change up to two weeks after toxin removal from the culture medium. The synaptic ultrastructure of electrically quiet neurons is not obviously altered. Preliminary studies with toxin-colloidal gold complexes indicate that the toxin is internalized into neuronal somata and into synaptic varicosities.

NOTE: This project was inactive last year. It has been reactivated this year.

Z01 HD 00073-13 Regulation of Immune Systems at the Cellular Level
Edgar E. Hanna, Ph.D.

Z01 HD 00918-03 Expression of Histocompatibility Antigens During Early Mammalian Development
Keiko Ozato, Ph.D.

Z01 HD 00920-03 Molecular Structure of Mouse Major Histocompatibility (H-2) Genes: DNA Sequence Basis for Immunological Parameters Associated with H-2 Antigens
Keiko Ozato, Ph.D.

Z01 HD 01300-02 Enhancement of Immunogenicity of Bacterial Polysaccharide and Protein Antigens
Rachel Schneerson, M.D.

Z01 HD 01301-02 Human Immune Response to Polysaccharide-Protein Conjugate Vaccines
Rachel Schneerson, M.D.

Z01 HD 01302-02 Toxins of Pertussis: Isolation, Characterization and Mechanisms of Action
Ronald D. Sekura, Ph.D.

Z01 HD 01304-02 Protective Effect of Vi Polysaccharide Against Typhoid Fever
John B. Robbins, M.D.

Z01 HD 01305-01 Characterization of the Group B Meningococcal (E. coli K1) Antibody Binding Site
John B. Robbins, M.D. (Inactive)

Z01 HD 01306-01 Pertussis Heat Labile Toxin HLT: Isolation and Characterization
R. D. Sekura, Ph.D.

Z01 HD 01307-01 Pertussis Toxin: An Approach to a New Pertussis Vaccine
R. D. Sekura, Ph.D.

Z01 HD 01308-01 Pneumococcal Cell Wall Polysaccharide Protein Conjugation
S. C. Szu, Ph.D.

Z01 HD 01309-01 Bacterial Polysaccharides Cross-reactive with Meningococcus Group A Polysaccharide
R. Schneerson, M.D.

NICHD ANNUAL REPORT

Laboratory of Developmental and Molecular Immunity

October 1, 1983 to September 30, 1984

Section on Bacterial Disease Pathogenesis and Immunity

Infectious diseases of newborns, young infants and children, especially those due to encapsulated bacteria such as Haemophilus influenzae type b, pneumococci, group B streptococci, Escherichia coli and meningococci continued to cause considerable morbitity and mortality despite effective antibiotics and supportive therapy. In the United States, so-called "treated" bacterial meningitis is the leading cause of acquired mental retardation. Prevention of these bacterial diseases, in addition to their control by epidemiologic and therapeutic measures, has now been considered a major objective of the National Institutes of Health. Further, treatment of many of these bacterial infections, including H. influenzae type b and pneumococci, is becoming more difficult because of the increasing emergence of antibiotic-resistant organisms especially those that can no longer be treated with ampicillin (a highly safe inexpensive antibiotic that has been effective against all common causes of bacterial infections in infancy and childhood). Prevention of these diseases by immunization requires basic insights into the components of the bacteria responsible for their pathogenicity, the nature of the host interaction with these bacterial components and the ontogenic mechanisms of immunologic maturation that are related to the unusual susceptibility of infants and children towards diseases caused by these organisms.

The capsular polysaccharides of these bacterial pathogens are essential for their virulence. Capsular polysaccharide antibodies confer a high degree of immunity against diseases caused by these organisms. The structure of these bacterial capsular polysaccharides associated with invasive diseases has been elucidated. The immunologic properties of these capsular polysaccharides are distinctive from those described for proteins, viruses or the intact bacteria. Vaccines composed of purified capsular polysaccharides have been shown to be safe, immunogenic, and protective in healthy adults. In infants, however, their usefulness as vaccines is limited by their 1) poor immunogenicity in this age group and, 2) their failure to induce a booster or "T cell dependent" serum antibody response in the age group most susceptible to invasive diseases caused by encapsulated bacteria.

Many attempts were made, including those in our laboratory, to both increase the immunogenicity of the capsular polysaccharides of H. influenzae type b, pneumococci and E. coli. Non-covalent complexes formed by electrostatic bonds with various molecules, fatty acid derivatives incorporated into liposomes and internal cross linking procedures designed to increase the molecular weight of these polysaccharides were unsuccessful when these experimental products were injected into laboratory animals as saline solutions by the subcutaneous or intramuscular route. In 1979, we reported the immunologic characteristics of a conjugate, composed of a covalent attachment between the capsular polysaccharide H. influenzae type b and model carrier proteins including heterologous serum albumins, diphtheria toxin and the hemocyanins of the horse-shoe crab. The use of a spacer molecule, adipic acid dihydrazide, greatly facilitated the formation of the covalent compound between the physical-chemically diverse capsular

polysaccharides and globular protein. In its original synthesis, the spacer was first attached to the protein molecule using the water soluble carbodiimide EDGE which formed an amide bond between the carboxyl of the protein and the azide and of the bivalent spacer molecule. These conjugates showed an increased immunogenicity of the polysaccharide but failed to induce a satisfactory immune response to the carrier protein. Later, we reported a synthesis of conjugates prepared by first derivatizing the polysaccharide with the adipic acid dyhydrazide and then attaching the polysaccharide derivative to the native protein. This synthetic scheme resulted in higher yields of the conjugate and the resulted products elicited a high level of antibodies to the carrier protein.

The concentration of the reactants, the temperature, pH and ionic conditions were found to be critical in determining both the composition of the conjugate and the yields. The immunologic properties of conjugates, prepared by this synthetic scheme, composed of H. influenzae type b polysaccharide and pneumococcus type 6A polysaccharide conjugated to tetanus toxoid or hemocyanin from the horse-shoe crab were studied in juvenile and infant rhesus. Another conjugate, proposed of H. influenzae type b polysaccharide covalently bound to cholera toxin was studied in juvenile and infant rhesus.

The major findings of the study is as follows: 1. Surprisingly, hemocyanin was a poor immunogen itself and its conjugate elicited low levels of polysaccharide antibodies. Although the levels elicited by the conjugates prepared with hemocyanin were greater than that elicited by polysaccharide alone, the serum antibody response was not considered wholly satisfactory. 2. A dosage response was seen when the serum capsular polysaccharide antibody response elicited by 5 and 50 µg doses of H. influenzae type b polysaccharide were compared. The polysaccharide antibody responses elicited by the H. influenzae type b-tetanus toxoid conjugates were greater than those elicited by the 5 µg dose. Booster responses were observed after the second and occasionally after the third immunization. Levels of polysaccharide antibodies in excess of 20-50 times that considered to be the protective level were observed in all the juveniles injected. The pneumococcus type 6A polysaccharide antibody responses elicited by the pneumococcus type 6A-tetanus toxoid conjugates were disappointing. Only 2 of the 18 juveniles injected with this conjugate responded with protective levels of pneumococcus type 6 antibodies. 3. In the second experiment, the effect of injecting 50 µg of H. influenzae type b-tetanus toxoid conjugates alone or with tetanus toxoid, DTP, or tetanus immunoglobulin was studied. In addition, the H. influenzae type b-cholera toxin conjugated was injected. The results can be summarized as follows:

The H. influenzae type b-tetanus toxoid conjugate elicited a protective antibody response in all juveniles - levels of type b polysaccharide antibodies increased after the second and third immunization.

1. Simultaneous injection of tetanus toxoid or DTP along with the 50 µg dose of H. influenzae type b-tetanus toxoid resulted in an increase of polysaccharide antibodies after each immunization. Simultaneous administration of tetanus immune globulin with the H. influenzae type b tetanus-toxin conjugate did not exhibit a suppressive effect upon capsular polysaccharide antibody synthesis. 2. The highest levels of capsular polysaccharide antibodies were elicited by the H. influenzae type b-cholera toxin conjugate. Injection of either tetanus-toxoid alone or H. influenzae type b polysaccharide alone elicited no change in the

levels of type b polysaccharide antibodies. The polysaccharide antibodies elicited by the conjugate had biological activities and in vitro assays correlated with clinical protection.

Injection of 5 or 50 µg of both H. influenzae type b polysaccharide-tetanus toxoid and pneumococcus type 6A tetanus toxoid conjugates into infants 1-3 months old or rhesus, alone or with tetanus toxoid injected simultaneously but at a separate site, was studied. In addition, infant rhesus also received 45 mg of H. influenzae type b-cholera toxin conjugate. The results of these experiments can be summarized as follows: 1. The 5 µg dose of H. influenzae type b-tetanus toxoid conjugates fail to elicit protective levels of antibodies in the infants after three injections. The 50 µg doses of the conjugates elicited protective levels of H. influenzae type b polysaccharide antibodies in most of the infants after the second injection and in all the infants after the third injections. These antibodies remained at protective levels for at least two months following the third injection. 2. Simultaneous injection of tetanus toxoid along with the non-immunogenic dose of 5 µg of the conjugate elicited protective levels of antibodies to the H. influenzae type b polysaccharide in all of the juveniles after the second and third immunization. These antibodies were also long-lived. 3. Only two of the 16 infant primates responded to pneumococcus type 6 antibodies. These animals were in the group receiving the 50 µg dose. 4. The conjugate composed of H. influenzae type b covalently bound to cholera toxin elicited protective levels of antibodies in all of the infants after two or three injections. These antibodies were also long-lived. 5. The H. influenzae type b and pneumococcous type 6A polysaccharide antibodies elicited in the juveniles, had biologic activities in in vitro reactions that have been correlated with protection against disease.

As a result of the data from these experiments a clinical experiment was conducted in which young college adults were divided into five groups that received either 1) 50 µg of H. influenzae type b-tetanus toxoid conjugate, 2) 100 µg of H. influenzae type b tetanus toxoid conjugate, 3) 50 µg of pneumococcus type 6-tetanus toxoid conjugate, 4) 50 µg each of H. influenzae type b and E. coli K100-tetanus toxoid conjugates at separate sites and, 5) 50 µg each of H. influenzae type b and pneomococcus type 6 tetanus toxoid conjugates injected at separate sites. The results of these experiments are as follows:

1. Side reactions: One of 22 volunteers that received the 50 µg dose of H. influenzae type b-tetanus toxoid conjugate had a systemic reaction with fever of 102° 24 hours following injection. The remainder in this group had no serious systemic or local reactions. The same results were obtained in the 100 µg dose. Local reactions were seen in all the recipients of the pneumococcus type 6A tetanus toxoid conjugate; no systemic reactions were observed in this group. Local and moderate systemic reactions were observed in the group receiving the H. influenzae type b conjugate along with either the E. coli K100 and pneumococcus type 6.

2. All recipients of the H. influenzae type b conjugate alone or in combination responded with at least a four-fold increase in serum and antibodies after the first injection. There was no booster response after the second injection. The geometric mean increase of antibodies was approximately 150-200 fold yielding the highest levels of polysaccharide antibodies in humans we have observed. A similar enhanced response was seen in pneumococcus type 6A tetanus toxoid recipients.

3. There was no relation between the presence of preexisting antibodies and local or systemic reactions. The volunteers are awaiting an additional bleeding approximately 2-3 months after their last injection. The isotype, subclass and biologic activities of these conjugate-induced antibodies is in study.

The effects of various adjuvants upon the immune response to the polysaccharide in laboratory mice has been studied. Fragments of the IgG Fc portion of the polypeptide chain enhanced the type b polysaccharide antibody response about two-fold over that elicited by the conjugates. MDP-N-butyl ester did not accelerate or enhance the type b polysaccharide antibody responses elicited by the conjugates.

Our future plans for this study are: To extend the immunogenicity studies of the H. influenzae type b and pneumococcus type 6A tetanus toxoid conjugates to children ages 2-3 years and then to infants. If the results are satisfactory, we plan to start an effectiveness trial. We will also study the immunogenicity of conjugates prepared with H. influenzae type b and pertussis toxin.

Serum antibodies have been known to confer protection against pneumonia and invasive diseases caused by pneumococci. In addition, several clinical studies have shown that serum antibodies will also confer against otitis media caused by pneumococci. Pneumococci presents its most serious effect upon the health of infants in the 3 months to 2 years age group. During this period of development, the polysaccharides of pneumococcal associated with otitis media also failed to induce protective levels of antibodies in most infant recipients. In addition to our program of both increasing the immunogenicity of and conferring the property of T-cell dependence to the pneumococcal capsular polysaccharides, we have also been studying the use of a species-specific rather than a type-specific antigen. Based upon studies of Briles, at the University of Alabama, we have been studying the purification and covalent attachment of the cell wall polysaccharide of pneumococcus (C-polysaccharide) to carrier proteins. The C polysaccharide is of too small a molecular weight to elicit antibodies even in adult volunteers. Methods for comparing active derivatives for the polysaccharide, using bi-functional "double agent" compounds have been studied. Preliminary evidence shows that the three amino groups, proported to be on the galactosamine of the C polysaccharide may be sterically hindered from interacting with N-hydroxy-succinimide esters of bi-functional reagents. We have succeeded in preparing one derivative of the C-polysaccharide and are in the process of studying the ability of this derivative to link covalently to carrier proteins.

Our preliminary evidence suggest that the immunogenicity of the polysaccharide covalently bound to the carrier protein may be related to its overall molecular weight within certain limits. Methods for preparing homogeneous polysaccharides of defined molecular weight have usually relied upon alkli or acid hydrolysis.

It seems probable that conjugate vaccines manufactured by several pharmaceutical firms, prepared by the synthetic scheme described by our laboratory, will be undergoing extensive clinical evaluation and licensure. We do not yet have an understanding of how to predict their immunogenicity (standardization). One variable in their composition is the molecular size of the polysaccharide used for the covalent binding to proteins. Sonication, which has been used to "shear" DNA, under controlled conditions of polysaccharide concentration, pH,

ionic strength and temperature has been used to reduce the molecular size of bacterial capsular polysaccharides including H. influenzae type b (as an example of a negatively charged phosphate ester polysaccharide), pneumococcal type 9N (as an example of a negatively charge polysaccharide containing uronic acid) and dextran (as an example of a neutral polysaccharide). In contrast to the low yields of heterogenous polysaccharides produced by either acid or alkaline hydrolysis, the products produced by sonication are homogeneous without loss of starting material. This process is being used to produce polysaccharides of various molecular sizes in order to compare the immunogenicity of conjugates prepared with these products.

Typhoid fever remains an important cause of morbidity and mortality in underdeveloped nations. Prevention by vaccination is an important control measure. Our U.S. licensed typhoid vaccine, composed of whole S. typhi cells inactivated with either phenol or acetone, elicits too many adverse reactions to be considered for routine use even in countries with a high attack rate of this disease. S. typhi is the only species of Salmonella that contains a capsular polysaccharide (Vi antigen). A collaborative program for the study of the immunopathogenic role of the Vi antigen in typhoid fever has been established with Dr. Hendrik Koornhof, Associate Director, The South African Institute for Medical Research, Johannesbrug, South Africa, Dr. I.L. Acharya, Infectious Diseases Hospital, Kathmandu, Nepal and Drs. Ramesh Kumar and I. Ghai, Departments of Microbiology and Pediatrics, The All India Institute of Medical Science, New Delhi, India. The aim of this program is to study the protective role of the purified Vi polysaccharide. Initial studies have been concerned with establishing the antiserum agar technique for the rapid and sensitive detection of S. typhi in human specimens including stool cultures and to characterize both the age-specific attack of typhoid fever and the prevalence of serum Vi antibodies in the health population. An inverse relation between the age-specific attack rate of invasive diseases due to encapsulated bacterial pathogens, including H. influenzae type b, meningococci and pneumococci, and of capsular polysaccharide antibodies has been established. The age-specific attack of typhoid fever differs from invasive diseases caused by these encapsulated bacteria. The at-risk age group for typhoid fever ranges in school age children (ages 4 through 15 years), in contrast to the maximum attack rate for the other encapsulated bacteria which is in infants and young children. The basis for this difference in age susceptibility between typhoid fever and other diseases due to encapsulated bacteria is under investigation.

Immunity to invasive diseases caused by encapsulated bacteria (such as meningitis), conferred by capsular polysaccharide antibodies, develops in almost all young adult individuals without contact with the homologous organisms. This age-related acquisition of "natural" antibodies has been shown to be due to a continuous interaction between the host and non-pathogenic cross-reacting bacteria in the respiratory tract and gastrointestinal tract. One of the most obvious examples of this acquisition of "natural" antibodies is the case of Group A meningococci. Group A meningococci are the cause of epidemic meningitis in many countries throughout the world. The last episode in the U.S. of epidemic meningitis due to Group A meningococci occurred in 1949. Since that time, there have been only several cases of Group A meningococcal meningitis reported in migrant individuals in the U.S. Northwest in the late 1970's. No Group A meningococcal strains have been reported in carriers. Despite the viral absence of Group A meningococci in the U.S., about 70% of U.S. Armed Forces recruits and about half of school age children in several studies have been

shown to have Group A meningococcal antibodies. Our laboratory searched respiratory and gastrointestinal samples for organisms cross-reactive with Group A polysaccharide antibodies. We found only one bacterial species, Bacillus pumilis, that precipitated with Group A meningococcal antibodies. The cross-reacting antigen of B. pumilis was identified and the presence of N-acetyl mannosamine phosphate, the monosaccharide component of Group A meningococcal polysaccharide, was identified in this gram-positive species. Yet, it was unlikely that B. pumilis, predominantly a soil bacillus, was a major antigenic stimuli for the wide prevalence of Group A meningococcal antibodies in the U.S. Dr. Nabil Guirguis, in a collaborative experiment with this laboratory and the Infectious Diseases Laboratory, Cairo, Egypt under the sponsorship of the PL480 program, studied pharyngeal and stool cultures of infants and children in several communities for bacteria cross-reactive with Group A meningococci using the antiserum agar technique. He found 11 strains of Escherichia coli, among approximately 600 samples, that yielded immunoprecipitates with Group A meningococcal antibodies. The antigens cross-reactive with Group A meningococcal polysaccharide have been identified as E. coli acidic capsular polysaccharides K93 and K51. Immunization of rabbits with formalin-fixed K93 and K51 strains elicited precipitating and bactericidal Group A meningococcal antibodies. Both K93 and K51 strains have been found among blood cultures and stool specimens from patients at the NIH Clinical Center indicating that these cross-reactive bacteria are present in the U.S. The structures of the two cross-reactive E. coli polysaccharides have been elucidated. K93 is composed of a disaccharide containing a pyrano glucuronic and galactose, but, unexpectedly no phosphate ester. The K51 polysaccharide is composed of the monosaccharide N-acetyl-glucosamine phosphate. The structural basis for the unexpected cross-reaction between K93 and Group A meningococcal polysaccharide, the prevalence of these cross-reacting strains in the U.S. and the reactivity of the two E. coli polysaccharide with adult sera containing "natural" and Group A meningococcal disease-induced antibodies will be studied.

There is convincing evidence that pertussis toxin is the major component of Bordetella pertussis responsible for both the symptoms of pertussis (whooping cough) and vaccine-induced and disease-acquired immunity.

Pertussis toxin is not a cytotoxin. Its mechanism of action is the enzymatic transfer of ADP-ribose to an acceptor protein involved with the regulation of adenylate cyclase. Its enzymatic and toxic actions, therefore, resemble that of cholera toxin. Two substrates have been identified to study this reaction; transducin (retinal protein involved in transduction of the photochemical effect) and Ni (cell membrane protein involved in the coupling of adenylate cyclase regulation). This latter system has been used to study the effect of pertussis toxin in a model system. Pertussis toxin-treated cells no longer respond to the actions of inhibitory hormones of adenylate cyclase regulation with high affinity. This finding serves to explain the in vivo toxicity of pertussis toxin, i.e. intoxinated cells of the pancreatic islet cells for example, do not respond to the hyperglycemic effect of adrenalin.

The nature of the binding of pertussis toxin to the cell surface has been investigated using the serum protein, fetuin, as a model compound. Fetuin, covalently bound to an insoluble support, has been utilized as an affinity resin during final stage of purification of pertussis toxin from the culture supernatant. Pertussis toxin binds to the carbohydrate component of this glycoprotein. Fetuin contains two distinct carbohydrates. The first, is a

sialoligosaccharide linked to aspartic acid. Pertussis toxin does not bind to this component. Unexpectedly, pertussis toxin was shown to bind to the asparagine linked mannose-containing oligosaccharide. A search of the literature showed that this carbohydrate was also found on the IgE immunoglobulin and direct binding studies confirmed that pertussis toxin reacted with this protein. This finding provides an important opportunity to study the effects upon histamine-sensitization and IgE synthesis that have been well known for B. pertussis. It is likely that this direct binding of pertussis toxin with the carbohydrate of IgE is not fortuitous and that this interaction occurs during active infection and after immunization with our current whole cell pertussis vaccine.

Pertussis toxin is sought by many laboratories interested in exploring its effect upon intracellular metabolism. The published methods for cultivation and purification, however, were not satisfactory for preparation of large amounts of homogenous toxin. In addition, pertussis toxin is most probably the major protective antigen of B. pertussis. B. pertussis is a fastidious gram negative; it is not fermentative and releases inhibitors of its growth during its cultivation in a fermenter. Some of these inhibitors form a pellicle which traps the organisms, causes their early death and release of toxic compounds into the media. Techniques to allow the growth the pertussis toxin production without pellicle formation in large scale fermenters were developed. This methodology uses controlled conditions of anti-foam, pH, oxygen tension, different iron concentrations for preparations of the inoculum and growth in the fermenter, etc. Yields of about 2 to 5 mg of pertussis toxin/L have been attained and the purification procedure using affinity resins, previously adapted for growth in large flasks, has also been adapted to extract and purify pertussis toxin from larger yields.

In addition to its unusual array of extracellular products with biological activity, B. pertussis possesses an unusual extracellular product, adenylate cyclase. This extracellular adenylate cyclase has been alleged to be a virulence factor exerting a depressant activity upon phagocytic cells of the lung. Another product with biological activity, the dermonecrotic toxin, is an intracellular product. This activity is inactivated by the heating processes used in vaccine manufacture. Some workers have proposed that this toxin exerts a pathogenic role by inducing local inflammation at the site of B. pertussis in the lung. The dermonecrotic toxin has been purified to close to homogeneity and has been found to be a protein of about 130,000. Treatment of this toxin with proteolytic enzymes causes the formation of adenylate cyclase activity. Our view of this finding is that intracellular adenylate cyclase exists as a proenzyme. This proenzyme has biological activity when injected into the skin. The nature of its dermonecrotic activity and the relation of this toxin to the pathogenesis of pertussis will continue to be under study.

Several approaches to preparing pertussis toxin as a vaccine for the prevention of pertussis have been studied. The first, has been to devise enzymatic assays, using the partial activity of the pertussis toxin, ADP-ribosylation, to monitor the toxicity of the intermediate products. The second, is to device a micro-assay of pertussis toxin antibodies in other to correlate the bioassay used to measure the potency of pertussis vaccines (mouse intracerebral challenge assay) with both the antigenicity and immunogenicity of the intermediate products. The third, has been to study various methods for inactivation of the toxicity (enzymatic activity) of pertussis toxin. The established methods for "toxoid-

ing" toxins such as formalin treatment are under study. In addition, the preparation of conjugates composed of the type b polysaccharide of H. influenzae of pertussis toxin is under investigation. The use of spacer molecules composed for NAD site specific analogues of NAD with a N-hydroxysuccinamide end and adipic acid hydrazide derivatives of the type b polysaccharide has been one approach to this problem.

Unit on Molecular Genetics of Immunity

This unit has devoted its energies to study two aspects of the major histocompatibility Class I antigens. Two approaches have been used. The first, is a structure/function analysis of these complex polymorphic surface structures that are critical in the expression and regulation of immune function. The H2-L^d antigen was studied as a model. Synthetic oligonucleotide directed mutagenesis was used to introduce site-specific mutations into potentially critical areas of the various H-2 domains. The effect upon the antigenic expression of these mutations was studied by transfer of the mutagenized genes into H-2 negative L cells. Monoclonal antibodies, characterized for their reactivities with the H2-L^d antigen and cytotoxic lymphocytes, generated by isoimmunization of isogenic mice, were used to characterize the expression of the new gene products in the L cells. The effect of the following mutations were identified: Mutants, shown by direct sequence analysis to have a deletion of the glycosylation site of the first domain had the same reactivities in the above two immunoassays as the native H-2 antigen. Unexpectedly, the H-2 lacking the glycosylation site was secreted and expressed on the surface of the L cells as the native antigen. Disruption of the S-S linkage in the second domain by replacement of the cysteine residue with serine did not detectably alter the immunological reactivities as L cells transformed with this mutant DNA. Replacement of phenylalanine with tyrosine at position 116, in contrast to the findings obtained with the deglycosylated Class I antigen, caused a considerable loss of reactivity with most of the monoclonal antibodies and cytotoxic T lymphocytes indicating the importance of the tertiary structure at this external portion of the H-2 antigen. The reactivities of new monoclonals specific for the H-2 site has allowed a preliminary assignment of antigenic activity to various regions of the first domains of the H-2L^k antigen.

The expression of the Class I major histocompatibility antigens occurs at as yet an undefined period during embryonic development. The conflicting reports of the embryonic development of expression and function of the H-2 antigens can be traced to the use of polyclonal antiserum and qualitative nature of the assays used to study these structures. The limitation of the previous studies have largely been circumvented by establishment of murine and rat monoclonal antibodies of defined structure and specificity and the use of both in vitro and in vivo embryonic tissue and cell suspensions. The reagents used to detect embryonic H-2 antigens were fluorocesinated monoclonals complemented by the flow fluorocytometer and ^{125}I-labeled antibodies. The sensitivity of these two assay systems was verified by several techniques. Class I antigens are essential for the recognition by the host of a foreign antigen that is associated with a cell surface such as pathogenic viruses. In addition, the Class I antigens are also responsible for most of the rejection phenomenon that occurs between incompatible tissue. These two functions pose important questions about the ontogeny of their expression and function during embryonic development. First, was mechanism(s) is involved in allowing the histoincompatible fetus to survive? And second, is the development of Class I H-2 gene expression related to the

unusual susceptibility of the fetus to teratological effects of intrauterine infection with some viruses (rubella for example) during early embryonic development?

Section on Immunoregulation and Cellular Control

Lymphoid cells in the tissues are in varied stages of their development and have highly differentiated functions. Thus, it has not been possible to isolate and characterize cells that express only one of the many immune functions of lymphocytes from healthy animals. Hybridomas, constructed from a stable malignant lymphoid cell line and lymphocytes from healthy animals have been used to isolate cell lines that are in arrested stages of differentiation and have specialized functions. T-cell hybridomas were constructed from spleen cells of immunized NFR/nude mice. Hybridomas, containing the genome of T-cells expressing one of two distinct functions have been isolated, cloned and characterized. The first, are helper T-lymphocytes at the precursor stage of their development. The second, are suppressor T-lymphocytes at their precursor stage of development. The hybridomas expressing the precursor T-cell suppressor function was investigated for its interaction with a exotoxin of a gram-positive pathogen, Group A streptococcal protein exotoxin. This exotoxin causes fever and altered immune function of lymphoid cells. While the streptococcal exotoxin binds to all T-cells and has been shown to exert a marked but transient effect only on the helper T-cells, the most profound effect is upon native suppressor cells at their precursor stage. This pathway of development is apparently altered and re-directed towards a helper function. This effect is associated with the diminution of the Lyt2 surface antigen (a marker associated with T-cell suppressor and cytotoxic activity). The relation between the alteration in function and the surface antigen is under study.

These T-cell hybridomas provide a valuable source of differentiated cells with regulatory function whose number and activity during development and immunization and are under study. These cells can also be used for study of the effect of immune function/regulation upon infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00073-13 LDMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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:	E.E. Hanna	Head	LDMI, NICHD
Others:	G. Webb	Staff Fellow	LDMI, NICHD
	P. Arora	Staff Fellow	LDMI, NICHD
	M. Walker	Biologist (Tech.)	LDMI, NICHD

COOPERATING UNITS *(if any)*

VR, DRS, NIH (C.T. Hansen, Geneticist)

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Immunoregulation and Cellular Control

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	2.4	.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.)*

Studies were carried out to attempt to understand processes for regulation and control of immune systems at the cellular level. Experiments were conducted using T-cell hybridomas as models to facilitate an understanding of regulatory mechanisms in A) the nude mouse immune system, including precursor cells and development of regulatory cell function. B) Development of regulatory cells for effector, cytotoxic T-cells (CTL). C) Modulation of T-cell function by microbial agents, e.g., streptococcal pyrogenic exotoxin (SPE). Monoclonal precursor T-cell hybridomas were constructed and cloned from antigen primed spleen cells of NFR/nude mice. These clones express several T-cell markers simultaneously (TLa, Thy1, Lyt1, Lyt2 and Lyt3) but in variable amounts as detected by flow cytofluorometry. The clones are hyperploid and expressed the allelic markers of both parental cell types (NFR-nu spleen cells and BW5147 thymic lymphoma cells of AKR origin). Clones derived from primed Pool 1 (eluted first from nylon wool columns) nude spleen cells express a helper function and those derived from primed Pool 2 (eluted second) nude spleen cells express a suppressor function. Neither type shows immunogen-carrier specificity and are thus developmentally blocked prior to a step required for the expression of "carrier specificity." During the development of T-cell function microbial agents such as SPE could divert/change the phenotypes of these precursor T-cell hybridomas. This effect is relevant to our understanding of the mechanisms by which microorganisms modulate (deregulate) immune systems. Cytotoxic or effector (CTL) clones recognizing and reactive with self + hapten determinants have been constructed. SPE was observed to suppress the function of cytotoxic effector T-cells (CTL) generated from precursors in 5 day spleen cell cultures recognizing NFR, $H2^{q}$-TNP (hapten-self immunogens). The relevance of these models is that the immortal cell clones which are capable of unlimited growth and survival and expressing functional phenotypes allow for authentic and meaningful study of pathways of regulation in immune systems and in biological systems in general. Alternative mechanisms for regulation and deviation of functions may be discovered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00918-03 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Histocompatibility Antigens During Early Mammalian Development

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

PI:	K. Ozato	Head	LDMI, NICHD
Others:	S. Adeniyi-Jones	Visiting Associate	LDMI, NICHD
	Y.-J. Wan	Visiting Fellow	LDMI, NICHD
	B. Orrison	Chemist	LDMI, NICHD
	R. Manjunath	Visiting Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*
H. Westphal, LMG, NICHD
A. Mukherjee, HGB, NICHD

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Unit on Molecular Genetics of Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.6	0.6

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

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

Major histocompatibility (MHC) Class I gene activation occurs during embryonic development, so that at birth most of somatic cells express the antigens derived from both paternal and the maternal MHC genes. The precise timing, and the types of Class I antigens expressed during embryonic development have been so far controversial. Class I antigen expression during fetal development poses another unsolved immunological question, i.e. even though the fetus carry histoincompatible paternal Class I antigens, allograft rejection is no detected during pregnancy. To determine Class I antigen expression in embryogenesis we studied cell surface expression of Class I antigens throughout mouse gestation. Two series of monoclonal antibodies, one reacting with all types of Class I antigen (rat xeno antibodies), another reacting with classic, polymorphic H-2 antigens (mouse alloantibodies) were employed. A significant antibody binding was noted in embryos at gestation day 10 (somite stage) and after. No binding was detectable in earlier embryos. Thus the time of the antigen expression is later than previously indicated. Only monoclonals reacting with all types of Class I antigens were positive on day 10 embryos. Studies on the onset of Class I gene transcription and that of the protein synthesis are under way. Studies of the effect of anti-paternal Class I antibodies (often produced in pregnancy) on the fetal immune development have been continued; radiolabeled monoclonal antibodies were injected into pregnant mice, radioactivity was found in various fetal tissues, much of which represented intact Ig as tested by SDS-gel electrophoresis, indicating active passage of the antibodies to the fetuses. Class I antigen expression and the immune function of the animals treated with the antibodies are being studied. To elucidate T cell activity in pregnancy, the ability of generating cytotoxic T cells in multiparous mice was examined. We found that during pregnancy T cell reactivity is markedly increased in allopregnant animals, but not in syngeneic matings indicating maternal allosensitization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00920-03 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI:	K. Ozato	Head	LDMI, NICHD
Others:	T. Shiroishi	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*
G.A. Evans, Slak Institute, San Diego, CA; N. Tada, Tokai University, Japan; R. Appella, LCB, NCI, NIH

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Unit on Molecular Genetics of Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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.)*

Major histocompatibility (MHC) Class I antigens are highly polymorphic. There are numerous alleles. Each molecule contains multiple amino acid substitutions; this polymorphism is required for recognition of viral pathogens by T cells. Our goal has been to delineate structure-function relationships of the Class I antigen. To this end DNA sequence encoding a mouse Class I gene is modified in vitro to examine the functions associated with the modification. In the past year oligonucleotide directed mutagenesis has been employed to introduce single amino acid substitutions into the H-2L^d gene. The following mutations were generated: [1] The S-S linkage in the 2nd domain was disrupted by replacing Cys with Ser[2] The glycosylation site was removed from the first external domain. [3] Phe at position 116 was changed to Tyr to replace the L^d residue with the K^b type amino acid. The mutant genes were transferred into L cells by DNA mediated gene transfer and the products of the mutants were examined for surface expression, antigenicity by antibody binding, and for T cell reactivity. The mutant gene with the disrupted disulfide bridge was expressed on cell surface, indicating that the disulfide bridge is not required for surface expression of the antigens even though it is highly conserved throughout mammalian species. However, numerous antigenic determinants for T cells and for antibodies were no longer found in the mutant antigen, indicating that the tertiary structure dictated by the disulfide bridge is important for the function of the Class I antigen. The mutants with the altered glycosylation site and that with altered amino acid at position 116 expressed antigens identical to those of the wild type gene products. Studies of domain specificities of numerous new monoclonal antibodies reacting with the H-2D^d antigen has been completed. Comparisons of amino acid sequences among different H-2 antigens including D^d led us to propose distinct amino acid positions to be the antigenic sites. Based on these predictions systematic introduction of mutagenesis is planned for the H-2D^d gene to identify the functional sites of the antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01300-02 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enhancement of Immunogenicity of Bacterial Polysaccharide and Protein Antigens

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

PI:	R. Schneerson	Medical Research Officer	LDMI, NICHD
Others:	D. Tietz	Visiting Fellow	LDMI, NICHD
	Z. Wang	Visiting Fellow	LDMI, NICHD
	N. Guirguis	Visiting Fellow	LDMI, NICHD
	S. Szu	Senior Staff Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*
A. Chrambach, ERRB, NICHD

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.3	

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

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

Conjugates were prepared by carbodiimide-mediated coupling of adipic acid dihydrazide derivatiaves of H. influenzae type b (Hib), pneumococcus type 6A (Pn6A) and E. coli K100 with tetanus toxoid (TT) and the immunogenicity characterized in laboratory mice and in primates. The Pn6A conjugates were less immunogenic than the Hib conjugates in laboratory animals. The immunogenicity of K100-TT was similar to the Hib-TT, but some variability was noted in both though their physical-chemical characteristics such as molecular size and polysaccharide/protein ratio were similar. Studies were initiated to further characterize the conjugates by physical-chemical methods to enable in vitro prediction of immunogenicity (standardization) Preliminary results of applying the Hib-TT to agarose gel electrophoresis showed that about two-thirds of the conjugate could be electrophoresed in a 2% gel; about one-third would not enter the gel. The electrophroesis was regulated by the ionic strength of the buffer used; low ionic buffer would aggregate the conjugate.

In search for methods of preparing conjugates reproducibly, a method of CNBr activation at neutral pH was adjusted to Hib, and sonication was utilized to produce polysaccharides of similar, lower molecular size.

Attempts at reproducibly derivatizing E. coli K1 polysaccharide were not successful.

Adherence to mucous membranes is an important initial event in bacterial colonization and may be related to their pathogenicity. Pili, shown to be the adherence mechanism in several bacterial species, have been recently demonstrated in H. influenzae. Studies were initiated to isolate and purify Hib pili, to evaluate their role in pathogenesis and immunity. Pili expression of several H. influenzae type b strains from disease isolates; CSF and epiglotitis and from carriers, was enriched utilizing their adherence properties to human RBC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01301-02 LDMI

PERIOD COVERED: October 1, 1983 to September 30, 1984

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:	R. Schneerson	Medical Research Officer	LDMI, NICHD
Others:	J.B. Robbins	Head	LDMI, NICHD
	Z. Wang	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS (if any)

A. Sutton, OoB; G. Schiffman, State Univeristy, New York; J.C. Parke, Charlotte Memorial Hospital, North Carolina; J. Schlesselman, USUHS, Bethesda, Maryland

LAB/BRANCH: Laboratory of Develomental and Molecular Immunity

SECTION: Section on Bacterial Disease and Molecular Immunity

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Haemophilus influenzae type b is the leading cause of bacterial meningitis in infants and children. It is also a major cause of septicemia, septic arthritis pneumonia and epiglottitis. Pneumococcus type 6A (Pn6A) is a major cause of otitis media and the most frequent pneumococcal type causing meningitis and pneumonia. Anticapsular antibodies are protective against disease but their induction by vaccines composed of purified capsular polysaccharide is hampered by both their poor immunogenicity in this young age group and lack of anamestic response. In contrast, conjugates composed these polysaccharides covalently bound to tetanus toxoid were immunogenic in laboratory mice and infant rhesus; and this response could be boosted by further injections. Simultaneous injections of both conjugates with tetanus toxid or with DTP enhanced the response to both polysaccharides.

Adult volunteers were immunized 2 times at 3 week intervals with conjugates composed of H. influenzae type b, the closely related E. coli K100 or Pn6A polysaccharides and tetanus toxoid in the following schedule: Group 1: Hib-TT, 50 µg/dose; Group 2: Pn6A, 50 µg/dose; Group 3: Hib-TT, 50 µg + Pn6A, 50 µg; Group 4: Hib-TT, 50 µg + K100-TT, 50 µg; Group 5: Hib-TT, 100 µg.

Local and systemic reactions were noted in about half of the vaccinees following the first immunization, especially in the groups that received the high dose (100 µg total) vaccines. No serious reactions occurred. 50 µg Hib TT alone was given to groups 3, 4, and 5 for the 2nd immunization. The antibody responses, assayed by RIA and ELISA, showed marked increases in antibody levels in > 95% of the volunteers. Hib and TT antibodies increased 10-1000 fold, Pn6A: 5-20 fold. A maximal response occurred in most volunteers after the 1st injection, with no booster response after the 2nd. No relation was found between the preimmune level of antibodies to the vaccine components or the rate of antibody rise to the side effects of the vaccines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01302-02 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Toxins of Pertussis: Isolation, Characterization and Mechanisms of Action

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

PI:	R.D. Sekura	Research Chemist	LDMI, NICHD
Others:	M.-J. Quentin-Millet	Guest Researcher	LDMI, NICHD
	Y.-L. Zhang	Visiting Fellow	LDMI, NICHD
	N. Tolson	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*
L. Birnbaumer, Baylor College of Medicine; H. Bourne, University of California; T. Reisine, J. Axelrod and W. Klee, NIMH; T. Cote and J. Kebabian, NINCHD; R. Downs, University of Virginia

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.7	0.5

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

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

Bordetella pertussis, the microorganism which causes the disease commonly known as whooping cough, produces several toxins (i.e., pertussis toxin (PT) and heat labile or dermonecratic toxin) (see project Z01 HD 01306 01) which appear to play important roles in pathogenesis of the organism. PT in addition is a major protective antigen which is a promising candidate for the development of a new acellular pertussis vaccine (see project Z01 HD 01307 01). The current project concentrates on elucidating the mechanisms by which pertussis toxin interacts with cells and elicits its diverse pharmacologic actions. The initial event in the interaction of PT with cells appears to be a rapid and essentially irreversible binding of toxin to cells. Using the interaction of PT with fetuin as a model, studies have been conducted which demonstrate that PT interacts with the carbohydrate moieties present in fetuin. Similar carbohydrate structures are present on cell surfaces and in a variety of serum glycoproteins including IgE and other immunoglobulins. The significance of these observations relative to the intoxication of cells and to specific adjuvant effects exhibited by PT are under consideration.

The mechanism of action of PT is the toxin catalyzed transfer of an ADP-ribose moiety from NAD to specific acceptor protein (N_i). N_i is a cell membrane protein, which couples hormone receptor interaction to modulation of adenylate cyclase. Most of the effects elicited by PT appear to be associated with the covalent modification of N_i. PT has served as an effective probe in purification of the regulatory N_i component and in elucidating the molecular mechanisms by which N_i acts in modulation of adenylate cyclase by inhibitory effectors. It has been established that N_i, in part, functions by changing the affinity of the hormone receptor for ligand, thus when cells are treated with PT high affinity of binding of ligand is eliminated. Additional studies are in progress attempting to define more clearly the role of N_i.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01304-02 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

PI: J.B. Robbins Head LDMI, NICHD

COOPERATING UNITS *(if any)*
H. Koornhof, South African Institute of Medical Research; I.L. Acharya, Infectious Diseases Hospital, Kathmandu, Nepal; R. Kumar, All India Institute of Medical Sciences; C.U. Lowe, OD, NICHD

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.9	0.9	

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

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

Typhoid fever remains a serious cause of morbidity and mortality throughout underdeveloped nations. The immunopathogenic role of the capsular polysaccharide of S. typhi, the causative agent of typhoid, is controversial. There is much indirect evidence that serum Vi antibodies could exert protein against typhoid fever. Typhoid is only a disease of humans; there is no satisfactory animal model. Collaborative studies with Dr. H. Koornhof, South African Institute of Medical Research, I.L. Acharya, Infectious Diseases Hospital, Kathmandu, Nepal and Dr. Ramesh Kumar, All India Institute of Medical Sciences have been established to study the prevalence of Vi antibodies in the population, the age-specific attack rate and the ultimately effectiveness of Vi polysaccharide vaccines in these various areas. The Vi polysaccharide has been purified and derivatized with tyramine for use as a ^{125}I antigen. The technique for preparing the tyramine derivatives and purification of the Vi is being prepared for publication. A clinical study of the Vi polysaccharide, prepared in our laboratory in 1974 BB-IND 660 was done in collaboration with Dr. Myrone Levine, University of Maryland. This Vi preparation passed the current FDA guidelines for pyrogen content of meningococcal cpasular polysaccharide vaccines. However, when administered by jet gun, one of 24 volunteers developed 102°C. In addition to Vi antibodies, O-specific antibodies were elicited in the volunteers. Accordingly, the high specific activity of S. typhi LPS and the effect of the jet gun upon immediate reactivity necessitates that Vi products of higher purity be prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01305-01 LDMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Characterization of the Group B Meningococcal (E. coli K1) Antibody Binding Site

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

PI: J.B. Robbins Head LDMI, NICHD

COOPERATING UNITS *(if any)*

J. Bierly, Cornell Medical College

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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 01306-01 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Pertussis Heat Labile Toxin (HLT): Isolation and Characterization

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

PI:	R.D. Sekura	Research Chemist	LDMI, NICHD
Others:	Y.-L. Zhang	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*

None

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Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

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

Bordetella pertussis produces several protein toxins including pertussis toxin (see project Z01 HD 01302-02) and dermonecrotic toxin or heat labile (HLT). HLT, when injected subcutaneously into rabbits or suckling mice, results in a local pronounced hemorrhagic lesion. The extent, localization and nature of this toxin-induced injury suggests HLT contributes to the pathogenesis of B. pertussis. Conditions for assay of the toxin using the suckling mouse model have been established. By use of conventional protein purification techniques and high pressure liquid chromatography, nearly homogeneous preparations of HLT have been obtained. These studies show that the toxin is a single polypeptide chain with a molecular weight about 150,000.

During the course of these studies it was observed that adenylate cyclase activity copurifies with HLT during the early stages of purification. However, these components can be resolved during later stages of purification. Studies with ATP analogs and other agents indicate that HLT and the adenylate cyclase exhibit similar sensitivity to inhibition. These data suggest a possible relation between adenylate cyclase and HLT, further studies are necessary. Preliminary data suggests that the action of proteolytic enzymes on purified preparations of HLT lead to an increased adenylate cyclase activity in these preparations. Thus, a proenzyme enzyme relation is possible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01307-01 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	R.D. Sekura	Research Chemist	LDMI, NICHD
Others:	Y.-L. Zhang	Visiting Fellow	LDMI, NICHD
	M.-J. Quentin-Millet	Guest Researcher	LDMI, NICHD
	S. Lerher	Guest Researcher	LDMI, NICHD
	N. Tolson	Biologist	LDMI, NICHD

COOPERATING UNITS *(if any)*
D. Rogerson, NIAMDD; C. Johnson, NIAID

LAB/BRANCH
Laboratory of Develomental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 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.)*

The incidence of pertussis infection has been effectively controlled by use of current whole cell pertussis vaccines. However, recent advances identifying pertussis toxin (PT) as a major protective antigen against B. pertussis infection and disease affords an opportunity to produce a new pertussis vaccine with improved safety and efficacy. Another project (Z01 HD 01302) is in progress directed at characterizing the biochemical action of PT. The current project concentrates on development of methods for production of larage amounts of toxin as well as methods for neutralization of toxic action and assessing the immune response to toxins thus neutralized. By this approach it should be possible to establish a scientific basis for the development of a new pertussis vaccine.

In order to obtain sufficient amounts of PT to permit studies on vaccine development, studies were initiated to establish conditions via which growth of the organisms could be achieved in large scale fermentators.

After extensive study, growth conditions and inoculum size have been established permitting large scale cultivation of the organisms. A suitable anti-foam was found which does not adversely effect bacterial growth or PT production, and optimal conditions for aeration during growth were established. Additional studies are in progress to determine factors which might lead to enhanced PT production.

In addition, enzyme linked immunoabsorbent assays (ELISA) are under development which will permit quantitation of the immune response elicited by the various preparations of inactivated pertussis toxin. By using this approach it should be possible to establish what methods of inactivaiton are suitable for preparation of effective immunogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01308-01 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pneumococcal Cell Wall Polysaccharide Protein Conjugation

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

PI: S.C. Szu Senior Staff Fellow LDMI, NICHD

The Principal Investigator on Project Z01 HD 01303-01 LDMI, John B. Robbins, has been changed to S.C. Szu and the project transferred to Z01 HD 01308-01 LDMI.

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

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

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

Antibodies against pneumococcal cell wall polysaccharide (C-ps), a species specific moiety, is protective against pneumococcal infection in mice. The C-ps alone is a poor immuogen and methods for preparing covalent conjugates with T-dependent proteins were sought. Since one of the major immuno determinants of the C-ps, phosphocholine, is alkaline labile, two bifunctional chemical reagents were chosen for the conjugation process that are reactive at neutral pH. Succinimidyl N-(2-haloacetyl)-β-alaninate iodine salt bears an SH-reactive haloacetyl and NH_2-reactive N-hydroxysuccinimide ester group which could react with the amino sugar on the C-ps. Using total iodine analysis and S-35 labeled glutathione as the haloacetylo substrate to measure derivatization, the optimal linker/C-ps binding molar ratio was shown to be 0.5% to 1%. The second cross-linking reagent tested, succinimidyl 3-(2-pyridyldithio) propionate (SPDP), similar to SIAP, has a NH_2-reactive N-hydroxy-succinimide ester group that could react with C-ps. It also has an "activated" disulfide bond, (2-pyridyldithio)-propionate which undergoes thiol-disulfide interchange with SH groups on proteins. The SPDP-C-ps reaction resulted in 4% to 6% derivatization with C-ps. We plan to use bacteria toxoids as the conjugating protein and then to study the immunogenicity of the C-ps in mice.

The size of the polysaccharides is an important factor in determining the immunogenicity and the ability to form conjugates with proteins. Further, low molecular weight polysaccharides can facilitate NMR studies of polysaccharide-ligand interaction. We have applied continuous sonication to polysaccharides. Results show that sonication of high molecular weight polysaccharide at optimized power can reduce the molecular weight of a model compound, dextran from 2,000,000 to 100,000 in 5 minutes and to 10,000 in 30 minutes independent of the type of the polysaccharides tested. This finding enables us to produce chemically unaltered lower molecular size polysaccharides at designated sizes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01309-01 LDMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bacterial Polysaccharides Cross-reactive with Meningococcus Group A Polysaccharide

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

PI:	R. Schneerson	Research Medical Officer	LDMI, NICHD
Others:	N. Guirguis	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*
J.D. Maclowry, CC, CP; W. Eagan, OoB; Ida and Frits Ørskov, Statens Serum Institute Copenhagen, Denmark

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
0.3	0.3	

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

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

Similar to other encapsulated pathogens, serum anti-capsular antibodies confer immunity to invasive meningococcal diseases. Group A meningococcal disease has a different epidemiological pattern than the other two major pathogenic meningococcal Groups B and C. In central Africa, Group A diseases are endemic with a high frequency. In other parts of the world Group A causes epidemics, lasting 1-2 years. In both cases asymptomatic carriage of Group A organisms is low. In the U.S., Group A meningococcal disease has been virtually non-detected in the past 25 years, yet most children and adults have protective levels of Group A antibodies. Investigations to elucidate the basis of the natural immunity to meningococcus Group A showed 11 E. coli strains of 645 stool isolates were cross-reactive with meningococcus Group A. These strains were serotyped, their polysaccharides purified and their structures and immunological cross-reactivities studied. The structure and the immunological properties of the previously identified cross-reactant, B. pumilis (SH17) were compared as well.

Ten of the 11 strains were found to be K93: O107: H27, one was K51: O7: H18. None produced enterotoxin or were invasive. Antisera were raised to the two E. coli types and to SH17. Double immunodiffusion and quantitative precipitin analysis showed partial identity between meningococcus Group A polysaccharide and each of the cross-reactants and non-identity among themselves.

Preliminary structural analysis showed the K93 polysaccharide to be composed of 3-D gal f-1-4 βD-glucuronic acid. The K51 is a polymer of αD-glucosamine phosphate. Antibodies elicited by these E. coli strains precipitated with and were bactericidal against meningococcus Group A.

Z01 HD 00040-09 Theoretical Studies and Modeling of Hormone Receptor Interaction
Peter J. Munson

Z01 HD 00165-09 Isolation and Characterization of Protein Hormones
Andreas Chrambach, Ph.D.

Z01 HD 00171-08 Electrophoretic Methodology
Andreas Chrambach, Ph.D.

Z01 HD 00188-04 Development and Evaluation of New Dimeric Analogs of Enkephalins
David Rodbard, M.D.

Z01 HD 00189-03 Development of Statistical Software for Use by Clinical Investigators
David Rodbard, M.D.

Z01 HD 01400-02 Clinical Applications of Stable Isotopes
Alfred L. Yergey, Ph.D.

Z01 HD 01401-02 Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry
Alfred L. Yergey, Ph.D.

Z01 HD 01402-01 The Volume and Surface Area of the Cytoplasmic Matrix and Intercellular Diffusion
Nahum D. Gershon, Ph.D.

Z01 HD 01403-01 The Three Dimensional Organization of Cells and Anatomical Components
Nahum D. Gershon, Ph.D.

Z01 HD 01404-01 Characterization of Opioid Receptors in Brain and Peripheral Tissues
David Rodbard, M.D.

Laboratory of Theoretical and Physical Biology
Annual Report, FY 1984
David Rodbard, M.D.

This laboratory is concerned with the application of biophysical, physical chemical, mathematical, statistical, and computational techniques to the study of fundamental biological processes, with clinical applications.

The interaction of hormones and neurotransmitters with their receptors has been analyzed by a combined theoretical and experimental approach. This has permitted the unequivocal demonstration of the existence of the mu-1 subtype of opiate receptor, which is responsible for analgesia and hypothalamic control of pituitary function. Mathematical modeling and computerized methods are used to permit improved analysis of ligand-binding systems. Clinical applications of computers are under development and evaluation, e.g. to assist with implementation of algorithms for self adjustment of insulin dosage.

Conformations of proteins and nucleic acid are studied on the molecular level using polyacrylamide gel electrophoresis, isoelectric focusing, and chromatographic techniques. New ultrasensitive methods including high performance liquid chromatography/thermospray mass spectrometry have been used to study intermediary metabolism in man, including glucose, steroid, carnitine and calcium kinetics in normal subjects and in a variety of disease states.

The activities of the laboratory are divided into three sections or units:

I. Section on Theoretical Biology;
II. Section on Macromolecular Analysis;
III. Unit on Metabolic Analysis.

I. Section on Theoretical Biology.

A. Computer Programs: We have continued development of computer programs for data analysis of interest to biochemists, pharmacologists, and multiple other disciplines. Our programs for analysis of ligand-binding systems have been expanded, improved, and widely distributed.

Siginificant activities include:

1. Development of a new and novel approach to characterize receptor system as a "two-dimensional affinity spectrum".

2. Development of a new method, the "Kd versus Kd plot", to characterize complex receptor systems, to identify distinct classes of binding sites, and evaluate reproducibility of findings between experiments.

3. Development of the theory and of computer programs to permit a "multi-ligand" approach, wherein two or more unlabeled ligands are present simultaneously with the labeled ligand. We show, both in theory and practice, that analysis of these "dose response surfaces" is a powerful and sensitive tool for demonstration of multiple types and subtypes of receptors.

4. Development of methods for optimization of experimental design and for improved hypothesis testing to detect multiple receptor sites or states.

5. Discovery of a potential source of error in the popular "Cheng-Prusoff correction", and development of an exact "correction to the correction".

6. Development of computer programs for analysis of enzyme substrate-inhibitor and transport systems, and their successful use for characterization of folate-FdUMP-polyglutamate-methotrexate interactions with thymidylate synthetase.

7. Development of programs for analysis of multicompartmental exponential decay processes and radiation inactivation processes.

8. Development of improved programs for analysis of immunoassays, using 5, 6 and 7 parameter extensions of the four parameter logistic equation.

9. Adaptation of a number of programs to operate on economical microcomputers.

10. Development of a new form of correlation analysis, to study the complex multivariate interactions among multiple steroids in response to ACTH stimulation. This analysis can reveal major biochemical pathways, and indicates the presence of multiple abnormalities and increased variability in patients with idiopathic hirsuitism.

11. Development of a computerized "card-file" system.

12. Development of software to facilitate retrieval of references and incorporation of bibliographies into manuscripts.

13. Development of programs for statistical estimation of normal ranges of results from the clinical laboratory.

14. Development of improved programs for RIA Quality Control.

The programs for ligand binding analysis have been used extensively in collaborative studies, including applications to thrombin receptors on platelets, histamine receptors on leucocytes and in lung, glucocorticoid receptors in resistant human cells, dopamine receptors, and monoclonal antibodies. The other programs have also been used extensively.

B. Characterization of opioid receptors in rat brain: We have utilized several of the new theoretical and computational methods described above, for the characterization of opioid receptors in rat brain. We have demonstrated the existance of a mu_1 subtype of receptor, defined operationally as a binding site with very high affinity ($K_d \approx 0.1$ nM) and lack of selectivity for mu- or delta-selective ligands (e.g. "DAGO"=D-Ala^{2}MePhe^{4}Gly-ol^5, enkephalin and "DADLE"=D-Ala^2 D-Leu^5 enkephalin, respectively). This receptor subtype had been proposed by Pasternak on the basis of its pharmacological properties.

We were able to demonstrate mu_1, by virtue of the computerized analysis and optimization of experimental design, and by refinement of experimental technique. We demonstrate that DAGO binds to two classes of sites, (μ_1,μ_2); that DADL binds to three classes of sites (μ_1,μ_2,δ); and that naloxone binds to at least four classes of sites (μ_1,μ_2,δ,κ). We

demonstrate that the dimer, naloxonazine, has very high affinity and is selective for the mu_1 subtype. Naloxonazine pretreatment of membranes will reduce the number of mu_1 sites, apparently irreversibly. In addition, it has competitive effects at both the mu_2 and delta sites. The competitive effects appear to predominate in producing the 'naloxonazine-shift'. These techniques for characterization of receptor systems should be widely applicable to other types of opiate receptors (e.g. kappa), and to other systems as well.

C. Dimeric Enkephalins: We have designed a series of dimeric analogs of enkephalins, which have been synthesized by Dr. Y. Shimohigashi in Dr. Chen's laboratory, ERRB, NICHD. These compounds have been analyzed for several activities, including mu and delta receptors in brain, delta receptors in brain, delta receptors in neuroblastoma-glioma hybrid cells (NG108-15 cells), adenylate cyclase in NG108-15 cells, muscle bioassays (guinea pig ileum, mouse vas deferens), and antinocioceptive assays involving intracerebroventricular (i.c.v.) or intrathecal injection. New findings this year include:

1. The dimeric tripeptides show a dramatic increase in affinity relative to monomer;

2. Dimeric tripeptide enkephalins show optimal mu-selectivity when the shortest cross linking methylene bridge (n=2) is used. In contrast, delta activity has an optimum for longer connecting chains (n=16-22).

3. Reduction of the density of receptors on membranes using an irreversible blocker, "FIT", results in no differential effect on the affinity of the dimeric pentapeptide DPE_2 relative to its monomer.

4. Dissociation studies fail to show an enhanced rate of dissociation of DPE_2 in the presence of unlabeled ligand.

The above 4 findings suggest that: a) The receptors for enkephalins are arranged as dimers; b) the dimeric enkephalins bind to 2 sites within a receptor, but not to two widely separated receptors; c) binding of two dimers, or of one dimer and one monomer, cannot occur simultaneously at any given receptor.

5. Alkylamides of enkephalins are being studied to evaluate the role of the hydrophobic crosslinking moiety, and monoacetyl-tyrosine enkephalin dimers are being studied to evaluate the role of bivalency.

D. Use of Computer Programs for Clinical Studies.

1. We have developed programs to assist with self-adjustment of insulin dosage. These programs, for small microcomputers, can be used in the physician's office, in clinics, or even in the patient's home. Our program is unique, providing considerable flexibility and versatility (6 regimens, 7 levels of control, 5 levels of aggressiveness) and provision of explanations for recommended changes in insulin dosage).

2. Three Dimensional Image Reconstruction.

An improved micro-computer system has been developed to permit three dimensional reconstruction of anatomical interrelationships on the ultrastructural, microscopic or gross-anatomical levels. This has been successfully applied to:

1) study of the surface/volume relationships of the intracellular trabecular network (cytoplasmic matrix);

2) Study of the cell central body and Golgi apparatus;

3) Study of the "whorl body" complex in selected nuclei of the hypothalamus in response to aging and hormonal manipulation:

4) Initiation of a project for illustrating the 3 dimensional interrelationships between neurotransmitters and their receptors in brain.

II. Section on Macromolecular Analysis.

This unit has pursued its goal of development of universally applicable, high resolution techniques and strategy for fractionation of macromolecules. The methods of polyacrylamide gel electrophoresis, isoelectric focusing, and chromatography have been refined and aplied to a number of important proteins, including the human growth hormone, receptors for steroids and protein hormones, bacterial proteins, and immunogens and components of the renin-angiotensin system.

1. Human growth hormone prepared from a bacterial source was isolated with high yield in a new large-scale, single step electrophoretic procedure.

2. Methods have been developed for fractionation of intracellular organelles (e.g. receptosomes).

3. Methods have been developed for other large particles, e.g. viruses and crosslinked, aggregated bacterial immunogens.

4. The properties of multicomponent chemically defined systems for isoelectric focusing have been studied and found to be in excellent agreement with the predicted properties using the theory and computer programs of Dr. L. Hjelmeland.

5. New methods are under development for two-dimensional macromolecular mapping of proteins. Combinations of pore limit gradient electrophoresis and Immoboline (TM) gels may provide a steady-state method, and hence more reproducible results.

This section has conducted extensive research to identify possible artifacts generated during isoelectric focusing and gel electrophoresis, and to develop techniques to prevent such artifacts and to alert the investigator to their presence.

The use of wide variety of detergents has been studied in a systematic manner, in collaboration with Dr. L. Hjelmeland who developed many of the detergents now extensively used for receptor purification of receptors and other integral membrane proteins.

III. Unit on Metabolic Analysis

This unit has pioneered the development of new techniques and approaches to mass spectroscopic analysis of biological molecules. The development of thermo spray apparatus and optimization of conditions has permitted continuous on-line analysis of the effluent from high performance liquid chromatography (HPLC),

with extraordinary simplicity, sensitivity and specificity. This technique has now been applied to glucuronides, acetylcholine, carbamyl choline, carnitines, phospholipids, platelet activating factor, and d-tubocurare analogs. A related method (thermal ionization) has been developed and applied to the measurement of calcium isotopes and other metals (zinc and magnesium in particular).

Methods have been developed to measure glucose, amino acids, and steroids. These new methods are rapidly being employed in collaborative studies with several number of groups of clinical investigators at NIH and throughout the country. Selected applications now in progress include:

1. Study of calcium metabolism in neonates and during normal growth and development, in pregnancy and lactation, in osteoporosis, and in response to growth hormone, sex steroids, normal puberty, Cushing's disease and other states of glucocorticoid excess, and in disorders of vitamin-D metabolism;

2. Studies of hepatic uptake and production of glucose in glycogen storage disease, type I (glucose-6-phosphatase deficiency);

3. Studies of acetycholine levels in brain and cultured cells (with LDN, NICHD).

4. Studies of acyl-carnitines in various organic acidurias.

These studies also involve multicompartmental analysis and mathematical modeling of metabolic pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00040-09 LTPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Theoretical Studies and Modeling of Hormone Receptor Interaction

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

PI:	P. J. Munson	Mathematical Statistician	LTPB, NICHD
Other:	D. Rodbard	Head	LTPB, NICHD
	R. Lutz	Visiting Scientist	LTPB, NICHD
	R. Cruciani	Visiting Fellow	LTPB, NICHD
	V. Guardabasso	Statistician	LTPB, NICHD
	M. Beveridge	Guest Worker	LTPB, NICHD

COOPERATING UNITS (if any)
N. Catholica del S. Cuore, Rome, Italy (M. Pocchiari), Max Planck Institute Fur Psychiatrie, Munich, W. Germany (T. Costa).

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

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

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

The development and refinement of analytical tools and methodology has continued for the analysis of receptor binding. These studies have focused on the opiate receptor system in rat brain where the need for models of multiple receptor subtypes is acute. Advances in the technique have allowed a more ambitious series of modeling studies to be undertaken. A rigorous demonstration of the Mu-1 receptor subclass has been achieved. Theoretical developments which have contributed to this include development of a graphical means of displaying multiple binding sites (the "Kd-Kd plot"), exploration of the mathematical properties of multiple site models including enumeration techniques, development of noniterative techniques for binding data analysis (the "2 dimensional affinity spectrum"), and enhancement of mathematical curve fitting techniques. Mathematical optimization of experimental design to optimize efficiency and precision of results also contributed to progress.

Studies of asymmetric dose-response curves for radioimmunoassay (RIA) were undertaken. New, more efficient computer software modeling tools have been implemented for description of hormone-receptor interactions and enzyme-substrate interactions (programs designated LIGAND-83, Fortran-LIGAND, ENZYME, PC-LIGAND, PC-ALLFIT, EXPFIT, ALLGRF).

An investigation into measures of randomness of residuals for multivariate non-linear models was begun as alternatives to the F-test.

An exact version of a very commonly used approximate calculation method ("Cheng-Prussof correction") was found. Use of the exact method may significantly reduce the errors in estimation of inhibition constants (Ki values).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-09 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Isolation and Characterization of Protein Hormones

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

PI:	A. Chrambach	Head	LTPB, NICHD
Other:	G. Kapadia	Guest Worker	LTPB, NICHD
	D. Tietz	Visiting Fellow	LMI, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Physical Biology, NIAMD, NIH (M. Gottlieb); Laboratory of Vision Research, NEI, NIH (B. An der Lan); Genentech, Inc., San Francisco, California (A. Jones).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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)*

1) Clathrin coated vesicles from brain and liver were fractionated by particle size, using agarose gel electrophoresis. The brain vesicle preparation consists of two components. Particle sizes were determined by Ferguson plot, using viruses as size standards.

2) Meningitis immunogen (prepared by crosslinking the bacterial coat carbohydrate with tetanus toxin) was fractionated on a 22 micron filter into a retained (66%) and a filtered (33%) species. The latter migrates as a single polydisperse zone on agarose gel electrophoresis. Immunogenicity of that zone and of the filterable component were tested to establish a physical criterion correlated with activity.

3) The procedure for the electrophoretic isolation of human growth hormone (hGH) from transformed bacteria was improved, and apparatus design for application of that procedure to the scale of at least 100 g per apparatus per man-year was initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00171-08 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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. Fawcett	Visiting Scientist	LTPB, NICHD
	D. Tietz	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*

Jate University, Szeged, Hungary (Zs. Buzas); Laboratory of Vision Research, National Eye Institute, NIH (L. M. Hjelmeland and B. An der Lan); Laboratory of Cellular and Developmental Biology, NIADD, NIH (R. Horuk).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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.)*

1) A 2-dimensional technique for native macromolecules was designed, using HPLC gel chromatography in the first dimension and Immobiline electrofocusing in the second.

2) Linear polyacrylamide solution and partially hydrolyzed agarose were tested as possible media for pore limit electrophoresis in the first dimension.

3) Agarose gel structure was found to comprise both a 0-D gel (under 0.9%) and a 1-D gel (above 0.9%), with characteristic difference of effective gel fiber radii by one order of magnitude.

4) Buffer Electric Focusing (BEF) pH gradients consisting of 13 acids or 16 bases were computed using a moving boundary model. Predicted and experimental pH gradients were found to agree in proportion to constituent multiplicity. Transient state Joule heat production was found to require BEF at very low initial voltages, independently of leading ion concentration between 0.1 and 1.0 M. Steady-state pHs in BEF of amphoteric buffers were found to be isoelectric. Similarly, pIs in BEF agreed with those found in conventional electrofocusing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00188-04 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Development and Evaluation of New Dimeric Analogs of Enkephalins

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

PI:	D. Rodbard	Head	LTPB, NICHD
Other:	R. Lutz	Visiting Scientist	LTPB, NICHD
	R. Cruciani	Visiting Fellow	LTPB, NICHD
	P. Munson	Mathematician/Statistician	LTPB, NICHD

COOPERATING UNITS *(if any)*

Instituto Superiore di Sanita, Rome, Italy (T. Costa); Max Planck Institute, Munich, Germany (T. Costa); Department of Pharmacology, USUHS (S. Krumins).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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.)*

Dimers of enkephalins using methylene crosslinking moieties were used as probes of the properties of opioid receptors in brain and cultured cells (NG108-15 cells). A new series of tripeptide dimers (designated DTRE) has been studied. The monomer is extremely weak and mu selective. The dimers have 200-400 fold increased potency. Short-chain dimers are mu selective; long-chain dimers are delta selective.

A new series of alkyl-amide derivatives of [D-Ala,2Leu5]enkephalinamide has been studied, and indicates the extent to which bivalency is responsible for the increased potency and specificity.

Kinetic studies indicate that 1) dimers have exactly half the molar binding capacity of monomers; 2) dissociation is not accelerated by the presence of unlabeled ligand; 3) reduction of receptor density by an affinity label does not appear to selectively reduce the affinity for the dimer. Collectively, these studies suggest a new model for interaction between the dimer and the receptor, involving binding to two sites within the same receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00189-03 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Development of Statistical Software for Use by Clinical Investigators

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

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	P. Munson	Mathematician/Statistician	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	R. Lutz	Visiting Scientist	LTPB, NICHD
	N. Pernick	Expert	LTPB, NICHD

(See attachment)

COOPERATING UNITS *(if any)*

Data Management Branch, DCRT (B. Cole) McMaster Univ., Hamilton, Ontario, Canada (W. Walker); University of Florence, Italy (M. Pazzagli and M. Serio).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	.9

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.)*

We have developed a series of computer programs to assist the clinical investigator and clinician: 1) The "Diabetes Data Management Program", in BASIC for the IBM-PC, provides data storage, retrieval, graphical and statistical analyses, advice regarding insulin dosage, and explanations. It is intended for patient and physician education, and is currently being evaluated in a number of medical centers; 2) Program "NORMAL" has been developed for estimation of normal ranges of laboratory tests; 3) We have continued development of the 'BRIGHT-STAT-PACK' system, to assist the investigator in selecting appropriate methods of analysis, and then implementing and interpreting the analysis. Life-table analysis has been introduced into BRIGHT. 4) Examining the between-subject correlation coefficients for multiple steroids in response to ACTH stimulation, we are able to identify major pathways and sequence of interconversion, and identify several differences between normal subjects and patients with idiopathic hirsuitism. Multiple, part, and partial correlation coefficients were also shown to be useful to sources of steroids; 5) Additional analyses were performed to examine interactions of thyroxine with binding proteins in plasma; interactions of steroids with binding proteins in amniotic fluid; serum beta-endorphin levels in pregnancy; and transketolase enzymatic activity in patients with familial alcoholism.

R. Staton	Statistician	LTPB, NICHD
G. Thornton	Computer Programmer	LTPB, NICHD
S. Shackney	Medical Officer	LTPB, NICHD
M. Evans	Medical Staff Fellow	HGB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01400-02 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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.) (Name, title, laboratory, and institute affiliation)*

PI:	Alfred L. Yergey	Head	LTPB, NICHD
Others:	Nora V. Esteban	Visiting Fellow	LTPB, NICHD
	James Sidbury M.D.	Senior Investigator	HGB, NICHD

COOPERATING UNITS *(if any)* Laboratory of Mathematical Biology, NCI (D. Covell); Department of Pediatrics, Washington University Medical School, St. Louis, Missouri (Laura Hillman); Department of Clinical Nutrition/Gastroenterology, University of Chicago Medical School (I. Rosenberg).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Unit on Metabolic Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The principle objective of this study is to elucidate the kinetics of calcium metabolism in normal children and to evaluate disease related changes in calcium metabolism in both children and adults. Stable isotopes make such studies possible in children and in women of childbearing age, for whom the risk of radioactive calcium tracers prevent such studies; stable tracers also permit repeated measurements. Thermal ionization isotope ratio mass spectrometry with a quadrupole mass filter are used to measure tracer enrichments in serum, urine, feces and food. Isotope ratio measurements are analyzed by using a multi-compartmental mathematical model from which mineral mixing kinetics and metabolic fluxes are determined. During the past year, 3 adolescent boys and 3 prepupertal girls were studied in collaboration with HGB, NICHD. The clinical protocol employed for these studies uses two stable isotopic tracers, one given i.v., the other orally. This use of two tracers allows direct measurement of several important parameters of calcium metabolism, principally the fraction absorbed and the endogenous fecal excretion. Mathematical analysis of results from two of the adolescents are complete. Comparison of the results from the two boys with a young patient with fibrodysplasia ossificans progressivia (FOP) shows that the metabolic parameters of the boys are consistent yet differ markedly from those of the FOP patient. The principal observations are that the fraction of dietary calcium absorbed is about the same for all three children, but the FOP patient excretes virtually no urinary calcium. The dimensions of the three compartments postulated for the non-skeletal internal calcium are about the same size for the two boys, and are about the same size as the most rapidly turning over compartment in the FOP patient; the remaining two compartments of the FOP patient are about 5-6 times larger than those determined for the adolescent boys. These observations are consistent with clinical observations, and may contribute to an understanding of normal calcium homeostasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01401-02 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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	Research Chemist	LTPB, NICHD
Others:	D. Liberato	Staff Fellow	LTPB, NICHD

COOPERATING UNITS *(if any)* Division of Pediatric Metabolism, Dept. of Pediatrics, Duke Univ. Durham, NC (D. Millington and C. Roe); University of Texas Medical Center, San Antonio, Texas (S. Weintraub); Laboratory of Oral Biology and Physiology, NIDR, NIH (J. Folk); and University of Kansas, Lawrence, Kansas (R. Borcherd).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Unit on Metabolic Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our principal objective is to develop and apply new, improved methods for analysis of biological materials that require mass spectrometric (MS) analysis, but which have not previously been amenable to such analysis by reason of volatility, thermal lability or charge state. Our basic approach involves a direct interface of high performance liquid chromatography (HPLC or LC) effluent with the mass spectrometer source, and permits use of conventional solvent flow rates and buffered aqueous solvents (1 ml/min 0.1 M ammonium acetate). Ions are desorbed directly from vapor droplets that are heated rapidly in passage from the HPLC capillary through the ion source by a mechanism that resembles other desorption techniques (field desorption, laser desorption, fast atom bombardment (FAB)). Thermospray LC/MS has the important advantages over these other methods of a) a chromatographic inlet, b) applicability to analysis of mixtures, and c) simplicity of sample preparation. Recent applications include: 1) Identification and quantification of novel fatty acid conjugates of carnitine in subjects with Reye's Syndrome, organic acidurias and valproic acid toxicity; 2) Separation and identification of the biosynthetic pathway of hypersine, an unusual basic amino acid that is a conjugate of spermidine and lysine, showing that the E-nitrogen comes from lysine; and 3) Separation and quantification of choline (Ch) and acetylcholine (ACh) from mouse brain. Sensitivity is comparable to electrochemical detection methods, but this work represents the first direct measurement of ACh. Quantitites of Ch and ACh found are comparable to earlier reports and show the biological variation expected; 4) Separation and quantification of catecholamines prior to working on a biosynthetic pathway problem; and 5) Separation and quantification of cortisol prior to developing a rapid clinical measurement for cortisol MCR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01402-01 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

The Volume and Surface Area of the Cytoplasmic Matrix and Intracellular Diffusion

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

PI: N. D. Gershon Visiting Scientist LTPB, NICHD

COOPERATING UNITS *(if any)* Cooperating Units: Division of Computer Research and Technology, NIH (N. Gershon and B. Trus); FIC, NIH, (K. Porter); Dept. of Molecular, Cellular and Developmental Biology, Univ. of Colorado, Boulder CO (K. Porter); Dept. of Biology, University of Maryland Baltimore County, Catonsville, MD (K. Porter).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.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 object of this work is to determine how much volume of the cytoplasm is occupied by the cytoplasmic matrix under different external osmotic environments, and how it affects the diffusional motion of proteins inside the cytoplasm.

The cytoplasmic matrix is composed of the microfilaments, intermediate filaments, microtubules and the microtrabecular lattice. We developed an image analysis method to study the volume and surface area of the cytoplasmic matrix. The low values (10%-30%) obtained for the fractional volumes indicate that by excluded volume alone, the cytoplasmic matrix cannot slow down the diffusion of proteins to such an extent as compared with the diffusion in water. Analysis showed that binding of the diffusing proteins to the cytoplasmic matrix could explain the results. The values of the binding constants obtained point out that although the association - dissociation process can occur very fast, at any given time, most of the proteins may be bound to the cytoplasmic matrix. We have initiated the study of the effect of the external osmotic environment on the fractional volume of the cytoplasmic matrix in the cytoplasm. The surface area attributed to the cytoplasmic matrix was calculated to be in the range of 43,000 - 140,000 m2 per cell.

The meaningfulness of this work lies in the fact that measuring the volume fraction of the cytoplasmic matrix can shed light on the physical and chemical constraints on molecular transport through the cytoplasm. The estimate of surface area associated with the cytoplasmic matrix is important in understanding the role of hydrated water on macromolecular surfaces in the physiology of the cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01403-01 LTPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Three Dimensional Organization of Cells and Anatomical Components

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

PI:	N. Gershon	Visiting Scientist	LTPB, NICHD
Others:	D. Mattison	Senior Investigator	PR, NICHD
	N. Esteban	Visiting Fellow	LTPB, NICHD

COOPERATING UNITS (if any) DCRT, NIH (N. Gershon); FIC, NIH (K. Porter); Dept. of MCDB, Univ. of CO, (K. Porter and M. McNiven); Dept. of Biol., Univ. of MD Balto. County (K. Porter and N. McNiven); Dept. of OB/GYN, Yale Univ. Sch. of Med. (F. Naftolin and H. Sakamoto); (See Attachment)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.9	0.9	0.0

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

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

1. The cell center (the microtubules organizing centers (MTOC) and the centrioles) was reconstructed from electron micrographs of serial sections and was found to be highly organized in the pigment cell of Holocentrus. This center is implicated in forming and maintaining the cell shape and its filamentous systems.
2. The organization of the endoplasmic reticulum (ER) and the Golgi apparatus in arcuate nucleus neurons of the hypothalamus was studied. Part of the ER, or whorl body, was reconstructed and found to resemble a combination of rough and smooth ER, which at some points is related geographically to the Golgi apparatus. A small pilot project of the structure of the Golgi apparatus in CHO cells has been initiated. The significance of our findings is that it will allow us to follow the mechanism of ER shape changes and its relation to the Golgi apparatus.
3. Many sections of rat brain were digitized and processed for developing a single full stereotaxic representation of the whole brain. We have initiated the steps that will allow us to represent the projection of neurotransmitters, peptides and receptors in color in their respective locations.
4. We digitized many sections of mouse embryos at different stages of development and initially processed them and are ready now for their three dimensional reconstruction. This will be used to evaluate the effects of teratogens and to follow the migration of germ cells in embryos.

These studies were made feasible by the development of a high resolution computer graphics system for three dimensional reconstruction. Algorithms and programs were devised to digitize serial sections, to align them and reconstruct them into a single three dimensional image.

The significance of these studies is that they will further our understanding as to how the cell gains its shape, and organizes its organelles and the tissue studies will reveal new functional interrelationships in the brain and embryos.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01404-01 LTPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Characterization of Opioid Receptors in Brain and Peripheral Tissues

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

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	R. Lutz	Visiting Scientist	LTPB, NICHD
	R. Cruciani	Research Chemist	LTPB, NICHD
	P. Munson	Mathematician/Statistician	LTPB, NICHD
	T. Costa	Visiting Associate	LTPB, NICHD

COOPERATING UNITS *(if any)*

University of Baltimore, Baltimore, Maryland (G. Pesce, J. Stolk)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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 was designed to characterize the ligand-binding properties of opioid receptors in brain and peripheral tissues, using quantitative ligand binding studies. We have demonstrated and characterized at least four classes of sites, present simultaneously: mu-1 (high affinity Kd = 0.2 nM and nonselective for mu- or delta- selective enkephalins); mu-2 (lower affinity, mu selective), delta, and kappa. Three of these sites (mu-1, mu-2, delta) were characterized in 10 consecutive experiments using rigorous statistical criteria and a new form of "Kd versus Kd" bivariate graphical analysis. A new "multiligand" experimental design was used to improve sensitivity for detection of mu-1 sites. Naloxonazine shows mu and mu-1 selectivity. It can irreversibly or noncompetitively block about 50% of mu-1 sites, while it shows significant competitive effects at mu-2 and delta sites. The existence of mu-1 sites has considerable implications for interpreting binding, pharmacological and biochemical studies of opioid mediated systems. These methods are now being extended, to consider epsilon, kappa and sigma receptors, to study receptors of hypothalamus, adrenal medulla and vas deferens to categorize subtypes of kappa receptors, and to examine opioid-adrenergic interactions.

HUMAN GENETICS BRANCH

Z01 HD 00131-10	Human Biochemical Genetics Michael A. Zasloff, M.D., Ph.D.
Z01 HD 00133-07	Study of Glycogen Storage Disease James B. Sidbury, Jr., M.D.
Z01 HD 00403-03	Magnesium Metabolism in Mothers and Neonates Joan L. Caddell, M.D.
Z01 HD 00404-02	Sulfur Metabolism in Fibroblasts Jean DeB. Butler, Ph.D.
Z01 HD 00405-06	Structure of the Methionine Initiator tRNA Genes in the Human Genome Michael A. Zasloff, M.D., Ph.D.
Z01 HD 00408-01	Pathophysiology and Treatment of Human Genetic Diseases Michael A. Zasloff, M.D., Ph.D.
Z01 HD 00409-01	Kinetics of Calcium Metabolism in Childhood and the Study of Prader-Willi Syndrome James B. Sidbury, Jr., M.D.
Z01 HD 00909-05	Effects of Ethanol on the Mother and the Fetus Anil B. Mukherjee, M.D., Ph.D.
Z01 HD 00910-05	Uteroglobin Anil B. Mukherjee, M.D., Ph.D.
Z01 HD 00912-05	Gene Regulation and Cellular Differentiation Janice Y. Chou, Ph.D.

NICHD ANNUAL REPORT

HUMAN GENETICS BRANCH

October 1, 1983 to September 30, 1984

The Human Genetics Branch conducts research which attempts to elucidate the pathophysiology of human genetics and developmental disorders through an understanding of basic biological mechanisms. Clinical activities include studies of the natural history, treatment, and methods of diagnosis of several heritable disorders of man.

I. Section on Molecular Biology

This section conducts research in both basic and clinical areas. These include: the organization and expression of tRNA genes in the human genome; the basic cellular mechanism involved in the transport of biological information from the cell nucleus to cytoplasm; the mechanism of action of thyroid hormone; the treatment and pathophysiology of hereditary heterotopic ossification disorders in man; the pathophysiology and treatment of the mucopolysaccharidoses.

Over the past several years we have studied various aspects of the organization and expression of the human $tRNA^{met}$ genes. Our studies have provided the first insight into the organization of this class of vital gene in higher vertebrates; the first localization to a specific chromosome of a tRNA gene; the demonstration of genetic variation at the DNA level for this class of eukaryotic gene. Systems have been developed for the transcription of this gene *in vitro* and new methodology has been developed utilizing the *in vivo* intact X. laevis system. The most striking finding of this series of studies has been the discovery of the existence of a transport mechanism in the eukaryotic cell which delivers tRNA from the nucleus to the cytoplasm of a cell. This process was first identified in our laboratory during studies of a naturally occurring variant human tRNA gene, the first of this class of eukaryotic mutation described. In addition, the nucleases which process the primary transcript of the tRNA gene, enzymes previously unidentified, have been purified, and studies characterizing these proteins have been underway for some time.

Over the past year we have generated some 30 point mutations in the human $tRNA^{met}$ previously cloned in our laboratory. These mutations were generated by hydroxlamine mutagenesis using a novel procedure developed in our laboratory. Using these 30 or so species, each bearing a single and unique mutation, we have explored the structural requirements of the tRNA transport system and the substrate specificity of the two processing nucleases. Transport studies have shown the striking finding that virtually every mutation placed into the wild-type tRNA molecule generates a species which is less efficiently transported than the wild type, demonstrating the extreme specificity of the transport system. Mutations which lead to severe disruption of the shape of the tRNA, i.e., those which disrupt tertiary structure, profoundly affect both the processing system as well as transport. Indeed, the processing nucleases appear to see general shape properties of the tRNA molecule. In contrast, mutations at several points in the tRNA which have no effects on processing, but profoundly affect transport have been identified. These, remarkably, cluster within the anti-codon loop.

This finding is of utmost significance, in that it shows us that portions of the tRNA molecule which are tRNA-specific (rather than general shape related) are being recognized by the transport system. Proteins which recognize one specific tRNA from another are very limited in the cell and include, for the $tRNA^{met}$ only two candidates L: amino-acyl-tRNA ligase and the initiation factor, EIF-2. Our current model of the transport system mechanism now required the participation of such a protein and to this end specific antibodies against these enzymes will be injected into the nucleus of the X. laevis oocyte to be assayed for specific tRNA inhibition activity.

Studies on the biochemistry of the $tRNA^{met}$ processing enzymes were continued. These enzymes were purified further from X. laevis oocytes and from human KB cells. The 3' processing nucleus now has been purified to apparent homogeneity. It acts on the 3' termius as an endonuclease, generating a 3' hydroxyl group on the tRNA product, and a 5' pG on the small trailer fragment. The enzyme is a single polypeptide of about 100,000 in molecular weight and functions as a monomeric activity.

Studies on the expression of the Alu sequence in the mouse genome have been continued over this interval and have yielded exciting new information about this class of eukaryotic gene. In mouse, as in most vertebrates, the Alu sequence is amongst the most ubiquitous gene present in the genome. In man about 300,000 copies of this sequence exist in scattered genomic loci. No function has yet been assigned. We had studied the expression of one particular gene, that contained in the first intron of the mouse alpha-fetoprotrin gene (αFP). We have shown that this gene is transcribed by RNA polymerase III. The primary transcript is processed by a specific endonuclease to yield a "core" Alu specific RNA and a non-specific 3' trailer. This core Alu is transported from the nucleus to the cytopasm by a specific transport system. In the cytoplasm it appears to be packaged into a ribonucleoprotein to which specific auto-antisera have been discovered. This pathway has been elucidated for the first time in our laboratory. We have now shown that the processed core Alu RNA can be found in only some mouse tissues and cultured cells, specifically liver tissues and hepatoma cell lines. We have concluded that the post-transcriptional pathway appears to be tissue-specific and the core Alu appears to be a "liver" specific RNA. This extraordinary specificity, the first so identified for this ubiquitous gene, suggests a role in specific gene expression in differentiated liver gene expression. Studies designed to determine the particular role being played by this newly discovered cytoplasmic species are underway.

Our previous studies of the mechanism of thyroid hormone action at the molecular level have been extended over the past year. We have focused on the development of new methodology for the identification of specifically induced low abundance mRNA species from a recombinant cDNA library, a technique blatantly missing in our current armamentarium of methodology. The technique developed is based on a contact hybridization method invented in our laboratory several years ago for quantitative determination of re-interaction frequency of DNA segments within a large cloned genomic DNA sequence. In this method, cloned cDNA species are nick-translated, restricted to release cDNA insert fragments, and electrophoresed in agarose. The gels are treated with alkalai to denature DNA and then neutralized. The agarose gels are then placed in contact with a sheet of DBM paper to which total cellular RNA had been bound. Blotting is performed under hybridization conditions. After washing, the nick-translated cDNA fragments are found to bind to the paper in proportion to the abundance of complementary in the total cellular

RNA. The method permits quantitative analysis of mRNA abundance for each corresponding cDNA cloned. It has been used successfuly to recover liver-specific cDNA species falling within a wide range of abundance and to identify those which vary in abundance in liver upon treatment of the rat with high doses of T3. We believe the method will have wide application in the screening of recombinant cDNA libraries.

Studies on the treatment of fibrodysplasia ossificans progressiva continue. After about 1 year, we now have demonstrated that 6 of 7 children treated with 13cis retinoic acid at 5 mg/kg/day have undergone remission of ectopic bone formation. Three of 3 children untreated have shown evidence of continued activity. As a result, we have expanded the study to a nation-wide scale. In addition, studies on the use of this agent as a therapeutic adjunct following surgery have begun.

We have identified a new disease entity this year, and some studies designed to understand mechanism are underway. Two children were referred to NIH with a condition characterized by the formation of ossification within the dermis. Eventually, ossified tissues appear within deeper fascial layers. The first evidence of disease is the appearance of a erythematous macular rash at birth. Lesions in the deep dermis arise during the first few years consisting of intramembraneous bone formation. The distribution of lesions is totally distinct from FOP. Recent evidence has suggested that cells grown from the ossifying lesions behave somewhat different from normal fibroblasts when propagated in tissue culture. Cells appear to pile up and present a different pattern of spreading and organizaton on the plate surface. Most recently, we have found that these cells are extremely sensitive to radiation damage. Several studies are suggestive of a viral basis for this process and definitive experiments are underway.

A new project was initiated this period, in collaboration with the Genetics and Biochemistry Branch, NIADDK. The study of the molecular basis of the Hunter Syndrome, a heritable lysosomal disorder resulting from defective expression of the enzyme iduronate sulfatase was begun. To this end, the enzyme has been purified from human plasma. This represents the first purification of this enzyme from any source, and amounted to a very technically demanding feat due to the low abundance of this enzyme. The pure enzyme, however, is necessary to generate specific polyclonal antibodies. These reagents are required for the study of the biosynthesis of this enzyme. Such a study is necesary to define the biological consequences of mutation in the protein, a goal of this project. In addition, this purified protein will be subjected to limited sequence analysis to provide sequence information on which to base the design of oligonucleotide probes. With these probes, isolation of the corresponding cDNA and gene should be forthcoming.

Therapeutic studies in children with the MPS syndromes focused on treatment of this group of human disorders by implantation of human amnion from normal newborns into children with MPS. The concept was based on the joint findings that the amnion is not acutely rejected in man and can be donated between otherwise histoincompatible individuals; and the biological observation that cells deficient in a lysosomal enzyme can nevertheless recapture normal enzyme from surrounding fluids and correctly deliver this enzyme into a functionally normal state in the cell's lysosomal compartment. Almost 20 children have been treated by this method over the past year. To date, no increase in circulating serum levels of the enzyme deficient in these patients has been detected. In several cases, however, objective improvement of certain clinical parameters such as joint range of motion

and frequency of upper respiratory infections has been noted. In at least two children a decrease in the size distribution of urinary mucopolysaccarides has been measured. The final evaluation of this approach will be gained over the coming year.

One striking finding which has fallen from our close observation of this population is the recognition of the very high incidence of hydrocephalus in MPS I and MPS II. Indeed, almost every child with these disorders appears to have physiologically significant increased intracranial pressure, perhaps secondary to a resorptive defect in the subarachnoid villi. The relationship between the frequently noted deterioration of CNS function and this process is not clear but the kinetics of both in several children have now suggested that management of hydrocephalus through surgical intervention may be indicated at a previously unrecognized stage in children with certain of the MPS syndromes.

II. Section on Developmental Genetics

During the past year we have found that Uteroglobin (UG) is a potent inhibitor of aggregation of both rabbit and human platelets. This is a significant finding since pregnancy induces produciton of thromboxane A_2, a potent mediator of platelet aggregation in the uterus and in the lung. It is possible that UG may counteract the hypercoagulable state of pregnancy. Preliminary data suggest that uteroglobin by its anti-platelet aggregation effects may prevent thrombosis in the microvasculature of the placenta, the uterus and of the lung. Using a sensitive radioimmunoassay, SDS-PAGE and isoelectric focusing we have discovered that a protein similar in molecular weight, PI and immunological crossreactivity to uteroglobin is present in the neonatal human lung. This is the first time the presence of a human counterpart of rabbit uteroglobin has been clearly documented. Experiments underway will delineate the distribution of this protein in different human tissues and its possible function. Furthermore, the exact mechanism of inhibition of platelet aggregation by this protein will be studied in more detail. In addition to the above findings, we have now established several rabbit alveolar and endometrial epithelial cell lines transformed by a temperature sensitive mutant of SV_{40}. These cell lines express both cytoplasmic and nuclear receptors for estradiol and progesterone. When stimulated with progesterone at 40°C the endometrial cells secrete uteroglobin in the medium. Thus, these cells, when fully characterized, will provide a unique tool to (i) determine the biological activity of various progestogenic agents in vitro which is unavailable at present and (ii) the regulation of expression of the uteroglobin gene in response to progesterone by c-DNA probe analysis.

Genetic studies on ethanol toxicity can be divided into two parts: (i) to what extent do inborn factors predispose individuals to abusing alcohol? and (ii) to what extent do inborn factors predispose individuals who abuse alcohol to the development of specific complications? Although recent studies suggest that there are at least some populations in which there are important genetics predispositions to the development of alcoholism, the first question remains controversial. It is clear, however, that some patients are biologically predisposed to developing one or another complication if they abuse ethanol. Transkeotlase abnormality has been suggested to be one of the concomitants of thiamine deficiency disease. A well known example is Wernicke-Korsakoff syndrome. Recently, we have found that this enzyme abnormality is present at a higher frequency among alcoholic men than in their non-alcoholic counterpart and in their male

progeny long before they abused alcohol. The inheritance pattern of this enzyme abnormality seems to be autosomal recessive in nature. Additionally, it appears that transketolase abnormality may also be responsible for many of the concomitants of fetal alcohol syndrome (FAS), namely, intrauterine growth retardation (IUGR), microcephaly and abnormal brain pathology. Preliminary data suggest that thiamine deficiency, a frequent complication in chronic alcoholism may contributre to IUGR and microscphaly in the rat and teratogenic effects of alcohol is dramatically increased when pregnant animals are rendered thiamine deficient. Since all chronic alcoholic pregnant women do not give birth to FAS children it is suggested that a genetic predisposition to thiamine deficiency (i.e. transketolase abnormality) may be responsible for this variability among individuals.

III. Section on Cellular Differentiation

Using the temperature-sensitive rat fetal liver cells, we have demonstrated that both qualitative and quantitative alternation in AFP gene expression occurred during transformation of fetal liver cells in vitro. At 40°C RLA209-15 fetal liver cells exhibit a differentiated phenotype that resembles fetal liver in vivo: they synthesize two AFP variants of 73,000 and 69,000 daltons and contain an AFP mRNA species of 20S. RLA209-15 cells exhibit a transformed phenotype at 33°C. Transformation is accompanied by the synthesis of reduce lead of AFP of 65,000 daltons and the detection of reduced level of AFP mRNA of 14S. The 14S AFP mRNA appears to be generated by the alternative RNA splicing pathway. The RLA209-15 cells prove to be a suitable model system to study the molecular basis of maturation. We found that retinoic acid is one of the regulators that induce maturation of fetal liver in vitro.

In studies on alkaline phosphatase gene expression, we found that both sodium butyrate and 5-bromo-2'-deoxyuridine (BrdUrd) induce a specific increase in the placental alkaline phosphatase mRNA leading to the observed enhancement of biosynthesis.

IV. Section on Biochemical Genetics

The Section on Biochemical Genetics has continued to investigate the carrier-mediated transport of cystine across leucocyte lysosomal membranes. We have shown that the lysosomal cystine carrier is saturable, stereospecific for the L-isomer of cystine, capable of exhibiting counter-transport, and deficient in cystinosis, a lysosomal storage disease generally resulting in renal death by age 10. Both egress and counter-transport measurements in polymorphonuclear leucocyte granular fractions demonstrated that normal cystine transport does not require lysosomal acidification, since transport was not inhibited by weak bases which neutralize the acid lysosomes, nor by the protonophore CCCP, which dissipates the proton gradient across lysosomes. Cystine transport was also shown to be stimulated by magnesium at pH 5.5.

In whole cell experiments using cultured fibroblasts, ^{35}S-cystine clearance from mucolipidosis II cells, or I-cells, was found to be impaired, resembling cystinotic cells in their rate of cystine loss. This explained the storage of large amounts of cystine within I-cell fibroblasts lysosomes, and has provided a model system in which to study defects in lysosomal cystine transport.

Clinically, the Section treats 20 cystinotic children with the cystine-depleting agent cysteamine in conjunction with the National Collaborative Cysteamine Study. Growth responses in young patients have been encouraging, and renal deterioration appears to have been delayed in several individuals. One patient, who tolerated cysteamine poorly, exhibited a 70% reduction in his leucocyte cystine levels during a three-month trial with oral pantethine, whose cystine-depleting efficacy had been demonstrated in cultured fibroblasts. In other investigations, a family with nephropathic cystinosis in one sibling and Fabry disease in her brother was described. The Section has also documented a deficiency of free plasma carnitine in 18 patients with cystinosis and two with Lowe syndrome, and has demonstrated that the deficiency resulted from failure to reabsorb carnitine due to renal Fanconi syndrome. A normal response of ketosis and free fatty acid mobilization was observed during a 24-hour fast of two of the carnitine-deficient subjects. The drug cysteamine was shown to blunt the prolactin response to thyroid releasing hormone in vivo, and, when given orally, to charge-shift the apolipoprotein E isoelectric focusing pattern of plasma very low density lipoproteins. Five homocystinuric patients, without x-ray evidence of osteoporosis, have exhibited decreased bone density on CT scan measurements of their vertebral bodies. This technique is being used to follow therapy of the bone lesion in homocystinuria.

V. Section on Disorders of Carbohydrate Metabolism

The study of the kinetics of calcium metabolism has only recently been initiated and the age span of patients proposed has not yet been completed. The comparison of endorphin response to a glucose load was not different in Prader-Willi syndrome patients and uncomplicated exogenous obesity. The results of glucose and insulin responses in these patients is not complete. The evaluation of the usefulness of raw starch in the management of type I glycogen storage disease has been gratifying and unexpectedly provided evidence of polymorphisms in the bytrolysis and absorption of starch.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00131-10 HG

PERIOD COVERED

October 1, 1983 to September 30, 1984

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)*

P.I.:	Michael A. Zasloff, M.D., Ph.D.	Acting Head	HGB, NICHD
Others:	William A. Gahl, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD
	Isa Bernardini	Technician	HGB, NICHD
	George Reed, Ph.D.	Math. Statistician	BB, NICHD
	Edward Fisher, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*

Section on Intermediary Metabolism, NIADDK (F. Tietze) - see attached

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Human Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.3	2.3	1.0

CHECK APPROPRIATE BOX(ES)

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

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

We continue to study inborn errors of metabolism with special emphasis on nephropathic cystinosis. Basic research into this disorder has been directed toward characterizing the normal lysosomal membrane's cystine transport system and comparing it with the system we have demonstrated to be defective in cystinosis. The intact system is stimulated by divalent cations such as magnesium at pH 5.5, and does not appear to require proton-pump mediated acidification for normal functioning. However, cystine storage in I-cell lysosomes suggests that the lysosomal cystine carrier may require either a mannose-6-phosphate recognition marker for placement in the lysosomal membrane, or processing by hydrolases deficient in I-cell disease. Clinical investigations into cystinosis have revealed a significant hypohydrosis in cystinotic children and a substantial deficiency of plasma free carnitine in all patients due to failure of the kidney to reabsorb carnitine. We have shown that cystinotics receiving cysteamine as a cystine-depleting agent exhibit an impaired prolactin response to thyroid releasing factor, and we have described nephropathic cystinosis and Fabry disease in a single sibship under our care. Pantethine has been investigated as a cystine depleting agent in cystinosis and patient recruitment has begun for a study of betaine's effects on bone density in homocystinuria. The transport of sialic acid across the lysosomal membranes of normal and sialic acid storage disease fibroblasts is being actively pursued.

COOPERATING UNITS

S.H. Mudd, NIHM

S. Goodman, University of Colorado

J. Schneider, University of California at San Diego

J. Thoene, University of Michigan

G. Thomas, Johns Hopkins University

R. Gregg, NHLBI

J. Hoeg, NHLBI

F. Tietze, NIAMDD

N. Bashan, Beersheva, Israel

D. Kurtz, CC, NIH

T. Triche, NCI

W. Den Tandt, Antwerp, Belgium

W. Rizzo, Medical College of Virginia

J. Barranger, NINCDS

M. Kaiser-Kupfer, NEI

B. Bercu, University of South Florida

H. Levy, Massachusetts General Hospital

D. Valle, Johns Hopkins University

J. Schulman, George Washington University

M. Evans, Wayne State University

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00133-07 HG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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, Jr., M.D.	Head	HGB, NICHD
Others:	Joseph Munzer, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD
	Abraham Karkowsky, M.D.	Medical Staff Fellow	LDP, NICHD

COOPERATING UNITS *(if any)*

Pamela Brye, RD, CC, NIH

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Disorders of Carbohydrate Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

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

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

The study has nearly completed the testing of uncooked starch vs the derivative food using corn, rice and potatoes in glycogen storage disease patients (GSD) and controls. We have evaluated corn starches with varying percentages of amylase vs amylopectin. We are currently beginning to evaluate absorption characterisitcs against arrow root starch, wheat starch, tapioca starch, sweek potato starch and cassava starch.

We have shown that some children under 3 years of age may not split and absorb uncooked starch. On the other hand we have one infant of 8 months who is readily regulated on uncooked starch. We believe this to be a difference in development of pancreatic amylase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00403-03 HG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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, M.D.	Guest Researcher	HGB, NICHD
Others:	James B. Sidbury, Jr., M.D.	Section Head	HGB, NICHD
	Barry Graubard	Math Statistician	BB, NICHD
	Howard Hoffman	Chief	BB, NICHD

COOPERATING UNITS *(if any)*

Joan Blanchette-Mackie, Ph.D., NIADDK

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Disorders of Carbohydrate Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

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.)*

The very young magnesium (Mg)-deficient mammal may experience a sudden, acute, self-limited shock-like syndrome characterized by apnea; bradycardia, with cardiac arrhythmia; pallor or cyanosis; neuromuscular hyperirritability; and sometimes respiratory distress; resulting in spontaneous recovery or sudden death. Studies in weanling rats have identified stresses that may provoke this state; biochemical changes during the acute episode (increased plasma osmolality, metabolic acidosis, increased plasma Mg, LDH, CPK, SGOT, SGPT, etc.); and post-mortem changes which are chiefly intrathoracic (pulmonary edema, hemorrhage, atelectasis, with overexpansion). A retrospective analysis of data from 249 human infants hospitalized with idiopathic apnea has identified two somewhat similar syndromes: 1) in the neonatal period, and 2) in the post-neonatal period, with a peak incidence at 2 or 3 months of age. Seventy-four % of all infants tested showed plasma Mg levels at or below the Hospital's lower limit of normal, 1.6 mEq per liter, despite concomitant biochemical events that would increase these levels, including acidosis, hypoxemia, and hemoconcentration. Parenteral Mg load testing usually showed high retention of Mg. One-third of the 249 infants received a minimum of 5 days of Mg therapy, while two-thirds did not. The premature infants constituted the largest subgroup of patients, and only their data achieved statistical significance. Among the premature infants, 61 were Mg-treated, receiving 11.4 ± 0.9 days of Mg therapy (mean ± SEM), while 140 received 0.54 ± 0.08 days of therapy. During an 18-month follow-up period, none of 60 treated infants followed were readmitted for apnea, while 29 of 134 untreated infants followed were readmitted for apnea ($P<0.0005$) 31 times. The Mg-treated infants survived; 6 of the 140 untreated infants died (N.S.), two during the first admission.

Future studies will focus on a controlled prospective clinical trial of Mg in human infants and on further anatomical and biochemical studies in the animal model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00404-02 HG

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Sulfur Metabolism in Fibroblasts

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

P.I.: Jean DeB. Butler, Ph.D. Senior Investigator HGB, NICHD

Other: William A. Gahl, M.D., Ph.D. Senior Staff Fellow HGB, NICHD

COOPERATING UNITS *(if any)*

Dr. Peter Pentchev, NINDS
Dr. Frank Tietze, NIADDK
Dr. Martin Zatz, NIMH
Dr. Stephanie Padilla, EPA

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

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

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

We study sulfur metabolism using skin fibroblasts and have concentrated on the following areas:

1. Study of the treatment of cystinosis with cysteamine, pantethine and WR-1065. The latter two compounds lower cystine levels in cystinotic cells, as does cysteamine but are less toxic than cysteamine. Pantethine is being used in a clinical trial, so methods for detection in serum and urine are being developed.

2. Efforts to define the source of cystinotic cystine by way of the study of metallothionine, a protein that contains one third cysteine residues and was found in ^{35}S-cystine labeled cystinotic cells at levels two fold that found in normal cells.

3. Discovery and investigation of a mutant mouse which stores cystine in lysosomes as do cystinotic patients. Possible anomalies in cholesterol metabolism are being investigated.

4. Continued study of the glutathione cycle which when manipulated with inhibitors or stimulators will directly vary the cystine levels in cystinotic cells.

5. Availability of diagnostic service for detection of cystinosis in new cases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00405-06 HG

PERIOD COVERED: October 1, 1983 to September 30, 1984

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

Structure of the Methionine Initiator tRNA Genes in the Human Genome

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

P.I.:	Michael A. Zasloff, M.D., Ph.D.	Head	HGB, NICHD
Other:	Samuel A. Adeniyi-Jones, M.D., Ph.D.	Visiting Associate	HGB, NICHD
	Janet A. Tobian, Ph.D.	Staff Fellow	HGB, NICHD
	Jose G. Castano, M.D., Ph.D.	Guest Researcher	HGB, NICHD
	Lee Drinkard	Biologist	HGB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH: Human Genetics Branch

SECTION: Section on Molecular Biology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.3	2.3	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies on the expression of human tRNA and tRNA-like genes were continued. Utilizing an in vitro mutagenesis method developed in our laboratory, both CtoT and G to A point mutants of the human $tRNA^{met}$ gene were generated. With these mutant genes we have explored the structural requirements of the tRNA nuclear transport mechanism, as well as the two nuclear processing nucleases involved in the biosynthesis of the human $tRNA^{met}$ species. We have shown, using micro-injection into the nuclei of intact X laevis oocytes that both the processing enzymes as well as the nuclear transport system handle all mutant forms of $tRNA^{met}$ less efficiently than the wild-type. The most surprising result is that mutations within the anticondon loop have profound effects on tRNA transport, suggesting that proteins which interact with specific tRNA species, such as the aminoacyl tRNA synthetase, may participate in tRNA transport.

Biochemical studies of the two processing nucleases continue. Studies on the Alu-family sequence, a small, ubiquitous gene present in the vertebrate genome have been extended. We had shown that a particular Alu is transcribed, processed, and transported into the cytoplasm after nuclear injection of the gene into the oocyte. We have now found that this pathway is expressed in a tissue-specific fashion, suggesting for the first time a role for the Alu sequence in specific gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00408-01 HG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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.:	Michael A. Zasloff, M.D., Ph.D.	Head	HGB, NICHD
Other:	Stuart A Stein, M.D.	Medical Staff Fellow	HGB, NICHD
	Joseph Muenzer, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD
	Joan Marini, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD
	Anthony Adams	Biologist	HGB, NICHD

COOPERATING UNITS *(if any)*

Elizabeth F. Neufeld, Ph.D., GBB, NIADDK
Roy Levitt, M.D., CC, NIH

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Molecular Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Studies were begun this year on the molecular defect in Hunter Syndrome and the use of human amnion in treatment of the MPS disorders. By classical techniques, iduronate sulfatase was purified several thousand fold from human plasma, representing the first purification to this extent of this enzyme. Plans for studies of the cell biology of this protein are underway. Some 20 children with MPS syndromes were implanted with human amnion epithelium. Initial studies have failed to demonstrate increases in circulating serum lysosomal enzymes, although subjective improvement has been observed in some patients. Studies of the natural history of the disease process in MPS I and II have demonstrated, for the first time, the high incidence of hydrocephalus in these diseases, a process which appears to accelerate CNS deterioration. Further studies on the mechanism of action of thyroid hormone continue. A new method has been developed for the identification of species present in moderate to low abundance in mRNA populations. The method utilizes a contact hybridization procedure previously developed in this laboratory for quantitation of genomic reiteration frequency. Studies on therapeutic utility of 13-cis retinoic acid in the treatment of fibrodysplasia ossificans progressiva (FOP) continue. After a one year pilot utilizing 5 mg/kg/day of this agent, experience with 7 children suggests that the drug is effective in inhibiting formation of new ectopic bone. Treatment studies have been expanded to include a larger population. The utility of this agent in inhibiting new bone formation after surgical intervention is being studied. A new disease process involving the formation of intramembranous bone in the dermal and deeper fascial planes has been delineated, representing a distinctly different condition than FOP. (Proj. 00406-05 & 00407-03 have been combined with this one).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00409-01 HG

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Kinetics of Calcium Metabolism in Childhood and the Study of Prader-Willi Syndrome

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

P.I.:	James B. Sidbury, Jr., M.D.	Head	HGB, NICHD
Other:	Joseph Muenzer, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD
	Nancy Vieira	Biologist	LTPB, NICHD
	Alfred L. Yergey, Ph.D.	Research Chemist	LTPB, NICHD

COOPERATING UNITS (if any)
Pamela Brye, RN, CC, NIH

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Disorders of Carbohydrate Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.6	1.1	.5

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 study utilizies two different stable isotopes of calcium to determine calcium kinetics. The fact that the isotopes are non-radioactive, they can be used in children and pregnant women. One isotope is given intravenously, the other by mouth. Since the isotopes have different atomic weights the two calciums can be determined in the mass spectrometer simultaneously. Blood sampling is required for 12 hours only. Urine collections are made continuously every 8 hours for 2 weeks. Using the modeling program designed by Dr. Mones Berman, one can determine the size of the several calcium pools.

Another component of the study involves patients with the Prader-Willi syndrome (PWS) and patients with simple exogenous obesity as controls. The PWS patients and obese control children were given loading tests with cornstarch vs cooked corn, rice starch vs cooked rice, and potato starch vs cooked potato. These tests will be repeated in these same individuals as they lose weight toward normal. The glucose and insulin responses are followed. The results on these patients will also be compared with those obtained in glycogen storage disease patients who lack the whole control loop of blood glucose regulation. It is much more difficult to interpret the data from obese individuals than in those with glycogen storage disease. Obese individuals who have a diabetic GTT do have a readily recognizable abnormal starch tolerance test.

Project No. Z01 HD 001341-07 HG has been combined with this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00909-05 HG

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Effects of Ethanol on the Mother and the Fetus

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

P.I.:	Anil B. Mukherjee, M.D., Ph.D.	Head	HGB, NICHD
Others:	A. Ghazanfari, Ph.D.	Visiting Fellow	HGB, NICHD
	Sondra Levin, M.D.	Clinical Associate	HGB, NICHD
	Kurt Schumacher	Chemist	HGB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

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.)

Genetic studies on ethanol toxicity can be divided into two parts: (i) to what extent do inborn factors predispose individuals to abusing alcohol? and (ii) to what extent do inborn factors predispose individuals who abuse alcohol to the development of specific complications? Although recent studies suggest that there are at least some populations in which there are important genetic predispositions to the development of alcoholism, the first question remains controversial. It is clear, however, that some patients are biologically predisposed to developing one or another complication if they abuse ethanol. Transketolase abnormality has been suggested to be one of the concomitants of thiamine deficiency disease. A well known example is Wernicke-Korsakoff syndrome. Recently, we have found that this enzyme abnormality is present at a higher frequency among alcoholic men than in their non-alcoholic counterpart and in their male progeny long before they abused alcohol. The inheritance pattern of this enzyme abnormality seems to be autosomal recessive in nature. Additionally, it appears that transketolase abnormality may also be responsible for many of the concomitants of fetal alcohol syndrome (FAS), namely, intrauterine growth retardation (IUGR), microcephaly and abnormal brain pathology. Preliminary data suggest that thiamine deficiency, a frequent complication in chronic alcoholism may contribute to IUGR and microcephaly in the rat and teratogenic effects of alcohol is dramatically increased when pregnant animals are rendered thiamine deficient. Since all chronic alcoholic pregnant women do not give birth to FAS children it is suggested that a genetic predisposition to thiamine deficiency (i.e. transketolase abnormality) may be responsible for this variability among individuals. Additionally, while investigating the effects of ethanol on opiate peptides we recently discovered that there are two pools of β-endorphin like immunoreactivity in blood of mice, rats, rabbits and humans. This is the first time an erythrocyte pool has been recognized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00910-05 HG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

P.I.:	Anil B. Mukherjee, M.D., Ph.D.	Head	HGB, NICHD
Others:	R. Fujita, M.D., Ph.D.	Visiting Fellow	HGB, NICHD
	Sondra Levin, M.D.	Clinical Associate	HGB, NICHD
	A. Ghanzanfari, Ph.D.	Visiting Fellow	HGB, NICHD
	Janice Chou, Ph.D.	Section Head	HGB, NICHD

COOPERATING UNITS *(if any)*
Soo Il Chung, Ph.D., NIDR
Elliott Schiffman, Ph.D., NCI

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

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.)*

During the past year we have found that Uteroglobin (UG) is a potent inhibitor of aggregation of both rabbit and human platelets. This is a significant finding since pregnancy induces produciton of thromboxane A_2, a potent mediator of platelet aggregation in the uterus and in the lung. It is possible that UG may counteract the hypercoagulable state of pregnancy. Preliminary data suggest that uteroglobin by its anti-platelet aggregation effects may prevent thrombosis in the microvasulature of the placenta, the uterus and of the lung. Using a sensitive radioimmunoassay, SDS-PAGE and isoelectric focusing we have discovered that a protein similar in molecular weight, PI and immunological crossreactivity to uteroglobin is present in the neonatal human lung. This is the first time the presence of a human counterpart of rabbit uteroglobin has been clearly documented. Experiments underway will delineate the distribution of this protein in different human tissues and its possible function. Furthermore, the exact mechanism of inhibition of platelet aggregation by this protein will be studied in more detail.

In addition to the above findings, we have now established several rabbit alveolar and endometrial epithelial cell lines transformed by a temperature sensitive mutant of SV_{40}. These cell lines express both cytoplasmic and nuclear receptors for estradiol and progesterone. When stimulated with progesterone at 40°C the endometrial cells secrete uteroglobin in the medium. Thus, these cells, when fully characterized, will provide an unique tool to (i) determine the biological activity of various progestogenic agents in vitro which is unavailable at present and (ii) study the regulation of expression of the uteroglobin gene in response to progesterone by c-DNA probe analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00912-05 HG

PERIOD COVERED
October 1, 1983 to September 30, 1984

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)

P.I.:	Janice Y. Chou, Ph.D.	Head	HGB, NICHD
Others:	Takeshi Sakiyama, M.D.	Visiting Scientist	HGB, NICHD
	Shori Takahashi, M.D.	Visiting Fellow	HGB, NICHD
	Vincenzo Zimarino, M.D.	Visiting Fellow	HGB, NICHD
	Kuo-Ping Huang, Ph.D.	Senior Investigator	ERRB, NICHD

COOPERATING UNITS (if any)

Drs. I. Sun and F.L. Carne, Purdue University

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Cellular Differentiation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
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.)

Our studies have concerned regulation of gene expression during normal and abnormal differentiation processes. Topics of current interest are: 1) control of expression of α-fetoprotein (AFP), albumin, and transferrin in liver and liver derived cell lines; 2) control of expression of human chorionic gonadotropin (hCG) and alkaline phosphatase in placental, normal and malignant nontrophoblastic cells. Using the temperature-sensitive rat fetal liver cells, we have demonstrated that both qualitative and quantitative alternations in AFP gene expression occurred during transformation of fetal liver cells in vitro. At 40°C, RLA209-15 fetal liver cells exhibit a differentiated phenotype that resemble fetal liver in vivo: they synthesize two AFP variants of 73,000 and 69,000 daltons and contain an AFP mRNA species of 20S. RLA209-15 cells exhibit a transformed phenotype at 33°C. Transformation is accompanied by the synthesis of reduced level of AFP with an apparent molecular weight of 65,000 and the detection of reduced level of AFP mRNA of 14S. The 14S AFP mRNA appears to be generated by the alternative RNA splicing pathway. The RLA209-15 cells also prove to be a suitable model system to study the molecular basis of maturation. We found that retinoic acid is one of the regulators that induce maturation of fetal liver in vitro.

In studies on alkaline phosphatase gene expression, we found that both sodium butyrate and 5-bromo-2'-deoxyuridine (BrdUrd) induce a specific increase in the placental alkaline phosphatase mRNA leading to the observed enhnacement of biosynthesis.

Z01 HD 00610-04 Puberty and its Disorders: Physiology, Pathophysiology and Therapy
Gordon B. Cutler, Jr., M.D.

Z01 HD 00613-04 Clinical and Basic Studies of Male Reproduction
Richard J. Sherins, M.D.

Z01 HD 00614-04 Biology of Hormone Binding Proteins
Bruce C. Nisula, M.D.

Z01 HD 00615-04 Steroid Antagonists
George P. Chrousos, M.D.

Z01 HD 00616-04 Structure, Function, and Physiology of Glycoprotein Hormones
Bruce C. Nisula, M.D.

Z01 HD 00618-03 Physiology and Clinical Applications of Corticotropin Releasing Hormone
George B. Chrousos, M.D.

Z01 HD 00619-03 Hypothalamic-Pituitary-Gonadal Interaction
D. Lynn Loriaux, M.D.

Z01 HD 00620-03 Steroid and Peptide Hormone Action
D. Lynn Loriaux, M.D.

Z01 HD 00621-02 Mechanism of Linear Growth
Fernando Cassorla, M.D.

Z01 HD 00622-02 Diagnostic and Therapeutic Applications of Growth Hormone Releasing Factors
George R. Merriam, M.D.

Z01 HD 00623-01 Adrenal Physiology and Pathophysiology
Gordon B. Cutler, Jr., M.D.

Z01 HD 00901-06 Endocrine Assays Laboratory
Wilbert E. Nixon, Ph.D.

Z01 HD 00916-04 Studies of Corpus Luteum Function in the Cycling and Pregnant Monkey: Relaxin Secretion
Wilbert E. Nixon, Ph.D.

ANNUAL REPORT
Summary

DEVELOPMENTAL ENDOCRINOLOGY BRANCH
National Institute of Child Helth and Human Development

The research aim of the Developmental Endocrinology Branch is to further our understanding of the role of the endocrine system in the complex processes of growth and development. The periods of research interest include fetal and neonatal life, puberty, and senility. The current focus of research is the pubertal period. The systems under study are three; the hypothalamic-pituitary-gonadal axis, the hypothalamic-pituitary-adrenal axis, and the system regulating skeletal growth which include growth hormone, its releasing factor, and somatomedin.

Studies on the hypothalamic-pituitary-gonadal axis are directed toward understanding the initiation of LH and FSH secretion which heralds the onset of puberty, the mechanism of action of these glycoprotein hormones, the gonadal response to these hormones, and the roles of the gonadal sex steroids in gametogenesis, central nervous system maturation, breast physiology, hair growth, and skeletal maturation.

Studies on the hypothalamic-pituitary-adrenal axis are directed at understanding the complex process of adrenarche, understanding the biochemical defects underlying the congenital adrenal hyperplasia syndromes, and clarifying the pathophysiology of the various causes of Cushing's syndrome and adrenal insufficiency.

Studies on the human growth hormone releasing hormone, human growth hormone, somatomedin C system are directed toward understanding the processes which regulate growth and maturation of the skeleton.

Specific areas of investigation are outlined in the following paragraphs:

Several ongoing studies are directed at understanding the phsyiology of human puberty. We have previously shown that central precocious puberty is mediated by LHRH and that this disorder can be treated with an LHRH analogue that desensitizes the pituitary response to endogenously secreted LHRH. Over 100 children with centrally mediated precocious puberty are being treated with this analogue. The results are promising. The secondary sexual characteristics regress and the accelerated rate of growth falls to normal. The altered behavior improves and peer group interactions tend to normalize. Some of these children have been treated now for as long as 4 years. No adverse reactions have occurred.

We have used the LHRH analogue as a probe to clarify the mechanism of puberty in two other forms of precocious puberty - the McCune-Albright syndrome and familial male isosexual precocious puberty. With this probe, we have shown that both of these forms of precocious puberty are independent of gonadotropin support and, hence appear to be gonadal disorders. Armed with this knowledge, we have designed rational treatment scheme for these two disorders. We have treated 6 patients with the McCune-Albright syndrome using testolactone, an aromatase inhibitor, to block estrogen formation in the gonad. All treated children have shown improvement in their secondary sexual characteristic and a

decreased rate of growth. We have treated 4 boys with familial isosexual precocity with the antiandrogen spironolactone. All but one have improved. These studies continue.

Adrenal maturation is an important feature of the pubertal process. A series of studies on the mechanism of adrenarche have come to fruition in the past year. These studies were done in the only known animal model of human adrenarche, the chimpanzee. The studies involved two groups of adult castrated male chimpanzees, one group of which was hypophysectomized and replaced with ACTH and thyroid hormone, the other group of which was sham hypophysectomized. Plasma and urine glucocorticoids remained the same in the two groups, while plasma DHA and DHA sulfate fell dramatically in the hypophysectomized group. The DHA to cortisol ratio following ACTH stimulation was significantly lower in the hypophysectomized animals. This study supports the hypothesis that a non-ACTH pituitary factor plays a role in supporting adrenal androgen secretion. Efforts to further identify this factor are underway.

The mechanism of action of the steroid hormones is of importance in understanding the effects of these hormones on development. Considerable work over the last year has been directed at better understanding the mechanisms of action of the glucocorticoids series of hormone.

Two experiments of Nature provided us with a unique opportunity: Two patients were identified who manifest primary cortisol resistance, a disease entity characterized by "hypercortisolism without Cushing's syndrome", hypertension and hypokalemic alkalosis, and second, the finding of very high free plasma cortisol in New World primate species. These monkeys also have other alterations of plasma steroid hormones such as increases os aldosterone, progesterone, estradiol and testosterone.

We have shown that both of these conditions can be explained on the basis of a receptor mediated resistance to glucocorticoid action. The abnormality appears to be one of affinity rather than receptor number. We have, in the past year, systemically investigated physico-chemical nature of these receptors. Their molecular weight, their pattern of activation, their interaction with chromatin, and their metabolism as reflected in the relative amounts and rate of appearance of the monoreceptor form of this receptor complex have been examined. In all respects, the receptors in these two models of resistance have been found to be identical to suitable controls: normal men in the human model, and Old World primates in the monkey model.

Complementing these experiments are a series of studies with a new discovered antiglucocorticoid, RU 38486, which competes with cortisol for binding to the glucocorticoid receptor. We have used this drug effectively to treat the ectopic ACTH syndrome and to prepare patients with this disorder for surgery. We are expanding our studies with this drug to explore the mechanism of the antiinflammatory action of glucocorticoids using a rodent model of inflammatory polyarthritis.

The regulation of ACTH is an important variable in the response of the hypothalamic-pituitary-adrenal axis to stress. The isolation, purification, sequencing and synthesis of corticotropin releasing factor (CRF) has provided an effective tool for exploring the physiology of ACTH regulation. We have developed a safe and effective CRF test for clinical use. This test has proved to be valuable

in the differential diagnosis of ACTH dependent Cushing's syndrome. Patients with Cushing's disease (ACTH secretion from a pituitary microadenoma) respond to CRF with an increase in circulating ACTH. Patients with the ectopic ACTH syndrome fail to respond to this stimulus and thus can be distinguished from patients with Cushing's disease with great confidence. CRF has also been used as a tool to help to understand the pathophysiology of increased cortisol secretion in depression. Increased CRF secretion has proved to be the underlying abnormality in this condition that is responsible for the increaed rate of cortisol synthesis and release.

The treatment of Cushing's disease is theoretically straight forward--excision of the ACTH secreting microadenoma. In practice, however, the efficacy of this procedure has been about 50%. We reasoned that preoperative localisation of the mciroadenoma within the substance of the pituitary gland might improve this record. In collaboration with Dr. John Doppman, we have shown that simultaneous cannulation of the inferior petrosal sinus allows preoperative determination of the side of the pituitary gland in which the microadenoma resides. CRF administration during the sampling amplifies the gradients and makes the determination simpler. Since we have initiated this diagnostic aid, our surgical success rate has climbed from 50% to nearly 100% in patients with Cushing's disease.

X-ray therapy is an important second line of treatment for Cushing's disease. It is, however, rather non-specific, ultimately impairing the synthesis of all of the anterior pituitary hormones. We wondered if we could alter the sensitivity of pituicytes to radiation by altering their metabolic activity during the course of radiotherapy.

To examine this question, we devised a rat model in which we could deliver a single dose of x-ray to the pituitary gland and monitor the alterations in hormone secretion that followed. Using the sex steroids in combination with the various releasing factors, we can selectively stimulate or suppress any given cell type or combination of cell types. We have shown that cells actively synthesizing and secreting peptide hormones are somewhat protected against the damaging effects of ionizing radiation. This model is being explored further for its potential clinical applications.

One of the interesting side effects of CRF is its ability to lower blood pressure. The effect is dramatic, about a 50% lowering of mean blood pressure, and prolonged, lasting over 3 hours. We have investigated the mechanism of this effect in primates and found that CRF causes an immediate and prolonged decrease in peripheral vascular resistance. The effect seems to be direct and not mediated through a secondary humeral mechanism such as ACTH or beta-endorphin. The possible applications of this finding are under study.

The sex steroid hormones, androgen and estrogens, are largely responsible for the secondary sexual changes that occur during the pubertal process. Some of these changes are desirable, some undesirable. One desirable change is the skeletal growth responsible for the pubertal growth spurt. Current evidence suggests that estrogen is the hormone most responsible for this effect. We have explored the dose-response relationship between estrogen and bone growth in children with gonadal dysgenesis in an effort to create a data base that would allow us to optimize this effect in children requiring the initiation and maintenance of puberty with exogenously administered hormone. The findings

were interesting in that the optimal dose was shown to be much lower than supposed and that the doses used and generally recommended may well lead to suboptimal growth. This finding is being further explored and applied.

Undesirable effects of sex steroids during puberty include the development of hirsutism, acne, and male pattern baldness. All of these effects are androgen mediated. Theoretically, they could be prevented or ameliorated with an antiandrogen. Systemic antiandrogen therapy, however, has several undesirable side effects which include loss of libido and potency. We have shown that these systemic effects can be avoided by the topical application of an antiandrogen and that the drug, applied in this way, is effective in treating hirsutism in women and in preventing male pattern baldness in the stump tail macaque, a primate model of male pattern baldness in men. These studies are being extended to the treatment of acne in adolescents.

Growth hormone plays a central permissive role in skeletal growth. The use of growth hormone, however, is limited by its short supply. Growth hormone releasing factor (GRF) is a 44 amino acid peptide that is readily synthesized and relatively inexpensive. We have shown that GRF releases growth hormone in over 80% of growth hormone deficient children and induces a growth response indistinguishable from that obtained with exogenous growth hormone administration. Thus, a new, more cost effective, and more widely available therapy for growth hormone deficient children now exists. We are beginning to apply this new therapy in a prospective fashion.

An RIA for growth hormone releasing factor (GRF) has been developed which is capable of determining GRF levels in serum of patients receiving GRF in experimental protocols. Efforts are continuing to improve sensitivity of the assay and to overcome nonspecific serum effects often encountered.

An important feature of the pubertal process is the initiation of gametogenesis. The mechanisms underlying this complex process in men and women are understood in only the most rudimentary way. We are studying the process in both men and women. Studies in women have centered about how the ovary and hypothalamic-pituitary unit interact and how a common disorder, proalctin secreting microadenomas, alter this interaction. We have shown that the ovary regulates the frequency of gonadotropin-releasing hormone secretory bursts from the arcuate nucleus of the hypothalamus and, in that way, regulates its own function. Prolactin interferes with the ability of the hypothalamous to sense the ovarian signals, estrogen and progesterone, and leads to a state of hypogonadotropic hypogonadism. The effect seems to be directly at the hypothalamic level. The ovary seems to signal the pituitary gland when a dominant follicle is ready to ovulate. This signal appears to be progesterone from the dominant follicle. How progesterone alters pituitary function is currently under study.

Studies aimed at understanding the hypothalamic-pituitary-thyroid axis have yielded significant new information about the mechanism of TSH action. Previously, TSH was shown to interact with two classes of binding sites in human thyroid tissue, one displaying a high affinity interaction with TSH, and the other a low affinity interaction. We conducted studies to evaluate the relative contributions of these two classes of sites to the action of TSH on adenylate cyclase. By use of a specific molecular probe developed in our laboratory, we demonstrated that the low affinity sites are not the sites through which TSH stimulates adenylate cyclase, and that the active role is

best ascribed to the high affinity sites. This insight has important implications for interpretation of earlier research on thyroid membranes and makes available a critical probe that can be expected to accelerate future research on the molecular mechanisms of TSH action.

Previous elucidation of human choriogonadotropin (hCG) as the thyrotropic factor that mediates the thyrotoxicosis of choriocarcinoma and hydatidiform mole has provided a molecular congener of TSH useful for structure function studies. In the current year, investigations of the role of the carbohydrate moieties of hCG in its thyrotropic activity have been especially fruitful in this area. Deglycosylation produced divergent effects--enhanced binding to the TSH receptor, but loss of intrinsic activity. In conjunction with our earlier studies, this finding strongly supports the concept that the TSH receptor has separate domains for its binding and activation functions. These results point to the potential for development of clinically applicable competitive antagonists for the treatment of Graves' hyperthyroidism, one of the most common endocrine disorders.

A number of studies have been aimed at elucidating the mechanisms of male reproductive disorders in an attempt to establish rational strategies of treatment. Studies have centered on the hormonal regulation of human spermatogenesis, testicular feedback regulation of gonadotropin secretion and the biology of sperm function.

The availability of men with selective gonadotropin deficiency has provided a unique opportunity to quantify the hormonal requirements for human spermatogenesis. We have shown during the past year that early exposure of FSH with hCG augments both testicular growth and appearance of sperm in men with complete hypogonadotropism. These data, together with our former observation that estradiol overproduction, resulting from exogenous hCG administration limits sperm production, suggest that the temporal relationships of FSH and LH in stimulating the testis are important in determining the level of gonadal response. Accordingly we going to study the gonadal response to changing algorhythms of FSH and LH by using a pulsatile pump to give GnRH exogenously to these subjects and assess the potential for augmenting testicular function.

Evaluation and management of men with idiopathic infertility continues to be a major focus in our Branch. It has been disappointing to recognize that endocrine manipulations have not improved testicular function and fertility in these patients, in contrast to the highly successful effects of replacing gonadotropins in hypogonadotropic men. We have just completed an assessment of androgen receptor binding in genital tissue fibroblast cultures from azoospermic men and find normal binding characteristics, in contrast to a recent report from another laboratory. Additionally, in a pilot study, we have treated these patients with hCG and Teslac to increase testicular testosterone bioavailability while reducing estradiol overproduction. In a word, testicular function was not improved.

In view of our observation that gonadotropin deficient men are highly fertile when they achieve sperm output of only 2-5 million following treatment we are now redirecting our laboratory efforts to assess sperm function in an attempt to develop a rational strategy for therapy. We have preliminary evidence now that sperm from men with idiopathic infertility have lower levels of several epididymal glycoproteins that appear to be required for normal fertilizing

potential. This area will be explored aggressively this year since the observation represents an important break through in our perception of sperm function and its relationship to fertility.

Considerable progress has been made in our studies of feedback regulation of gonadotropin secretion in the male. The mechanism underlying regulation of FSH secretion (the inhibin concept) has been very controversial. We have previously shown that testosterone alone can maintain FSH and LH secretion within the normal range in the absence of other testicular factors, that the increased FSH level in men with germ cell depletion is associated with a 50% decrease in testosterone production and that in the experimental animal selective increase in FSH can be accomplished when androgen production is reduced in association with increased estrogen levels. These data provide an important alternative to the inhibin hypothesis. Accordingly, further studies in men are designed to assess the production rates of estradiol in such subjects and to determine whether FSH concentrations can be reregulated into the normal range by testosterone alone.

The mechanisms by which sex steroids regulate gonadotropin secretion have been of particular interest. Techniques have been developed to quantify the biosynthesis of LH subunits in rat pituitary tissue and considerable data collected which show that castration increases the LH subunit biosynthetic rate as well as secretion. Additionally we have found that GnRH increases specific glycosylation of LH subunits. These methods will now be used to explore the biochemical mechanisms of sex steroidal regulation of gonadotropin secretion. We have been hampered in our abilty to assess FSH biosynthetic events by lack of potent antiserums to the FSH subunits, but are increasing sensitivity of the methods used to allow studies of both gonadotropins soon. These studies are promising with regard to defining the biological and physiological mechanisms involved in gonadotropin synthesis, processing, storage and release.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00610-04 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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 list)

COOPERATING UNITS *(if any)*

National Institute of Mental Health; Stanford University Department of Pediatrics; see attached list

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	6	.5

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

The objective of this project is to 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 investigation include the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the treatment of central precocious puberty with an analog of luteinizing hormone releasing hormone, the development of luteinizing hormone releasing hormone agonists that can be administered by an intranasal route, the treatment of central precocious puberty secondary to congenital adrenal hyperplasia, the treatment of the McCune-Albright syndrome with an aromatase inhibitor, and the treatment of familial male isosexual precocious puberty with an antiandrogen.

Others:	D. L. Loriaux	Chief	SSH, DEB, NICHD
	B. Albertson	Staff Fellow	DEB, NICHD
	K. M. Barnes	Bio Lab Tech	DEB, NICHD
	J. Booth	Visiting Associate	DEB, NICHD
	F. Cassorla	Visiting Scientist	DEB, NICHD
	G. Chrousos	Visiting Scientist	DEB, NICHD
	F. Comite	Med. Staff Fellow	DEB, NICHD
	P. Feuillan	Med. Staff Fellow	DEB, NICHD
	J. Levine Ross	Med. Staff Fellow	DEB, NICHD
	G. Merriam	Clinical Associate	DEB, NICHD
	A. Munabi	Med. Staff Fellow	DEB, NICHD
	O. Pescovitz	Med. Staff Fellow	DEB, NICHD
	D. Risin	Biologist	DEB, NICHD
	M. Uriarte	Guest Worker	DEB, NICHD
	J. Winterer	Clinical Associate	DEB, NICHD

Cooperating Units

LDP, National Institute of Mental Health (E. Susman, G. Inoff, J. Blue); Dept. of Pediatrics, Stanford Univ. (R. Rosenfeld, R. Hintz); Human Genetics Branch, NICHD, NIH (S. Adeniyi-Jones); Child and Family Research Branch, NICHD, NIH (W. Sonis, R. Klein); Clin Center, NIH (M. Skerda, A. McNemar, K. Hench); Developmental Pharmacology Branch, NICHD, NIH (C. Foster); Pregnancy Research Branch, NICHD, NIH (D. Kenigsberg)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00613-04 DEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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)*

P.I.: R. J. Sherins Head, SRE DEB, NICHD

Others: (see attached list)

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

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Reproductive Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of this study are to ascertain biological, physiological and clinical mechanisms of male reproductive disorders and to provide rational strategies of treatment for men with reproductive disease.

This project represents a continuum of research begun in 1970 and includes studies of 1) the hormonal regulation of spermatogenesis in gonadotropin deficient men, 2) biology of sperm function 3) adverse effects of 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 during the past year have shown 1) that early exposure of hypogonadotropic men to FSH (with hCG) augments testicular growth and onset of sperm production, 2) that epididymal glycoproteins, which normally coat sperm and which are required for fertilization, are reduced on sperm from some men with idiopathic infertility, 3) that testicular exposure to fractionated radiation above 50 rads leads to germinal depletion with increased serum FSH levels and that above 100 rads leads to prolonged testicular injury, 4) that, contrary to a recent report, no subtle androgen receptor deficiency is found in cultured genital skin fibroblasts from men with idiopathic azoospermia and that treatment with human chorionic gonadotropin plus testolactone (an aromatase inhibitor) to augment testicular androgen production does not improve sperm production; and 5) that GnRH and orchiectomy differentially regulate LH subunit apoprotein and carbohydrate biosynthesis in studies of rat pituitary tissue.

The current project "clinical and basic studies of male reproduction" combines two former projects "role of sex steroids in regulation of FSH and LH levels in man" (Z01 HD 00612-03 DEB) and "clinical studies of male reproductive disorders" (Z01 HD 00613-03 DEB) but retains one of the original project numbers.

Others	D. L. Loriaux	Chief	DEB, NICHD
	B. C. Nisula	Senior Investigator	DEB, NICHD
	D. Vogel	Medical Staff Fellow	DEB, NICHD
	R. V. Clark	Medical Staff Fellow	DEB, NICHD
	J. Booth	Guest Researcher	DEB, NICHD
	G. R. Merriam	Junior Investigator	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	J. Winterer	Medical Staff Fellow	DEB, NICHD
	S. Rose	Medical Staff Fellow	DEB, NICHD
	D. Brightwell	Technician	DEB, NICHD
	J. Blaquier	Serono Fellow	DEB, NICHD

Coooperating Units:

Applied Physics Laboratory, Johns Hopkins University, Laurel, MD; Surgery Branch, NCI, NIH (S. Rosenberg); Radiation Oncology Branch, NCI, NIH (T. Kinsella); Pediataric Oncology Branch, NCI, NIH (D. Poplack); Clinical Endocrinology Branch, NIADDK, NIH (B. Weintraub); Pharmaceutical Development Service, CC, NIH; Division of Endocrinology, National Naval Medical Center, Bethesda, MD (C. Eil); Laval University, Quebec, CANADA (C. Gagnon)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00614-04 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Biology of Hormone Binding Proteins

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

PI:	B. C. Nisula	Head	DEB, NICHD
Others:	G. Chrousos	Visiting Scientist	DEB, NICHD
	R. Hiramatsu	Visiting Fellow	DEB, NICHD
	D. Loriaux	Head, SSH	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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.)*

The general goals of this project are to understand the biology of the circulating hormone binding proteins and to delineate the role that they play in human disease. Recent research findings include demonstration of striking evolutionary divergences in corticosteroid-binding globulin and testosterone-binding globulin in primates, and observation of apparent androgen resistance in primate species that are resistant to cortisol. Future directions of the project will emphasize the role of adrenal function in modulation of circulating CBG and the application of sex-hormone binding globulin as a biochemical index of thyroid hormone action in patients with resistance or pseudo-resistance syndromes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00615-04 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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:	G.P. Chrousos	Head	DEB, NICHD
Others:	G. B. Cutler, Jr	Senior Investigator	DEB, NICHD
	D. L. Loriaux	Chief	DEB, NICHD
	G. Merriam	Medical Officer	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

Pregnancy Research Branch, NICHD (D. Healy)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Hypothalamic Releasing Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1	.4

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 38486) developed recently by Roussel-UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects. Given to nonhuman primates or man it causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of glucocorticoid (dexamethasone). Thus, antiglucocorticoids could be used for challenging the hypothalamic-pituitary-adrenal axis when clinical testing is required in patients with disorders of this axis. We also used RU 38486 to treat a patient with severe hypercortisolism due to ectopic ACTH secretion. The therapy caused remission of the clinical manifestations of Cushing's syndrome in this patient. We are planning to enlarge the therapy series. We are currently studying the molecular mechanisms of action, and the effects and the pharmacokinetic properties of this drug in nonhuman primates and man. Four new, potentially better analogs of RU 38486, will be provided by Schering Co. for both basic studies and potential clinical applications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00616-04 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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:	S. Amr	Visiting Associate	DEB, NICHD
	D. Blithe	Staff Fellow	DEB, NICHD
	J. P. Caron	Visiting Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.7	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The 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 progress includes the following: delineation of the biological relevance of low and high affinity TSH binding sites in human thyroid membranes and elucidation of the role of the carbohydrate moiety of hCG in its thyrotropic activity. Future investigations will evaluate the role of the galactose-terminated glycoprotein pathway in the metabolism of fully glycosylated proteins, explore the nature and biological relevance of hCG alpha-subunit heterogeneity in pregnancy, and develop clinical applications for ultrasensitive glycoprotein hormone assays.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00618-03 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Physiology and Clinical Applications of Corticotropin Releasing Hormone

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

PI: G. P. Chrousos Head DEB, NICHD

Others: (see attached list)

COOPERATING UNITS *(if any)* Clinical Neuroendocrinology Section, BPS, NIMH (P. C. Avgerinos); Surgery Branch, NCI (R. Udelsman); CNS, BPB (P. Gold); Pregnancy Research Branch, NICHD (D. Healy); SNB, NINCDS (E. Oldfield).

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Hypothalamic Releasing Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

In this project we seek to advance understanding of the role of corticotropin releasing hormone (CRH) in normal and stress physiology and in disorders of hypothalamic-pituitary-adrenal function. Rapid progress in this area has been made possible by the recent discovery of the chemical structures of, first, ovine CRH (oCRH) and, more recently, of human CRH (hCRH). Our objectives have been to determine the dose-response relationship for ovine and human CRH in nonhuman primates and in man, to study the metabolic clearance rates of these peptides, to develop methods to measure CRH accurately in tissues and in biological fluids of patients with abnormalities of the hypothalamic-pituitary-adrenal axis, to develop a clinical CRH test, and to evaluate its usefulness in adrenal insufficiency, Cushing's syndrome, and pseudo-Cushing's states. Our studies to date have shown that both ovine and human CRH are active in nonhuman primates and man. The appropriate dose and mode of testing man have been established and the pharmacological parameters have been determined in both primates and men. CRH stimulation appears to be a useful test in the differential diagnosis of adrenal insufficiency, Cushing's syndrome and pseudo-Cushing's states. Physiological experiments suggest that Cushing's disease is pituitary whereas hypercortisolism in depression is hypothalamic in origin. Successful treatment of Cushing's disease with surgery is followed by normalization of the CRH stimulation test. Human CRH causes brief plasma ACTH and cortisol elevations in human subjects that are pulse-like and mimic the spontaneously occurring physiologic ACTH and cortisol secretory episodes. This is explained by the brief plasma half-life and the high MCR of this peptide. These properties of hCRH make it an important means for the study of the physiology of the hypothalamic-pituitary-adrenal axis.

Others:	G. B. Cutler, Jr.	Senior Investigator	DEB, NICHD
	W. T. Galluci	Guest Researcher	DEB, NICHD
	D. L. Loriaux	Chief	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	R. Rittmaster	Medical Staff Fellow	DEB, NICHD
	T. Tomai	Guest Researcher	DEB, NICHD
	T. Schuermeyer	Guest Researcher	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00619-03 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Hypothalamic-pituitary-gonadal interaction

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

P.I.	D. L. Loriaux	Head	DEB, NICHD
Others:	G. R. Merriam	Junior Investigator	DEB, NICHD
	R. Collins	Guest Worker	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	C. Coddington	Guest Worker	DEB, NICHD
	B. D. Albertson	Staff Fellow	DEB, NICHD
	D. G. Pfeiffer	Visiting Fellow	DEB, NICHD
	J. Booth	Visiting Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

Dept. of Obstetrics and Gynecology, Stanford University (S.A. Brody);
The Population Council of New York (I. Spitz).

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Steroid Hormones

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Studies this year have centered about how the ovary and the hypothalamic-pituitary unit interact in women. We have shown that the hypothalamic release of GNRH is modulated by both estrogens and progestins of ovarian origin. The mechanism of modulation has been clarified. It has been shown that the ovary signals the hypothalamic-pituitary unit when it has a dominant follicle ready for ovulation, and that the signal is progesterone from the dominant follicle. The mechanism of the preovulatory progesterone surge has been clarified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00620-03 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Steroid and peptide hormone action

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

P.I.	D. L. Loriaux	Head	DEB, NICHD
Others	R. Rittmaster	Medical Staff Fellow	DEB, NICHD
	P. Feuillan	Medical Staff Fellow	DEB, NICHD
	V. Goh	Visiting Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

Brown University (Stump-trailed Macaque Colony)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Steroid Hormone

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

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

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

Studies over the past year have concentrated on disorders of masculinization and feminization. The topical application of an antiandrogen has been shown effective in the treatment of hirsutism. The topical application of a 5-α reductase inhibitor has been shown to prevent or retard male pattern baldness in stump tailed macaque monkeys. An investigation of an outbreak of gynecomastia in Haitian refugees has been concluded with the findings that a delousing agent, R & C spray, contains an antiandrogen, phenothrin, which can account for the findings.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00621-02 DEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

P.I.:	F. Cassorla	Visiting Scientist	DEB, NICHD
Others:	G. B. Cutler, Jr.	Head	DEB, NICHD
	O. Pescovitz	Medical Staff Fellow	DEB, NICHD
	S. Rose	Medical Staff Fellow	DEB, NICHD
	S. Malozowski	Visiting Fellow	DEB, NICHD
	M. Nicoletti	Guest Worker	DEB, NICHD
	D. L. Loriaux	Chief	DEB, NICHD

COOPERATING UNITS *(if any)*
Clinical Center, NIH (M. Skerda); Catholic University of Nijmegen, The Netherlands (I. M. Valk); Hahnemann Medical School, Philadelphia, Pennsylvania (J. L. Ross); Stanford University, Stanford, California (R. Rosenfeld)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Developmental Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include studying short term growth in normal children. In addition, we are investigating the growth of patients with precocious puberty, and the effects of growth hormone and sex steroid administration on linear growth in patients with Turner's syndrome and delayed puberty. We are also attempting to define the optimal dose of hydrocortisone for growth in patients with adrenal insufficiency. 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. Finally, we are 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00622-02

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diagnostic and Therapeutic Applications of Growth Hormone Releasing Factors

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

PI:	G. R. Merriam	Medical Officer	DEB, NICHD
Others:	O. F. Almeida	Visiting Fellow	DEB, NICHD
	F. G. Cassorla	Visiting Scientist	DEB, NICHD
	M. C. Gelato	Medical Staff Fellow	DEB, NICHD
	D. L. Loriaux	Head	DEB, NICHD
	S. Malozowski	Visiting Fellow	DEB, NICHD
	O. H. Pescovitz	Medical Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, NINCDS, NIH; Biological Psychiatry Branch, NIMH; Dept. of Medicine, Univ of Virginia, Charlottesville, Va.

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Steroid Hormones

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.55	.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.)*

Growth hormone (GH) secretion is regulated by two hypothalamic peptides: somatostatin, which inhibits GH release; and growth hormone-releasing factor (GRF), which stimulates the synthesis and release of GH. We are using GRF as a tool to study the neuroendocrine regulation of GH, and exploring the potential uses of GRF in the diagnosis and treatment of diseases including GH deficiency (dwarfism) and GH excess (gigantism and acromegaly). During the past year we have determined the dose-response curve for the stimulation of GH by GRF (1-44) NH_2 (GRF-44) in normal men and women, and on this basis have developed a standardized GRF test. We have tested children in various developmental stages and aging adults to provide a normal range throughout life. Using the GRF test, we have studied patients with acromegaly to determine what proportion respond to GRF, and whether the response could be used to assess their clinical status. The majority responded to GRF with a prompt rise in GH. Most responses overlapped with the normal range, and the response did not correlate with clinical or biochemical featues of the disease. We have compared the responses of GH deficient (GHD) children with those of age-matched normal control children. The majority (80%) of children with GHD have measurable GH responses to GRF; they are lower as a group than those of controls, but overlap with the normal range. This indicates that GHD is usually due to GRF deficiency, and that GRF might therefore be used as a therapy for GHD. To test this possibility, we have administered GRF or placebo repeatedly to 9 children with GHD. In 6 of the 9, each dose of GRF stimulated a burst of GH secretion. Over the course of treatment plasma somatomedin-C rose toward normal, and linear growth rates were accelerated to a degree comparable to or greater than that of standard doses of hGH. Thus it appears that GRF could become an alternative therapy of GHD in the majority of cases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00623-01 DEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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: G. B. Cutler, Jr. Head DEB, NICHD

Others: (see attached list)

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

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Developmental Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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.)*

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 secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing's syndrome.

Other professional personnel

Others:	D. L. Loriaux	Head	SSH, DEB, NICHD
	B. Albertson	Staff Fellow	DEB, NICHD
	P. Avgerinos	Visiting Fellow	DEB, NICHD
	K.M. Barnes	Bio Lab Tech	DEB, NICHD
	J. Booth	Visiting Associate	DEB, NICHD
	F. Cassorla	Visiting Scientist	DEB, NICHD
	G. Chrousos	Visiting Scientist	DEB, NICHD
	F. Comite	Clinical Associate	DEB, NICHD
	P. Feuillan	Medical Staff Fellow	DEB, NICHD
	A. Munabi	Medical Staff Fellow	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	O. Pescovitz	Medical Staff Fellow	DEB, NICHD
	D. Risin	Biologist	DEB, NICHD
	R. Rittmaster	Medical Staff Fellow	DEB, NICHD
	J. Levine Ross	Medical Staff Fellow	DEB, NICHD
	J. Winterer	Clinical Associate	DEB, NICHD

Collaborating Investigators

Chief, Radiology, Clinical Center, NIH (J. Doppman); Chief, SNE, BPB, National Institute of Mental Health (P. Gold); New Mexico State University, Holloman AFB, New Mexico (W. C. Hobson); Rush University College of Health Sciences, Chicago, Ill. (J. Ogden); Senior Investigator, SNB, NINCDS, NIH (E. Oldfield); Staff Radiologist, Radiology, CC, NIH (N. Petronas)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00901-06 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Endocrine Assays Laboratory

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

PI:	W. E. Nixon	Senior Investigator	DEB, NICHD
Others:	O. Pescovitz		DEB, NICHD
	R. Reid	Technician	DEB, NICHD
	J. Posey	Summer Aid	DEB, NICHD
	E. Witter	Stay-in-School	DEB, NICHD
	M. Nicolette	Guest Worker	DEB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Endocrine Assay Unit

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

An enzyme-linked immunosorbent assay (ELISA) for human chorionic gonadotropin has been developed. Results with this hCG ELISA have been compared with those from an established radioimmunoassay (RIA) and correlation coefficients of R=0.95 and R=0.93 were obtained for serum and urine, respectively.

An RIA for growth hormone releasing factor (GRF) has been developed which is capable of determining GRF levels in serum of patients receiving GRF in experimental protocols. Efforts are continuing to improve sensitivity of the assay and to overcome nonspecific serum effects often encountered.

Teaching of radioimmunoassay methodology for polypeptide hormones as well as other laboratory procedures required for ongoing projects has been provided for Branch Fellows and others.

Sheep anti-rabbit gamma globulin sera (second antibody) was generated, titered and distributed to NICHD Intramural Investigators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00916-04 DEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Studies of Corpus Luteum Function in the Cycling and Pregnant Monkey: Relaxin Secretion

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

PI:	W. E. Nixon	Senior Investigator	DEB, NICHD
Others:	R. Reid	Technician	DEB, NICHD

COOPERATING UNITS *(if any)*

University of Arizona Medical School (R. L. Stouffer)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Endocrine Assay Unit

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.4	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Previous investigations have demonstrated an induction of relaxin secretion in monkeys administered hCG during the luteal phase of the menstrual cycle. Recent studies utilizing hCG at levels that mimic early pregnancy indicate that the pattern of hCG induced relaxin secretion is dependent on the age of the corpus luteum at the time of initial hormonal administration. During early, middle and late luteal phases (days 5, 8 and 12 following LH surge, respectively), hCG administration resulted in detectable levels of relaxin in 9.0 ± 1.0 days, 6.6 ± 1.4 days and 4.7 ± 1.9 days, respectively.

Serum progesterone decreased to minimal levels (<2 ng/ml) prior to relaxin secretion.

Relaxin secretion was sustained only in those animals receiving hCG at midluteal phase, the time of expected implantation in a fertile cycle. Thus, these observations may support a role for relaxin during early pregnancy.

ENDOCRINOLOGY AND REPRODUCTION RESEARCH BRANCH

Z01 HD 00022-11 Renin-Angiotensin System and Aldosterone Regulation
Greti Aguilera, M.D.

Z01 HD 00035-12 The Structure and Function of Biologically Active Molecules
Hao-Chia Chen, Ph.D.

Z01 HD 00146-09 Structure and Function of Chorionic Gonadotropins
Hao-Chia Chen, Ph.D.

Z01 HD 00147-09 Mechanism of Action of Peptide Hormones in Steroidogenic Cells
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00148-09 Ontogeny of Gonadotropin Receptors and Gonadal Function
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00149-09 Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00150-09 Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00151-09 Receptor-mediated Regulation of Gonadal Function
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00160-09 Regulation of Adrenal Steroidogenesis
Charles A. Strott, M.D.

Z01 HD 00184-06 Regulation of Pituitary Hormone Secretion
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00187-05 Hormonal Regulation of Cellular Metabolism
Kuo-Ping Huang, Ph.D.

Z01 HD 00190-02 Development and Regulation of Cellular Zonation of the Adrenal Cortex
Charles A. Strott, M.D.

NICHD ANNUAL REPORT
Endocrinology and Reproduction Research Branch
October 1, 1983 to September 30, 1984

The research programs of the Endocrinology and Reproduction Research Branch are directed at the elucidation of cellular mechanisms involved in hormone secretion and action, and at the investigation of normal and disordered function of the hypothalamic-pituitary system and its effects upon gonadal and adrenal function. 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. Of particular interest are the analysis of pituitary-gonadal relationships, the regulation of ovarian function during the reproductive cycle and pregnancy, and the participation of hormone receptors in the regulation of gonadal function. In the current year, research has continued in several areas of hormone secretion and action, and on the receptor-mediated processes that are responsible for the control of steroid production in endocrine target cells. The role of hormones in cellular regulation has also been examined in selected areas of normal and disordered human endocrine function, and in appropriate animal model systems for the analysis of peptide secretion and the stimulatory and inhibitory control of target-cell function. The staff of the ERRB share common interests in the secretion and mechanisms of action of peptide and glycoprotein hormones, the basic endocrinology of hypothalamic-pituitary regulation, the control of gonadal and adrenal function by pituitary hormones, the renin-angiotensin system, and the role of 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/Dr. Greti Aguilera) performs research on the control of endocrine target cells by peptide hormones, in particular the characterization, regulation, and activation mechanisms of membrane receptors for gonadotropins, angiotensin II, gonadotropin-releasing hormone (GnRH), and corticotropin-releasing factor (CRF). Current studies on the development of testicular function have explored the fetal development of gonadotropin receptors, and differences between fetal-neonatal and adult Leydig cells in response to gonadotropic stimulation and treatment with GnRH agonists. The expression of receptors for LH and FSH has been analyzed in the fetal rat, and shows notable correlations with the changes in testicular endocrine function that occur during fetal life. The previously demonstrated difference in response of the fetal-neonatal testis (mainly stimulatory) and the adult testis (stimulation followed by receptor loss and desensitization) was further analyzed to show that the adult-type steroidogenic lesions thus follow LH/hCG stimulation in adults do not occur in the fetal Leydig cell. However, such lesions (at the level of 17α-hydroxylase and 17-20 desmolase) were produced in neonatal rats by GnRH agonist treatment, via activation of GnRH receptors present in the fetal-neonatal generation of Leydig cells. In the ovarian granulosa cell, the inhibitory effects of GnRH on FSH-induced cAMP production and cellular differentiation were shown to be associated with a calcium-dependent mechanism, as in the stimulatory actions of GnRHa at the pituitary level. In addition to inhibiting the expression of LH and prolactin receptors, GnRHa also suppressed FSH receptors and impaired the ability of FSH to activate adenylate cyclase. The maintenance of FSH receptors, similar to the induction of LH and prolactin

receptors, was found to be dependent upon cyclic AMP action within the granulosa cell.

In the pituitary gland, GnRH receptors were shown to undergo agonist-dependent endocytosis prior to a secondary phase of replenishment and up-regulation that was dependent on protein synthesis. The early actions of GnRH were found to include increased phospholipid turnover and arachidonic acid release. Further evidence was obtained for a role of arachidonate metabolites, probably formed via the lipoxygenase pathway, in pituitary gonadotropin release. A potential role for protein kinase C in this process was indicated by the demonstration of the enzyme in pituitary cells, and by its activation by phorbol esters that stimulate LH release. Studies on the binding of GnRH in rat brain were commenced, together with that of CRF, to analyze the loci at which these peptides exert actions in the central nervous system. The CRF receptors of the pituitary gland, previously shown to elicit ACTH secretion by a cyclic AMP-dependent process, were further analyzed to determine the mechanisms involved in their marked down-regulation in the adrenalectomized rat.

The actions of angiotensin II (AII) in the adrenal zona glomerulosa cell were shown to be selectively blocked by dihydropyridine calcium channel antagonists, and the high concentration of [^{3}H]nitrendipine binding sites in the adrenal glomerulosa zone suggested a close relationship between the AII receptor and calcium channels. This is consistent with the need for increased cytosolic calcium in the acute steroidogenic response to AII, and the selective actions of calmodulin antogonists upon AII-stimulated aldosterone production. Current studies also suggest that calcium-calmodulin dependent protein kinase is involved in the adrenal actions of AII. The central actions of AII within the brain have been correlated with the presence of specific AII receptors, localized by topical autoradiography in specific brain area including circumventricular organs, paraventricular nucleus and regions related to the limbic system. AII receptors in the subformical organ were selectively increased during dehydration, indicating that positive regulation of sites by the high plasma AII levels may enhance the drinking response to water deprivation.

(b). 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. In the adult Leydig cell, the activation of adenylate cyclase by LH is accompanied by guanyl nucleotide binding to membrane components and cAMP-independent phosphorylation of a 42000 Mr protein. In cell membranes, guanyl nucleotide dependent phosphorylation is highly sensitive to calcium, being enhanced by low but inhibited by high concentrations of calcium, and is characterized by a shift from phosphothreonine to phosphoserine. The relation between membrane phorphorylation and the activation/desensitization process in Leydig cell membrane is under investigation. Of interest was the finding that a deglycosylated antagonist analog of hCG caused neither receptor down-regulation nor steroidogenic desensitization, consistent with the need for receptor activation to elicit such responses. Studies on LH action in purified Leydig cells were facilitated by development of an elutriation procedure for isolation of Leydig cells, and revealed no functional differences within the classes of cells of different density. Fetal Leydig cells were maintained in culture with

prolonged retention of LH-responsiveness, and were shown to be inhibited by GnRH as well as stimulated by gonadotropins, suggesting that GnRH-like factors could modulate the action of LH in the fetal testis.

Studies on ovarian receptors for LH and Prl revealed that the ovarian LH/hCG receptor exhibits multiple binding forms of Mr 16,000 to 180,000, in contrast to the dimeric form proposed for the testicular LH receptors. However, affinity purification of the ovarian LH receptor has shown a single form with Mr of 60,000 on SDS gel analysis. The ovarian LH receptor was also affinity-purified to near-homogeneity and contains Mr 40,000 and 80,000 components on SDS analysis. The purification of gonadal receptors for LH and prolactin will permit further physico-chemical characterization and investigation of their functional relationships to membrane effector systems.

Application of the sensitive bioassay for plasma LH has shown that LH is secreted in pulses of high biological activity, a feature that is enhanced when the endogenous GnRH signal is amplified by opiate receptor blockade. Significant modulation of LH pulse frequency occurs during the menstrual cycle, with increased rate during the late follicular phase, a finding which provides a basis for optimal treatment with GnRH for induction of ovulation. The occurrence of significant discordance between immuno- and bioactive LH pulses indicates that estimates of bioactive LH are necessary to fully characterize physiological patterns of LH secretion during the menstrual cycle. This work will be extended to define the effects of gonadal steroids upon the bioactivity of pituitary and circulating LH, and to characterize bioactive prolactin profiles in normal women.

(c). Section on the Adrenal Cell Biology. (Dr. C. Strott) investigates the physiology and regulation of adrenal steroidogenesis, 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. Current research has employed the guinea pig as a model for analyzing the differential function of the adrenocortical zones. In this cortisol-producing species, the zone reticularis is insensitive to ACTH stimulation, and appears to be much less dependent than the zona fasciculata upon pituitary ACTH secretion for its maintenance. Thus, dexamethasone suppression causes selective atrophy of the zona fasciculata, with loss of ascorbic acid and cholesterol side-chain cleavage activity, but has no such effects in the zona reticularis. The lack of steroidogenic responsiveness of reticularis cells to ACTH and cAMP, in the face of increased cAMP production in ACTH-treated cells, indicate that the ACTH insensitivity of the reticularis zone is not due to a receptor defect but to a deficient cellular response beyond the formation of cAMP. Also in the guinea pig, studies were commenced on the uptake of cholesterol and LDL, and the intracellular metabolism of cholesterol. Attempts are in progress to purify and raise antibodies against specific adrenal steroid-binding proteins, including these for pregnenolone, pregnenolone sulfate and cholesterol. It is expected that these studies will contribute to clarification of the processes by which cholesterol and pregnenolone are mobilized to and from the inner mitochondrial membrane during stimulation of the rate-limiting step in steroid-genesis, the cholesterol side-chain cleavage reaction.

(d) 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 synthesis of unusual structure and sequences in amino acids and peptides, and the development of new techniques for peptide sequencing and synthesis. Correlations between peptide structure and function are analyzed in hypothalamic releasing hormones, including gonadotropin-releasing hormone (GnRH) and the recently discovered corticotropin-releasing factor (CRF). The 41 amino acid CRF molecule and its fragments have been synthesized and employed for receptor binding analysis and structure-function studies. Attempts to prepare a useful derivative for pituitary imaging included the use of a DTPA deviation for conjugation with ^{111}In. Specific peptide sequences of the CRF molecule will be analyzed to define the features necessary for agonist and antagonist activity. Dimeric preparations of GnRH agonists are also being synthesized and analyzed for bioactivity, in an approach to the role of receptor micro-aggregation and cross-linking in the stimulation of hormone responses. A pentadecapeptide segment of a 68,000 MW protein coded by cytoplasmic RNA differentally expressed in gastrula embryos of Xenopus laevis was synthesized and conjugated to thyroglobulin for use in raising antibodies to the differentiation- related protein. Further studies on hCG included the analysis of α subunit secretion in a large series of patients with trophoblastic neoplasms, and the development of a method for large-scale assays of urinary hCG excretion for the direction of fetal loss in early pregnancy. The hCG-like substance (hCG') of putative pituitary origin, detected during the late luteal phase in normal women with or without an intrauterine device, was found to be biologically active in the Leydig cell testosterone assay, whereas the hCG' secreted episodically after steroid contraceptive withdrawl was inactive and resembled an hCGβ subunit. These and earlier findings suggest that steroid hormone may modify the quantity and activity of hCG' secreted by the pituitary gland.

(e). The Section on Metabolic Regulation (Dr. K.-P. Huang) studies the regulation and hormonal control of glycogen metabolism in normal and diabetic tissues, and the activity of glycogen synthease and phosphorylase kinase. Phosphorylation and dephosphorylation of enzymes controlling rate-limiting metabolic steps is one of the major mechanisms by which cellular metabolism is controlled, and glycogen synthase and phosphorylase kinase are regulated by this mechanism in response to hormonal stimuli. These enzymes are phosphorylated and dephosphorylated by multiple forms of protein kinases and phosphatases, but the way in which hormones affect the various protein kinases and phosphatases is not completely understood. Studies have been performed to define the regulation of glycogen synthase and phosphorylase kinase activities by protein kinases and phosphatases; to determine the defects in glycogen metabolism resulting from streptozotocin-induced diabetes in rats; and to elucidate the hormonal regulation of glycogen metabolizing enzymes in cultured rat hepatocytes. Recent findings include the observation that a unique protein kinase, whose regulatory function has yet to be defined, may be a potential target for the action of glucagon. This protein kinase not only phosphorylates glycogen metabolizing enzymes, but also muscle contractile proteins and the components of microtubules. It has also been found that a detectable alteration in the immunological properties of rat hepatic glycogen synthase occurs in streptozotocin-induced diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00022-11 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	Greti Aguilera	Visiting Scientist	ERRB, NICHD
	Kevin J. Catt	Head	ERRB, NICHD
Others:	John X. Wilson	Guest Researcher	ERRB, NICHD
	Frederick O. Mendelsohn	Visiting Scientist	ERRB, NICHD
	Takako Hirota	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	3.0	1.25

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to study physiological and pathological aspects of the renin-angiotensin system, with emphasis in the role of AII in the regulation of aldosterone secretion and circulatory homeostasis. AII mediates the increases in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on the sensitivity of the glomerulosa cell to AII. The adrenal sensitivity to AII is increased during sodium restriction and decreased during sodium loading through mechanisms involving the dopaminergic system. In vivo and in vitro studies are in progress to analyze the mechanisms by which dopamine, atrial natriuretic peptide and other factors modulate the actions of angiotensin II in the glomerulosa cell and determine the adrenal sensitivity to AII during changes in sodium intake. The action of AII is highly calcium-dependent, and studies in rat, dog, and bovine adrenal cells and membranes have demonstrated that nitrendipine binding sites are preferentially located in the glomerulosa zona. Their high correlation with the content of AII receptors suggests a structural relationship between the AII receptor and calcium channels. In addition, dihydropyridine calcium antagonists selectively inhibited AII and K^+ stimulated aldosterone production in rat adrenal glomerulosa cells, indicating a role of voltage-dependent calcium channels in the mechanism of action of these stimulators. Studies in progress also indicate the involvement of calcium-dependent protein kinases in the action of AII. Autoradiographic analysis of angiotensin II binding in frozen brain sections has permitted the identification of AII receptors in specific brain areas involved in circulatory homeostasis, including circumventricular organs, paraventricular nucleus and regions related to the limbic system. Following 48 hr water deprivation, angiotensin II receptors were significantly increased in the subfornical organ (SFO). This increase may represent positive regulation of SFO receptors by the high plasma AII levels during dehydration, with consequent enhancement of the drinking response to water deprivation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00035-12 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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	ERRB, NICHD
	J.L. Morell	Research Chemist	ERRB, NICHD
	J.H. Brown	Research Chemist	ERRB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION
NICHD, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	0.5	2.0

CHECK APPROPRIATE BOX(ES)

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

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

This project focuses on structural design, chemical synthesis and modification of molecules important to reproductive and developmental biology, with particular emphasis on polypeptide hormones.

A. A new diethylene triamine penta-acetyl^{1}-ovine corticotropin releasing factor (DTPA-oCRF) was synthesized, purified and radiolabeled by coordination with In^{111}. The In^{111}-DTPA-oCRF exhibited antibody binding properties similar to the native hormone. However, the large DTPA group was found to perturb the peptide molecule for receptor binding activity.

B. A pentadecapeptide segment of a 68,000 dalton protein coded by cytoplasmic RNA differentially expressed in gastrula embryos of Xenopus laevis was synthesized, purified and conjugated to bovine thyroglobulin. The conjugate is being used to immunize rabbits for the production of antibodies.

C. A peptide corresponding to residues 15-41 of oCRF was synthesized and shown to exhibit full agonist activity but three orders less potency than the native hormone. Ten anticipated peptides elongated from residue 17 of human CRF are being synthesized and purified, and will be studied to identify the structural requirements for agonist or antagonist activity.

D. The dimeric [D-Ala^{6}]GnRH cross-linked at the carboxyl terminus by methylene chains of various length [-(CH_2)n-, n=4,6,8,12,14] was two orders less active than the monomeric peptide in both receptor binding and LH releasing activities. The low activity of these dimeric hormones is attributed to the distortion of β-turn structure of peptide sequence which is essential for full activity. The anticipated less constrained dimers cross-linked at ε-amino group of [D-Lys^{6}, Pro^{9}-NEt]GnRH with malonic acid (Mal) and (Gly)n-Mal-(Gly)n, n=2 and 4, were synthesized and purified. These dimeric GnRH derivatives will be used to study the effect of cross-linking or microaggregation of receptors on hormonal responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00146-09 ERRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

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:	A. Bartkiewicz	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

National Taiwan University Hospital (P.C. Ouyang)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	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.)*

Human chorionic gonadotropin (hCG) and hCG-like substance (hCG') in tissues and body fluids of normal subjects and patients with neoplasms were investigated for their physico-chemical and immunological and biological properties.

A. HCG. (1) Derangement of hCG secretion in terminal choriocarcinoma patients was revealed by marked heterogeneity of hCG and hCGα subunit. (2) Radioimmunoassays of 748 serum samples from 139 patients under treatment for gestational trophoblastic neoplasms showed no general trend towards high hCGα levels, a proposed indicator of disease recurrence. (3) A simple procedure for collection, concentration, and assay of urinary hCG was designed and applied successfully in a large scale study on the detection of fetal loss in early pregnancy. (4) Chemically deglycosylated hCG was shown to have increased binding affinity for both LH and FSH receptors in granulosa cells, and to antagonize hCG-stimulated progesterone production during the early appearance of LH receptors in the cell.

B. HCG-like substance. HCG' secreted during the late luteal phase of normal women with and without an intrauterine device as a contraceptive measure was found to be biologically active in a Leydig cell testosterone assay. In contrast, the episodically secreted hCG' after scheduled withdrawing of steroid contraceptives was biologically inactive and appeared to be mostly an hCGβ-like subunit. These and our previous findings suggest that steroid hormones may modulate pituitary hCG' secretion in both quantity and quality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00147-09 ERRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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:	Maria L. Dufau	Head	ERRB, NICHD
Others:	Chon-Hwa Tsai-Morris	Staff Fellow	ERRB, NICHD
	Michael S. Blank	Expert	ERRB, NICHD
	Daniel R. Aquilano	Visiting Fellow	ERRB, NICHD
	Christine A. Winters	Chemist	ERRB, NICHD
	Mary L. Castellon	Chemist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions
DHEW-275-82-2823

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.75	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 control of androgen production by the Leydig cell is directly regulated by luteinizing hormone via specific receptors for LH/hCG. In vivo treatment with endogenous or exogenous gonadotropin causes initial LH receptor up-regulation followed by down-regulation. Large doses of hCG cause "early" (prior to pregnenolone) and "late" (17^3-hydroxylase, 17-20 desmolase) lesions that are independent of receptor loss or protein kinase activation. Such negative control by LH of receptors and responses is not observed in immature or fetal Leydig cells in vivo or in vitro, where only up-regulation was demonstrated. The goal of this project is to understand the steps involved in the hormonal control of steroidogenesis. We have extended the studies and have developed rapid and convenient methods using combined centrifugal elutriation and metrizamide gradients for complete purification of large quantities of functionally intact Leydig cells, to facilitate analysis of the mechanisms of gonadotropin action in vitro. The Leydig cell population is composed of cells with different densities and sedimentation velocities but with similar morphology, biological activity, and susceptibility to desensitization by gonadotropins. The latter caused considerable reduction of cellular density, possibly related to changes in lipid content. Exposure of cultured adult Leydig cells to chemically deglycosylated hCG caused no receptor down-regulation or steroidogenic lesions due to the predominant antagonist activity of this derivative. We have also shown that the fetal rat Leydig cell can be maintained in culture with retention of their LH-mediated steroidogenic responses and with expression of functional GnRH receptors. The demonstrated inhibitory actions of GnRH on steroidogenesis, with expression of GnRH receptors in the fetal and post-natal testes, are in contrast to the up-regulatory functions of the microsomal enzymes by gonadotropins, and indicate that GnRH could influence the actions of gonadotropins upon Leydig cell function in the neonatal testis. We will proceed with studies on the biochemical definition of hormone-induced steroidogenesis in fetal and adult Leydig cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00148-09 ERRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Ontogeny of Gonadotropin Receptors and Gonadal Function

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

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

COOPERATING UNITS *(if any)*

D. Warren	Department of Physiology, UCLA
I. Huhtaniemi	Department of Clinical Chemistry, Univ. of Helsinki

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Sections on Hormonal Regulation and Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The endocrine function of the fetal and neonatal rat testis has been studied with particular reference to the development and regulation of the Leydig cell and its receptor-mediated responses. The effects of gonadotropin stimulation upon fetal-neonatal Leydig cells differ markedly from those in the adult, with mainly stimulatory responses in contrast to the marked receptor loss and desensitization that is characteristic of the adult Leydig cell.

A. Detailed analysis of gonadotropin receptors in the fetal rat testis revealed a close correlation between the appearance of LH receptors and gonadotropic responsiveness at 15.5 days of gestation, with a further rise at 18.5 days coincident with a marked increase in testosterone (T) production. FSH receptors appeared at 17.5 days and rose sharply after 19.5 days. These findings are consistent with the presence of Sertoli cells at this stage of gestation, and suggest that FSH has a role in gonadal development during the last 2 days of fetal life.

B. In the neonatal rat, in vivo treatment with hCG elevated serum and testicular T levels with little change in progesterone and 17-hydroxyprogesterone, is contrast to the marked increase in these precursors in the adult rat. In the latter, the role of a steroidogenesic lesion in the biphasic serum T response to hCG was indicated by a marked rise in serum and testicular progesterone levels during the nadir of T production. The adult-type steroidogeic lesions that follow LH/hCG stimulation are not operative during the fetal-neonatal phase of testicular development.

C. GnRH receptors were demonstrated in cultured fetal testes and neonatal gonads. In the former, expression of GnRH receptors was correlated with inhibitory effects of GnRH agonists upon LH-stimulated steroidogenesis. In the neonatal rat, GnRHa treatment markedly enhanced testicular progesterone responses to hCG or 8-BrcAMP, indicating that the adult-type steroidogenic lesion can be reproduced by GnRH acting through its Leydig-cell receptors in the neonatal testis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00149-09 ERRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters 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:	Maria L. Dufau	Head	ERRB, NICHD
Others:	Michael S. Blank	Expert	ERRB, NICHD
	Mary L. Castellon	Chemist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Department of Medicine, Hershey, PA and Charlottesville, VA.
Department of Pathology, University of New Mexico, Alburqueque.
Contract for preparation of gonadal cells and cell fractions DHEW 275-82-2823

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.25	1.0	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 highly sensitive in vitro bioassay for measurement of luteinizing hormone (LH) and chorionic gonadotropin (hCG) in blood, developed in our laboratory and termed the RICT assay (rat Leydig cell testosterone bioassay), has been applied to the measurement of serum LH and LH-like gonadotropins in man and several animal species. This technique has indicated that the biological activity of LH secreted in man, rhesus monkey and rat is modulated by gonadal steroids. Studies on the pulsatile release of LH in men and postmenopausal women have demonstrated that LH is secreted in pulses of high biological activity. When the endogenous GnRH signal is amplified by opiate receptor blockade, bioactive LH is released in more frequent pulses of high biological activity, with a significant increase in androgen production. Long-term studies on the in vitro bioactivity of LH and CG in man and other species were continued.

We have demonstrated in normal cycling women that biologically active LH is secreted in discrete episodic pulsations preferentially enriched in biologically active hormone, and that modulation of the frequency of bioactive pulses occurs during the late follicular phase of the menstrual cycle. This is an important physiological mechanism for regulating the blood concentration of LH available to the ovary and provides a basis for optimal treatment during the use of GnRH for the induction of ovulation. Our finding of significant discordance between immuno and bioactive LH pulsations indicates that estimates of bioactive LH are necessary to fully characterize physiological patterns of LH secretion during the menstrual cycle. We have also shown that the infertile mink is a useful model of human infertility, involving both endocrinological and immunological mechanisms. Our future goal is to define the effects of gonadal steroids and altered secretion rate upon the biochemical properties of pituitary and circulating LH, and to characterize the bioactive profiles of prolactin in cycling women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00150-09 ERRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

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:	Maria L. Dufau,	Head	ERRB, NICHD
Other:	Christine Winters	Chemist	ERRB, NICHD
	Mitsuaki Mitani	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions
DHEW-275-82-2823

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
1.5	0.75	0.75

CHECK APPROPRIATE BOX(ES)

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

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

(A) Multiple forms of ovarian LH/hCG receptors have been reported by our laboratory and confirmed by others (Mr range 16,000-18,000 under non-denaturing conditions). This contrasts with our previous observations on the LH receptors of Leydig cells, for which a dimeric form was demonstrated. (B) LH receptors were resolved functionally from adenylate cyclase. Rapid membrane events that are initiated after hormone interaction with specific receptors include increased binding of guanyl nucleotide (G) and G-induced phosphorylation, a cAMP independent process. This stimulatory event requires a low Ca++ concentration but is inhibited by high Ca++ concentrations. Similar reduction of basal and hCG-stimulated adenylate cyclase in the presence of G indicated interdependence between these events during membrane activation. (C) Initial studies have indicated the existence of masked ovarian particulate receptors. Crosslinking studies have demonstrated that the prolactin receptor is a protein of Mr 80,000 containing a 40,000 Mr subunit. The above studies were extended as follows: (A) Studies on labeling affinity purified ovarian LH receptors have yielded receptor with low bindability to a subsequent affinity column step. The eluted tracer gave a single form of Mr 60,000 on SDS analysis. (B) During guanyl nucleotide-induced phosphorylation, not only an increase but also an apparent shift from phosphothreonine to phosphoserine takes place. G nucleotides affect membrane phosphorylation in the same potency order with which they bind to membranes. (C) The ovarian prolactin receptor has been purified to near-homogeneity in low yield, and contains Mr 40,000 and 80,000 protein bands after SDS analysis and silver staining. We will proceed with the characterization of gonadotropin and prolactin receptors of the testis and ovary, and with studies on the physical and functional relationships of the LH receptor site and the individual adenylate cyclase components.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00151-09 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Receptor-mediated regulation of gonadal 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	Staff Fellow	ERRB, NICHD
	T. Ranta	Visiting Scientist	ERRB, NICHD
	J.M. Darbon	Visiting Fellow	ERRB, NICHD
	A. Baukal	Biomedical Engineer	ERRB, NICHD

COOPERATING UNITS *(if any)*
M. Korhonen, Dept. of Ob/Gyn, Baylor College of Medicine, Houston, Texas

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

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

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

Current research in this project has focused on the actions of gonadotropins and GnRH agonists upon the maturing ovarian granulosa cell. We have previously shown that FSH induces granulosa-cell maturation via an increase in cyclic AMP production, and that the inhibitory effects of GnRH agonists (GnRHa) are associated with impairment of adenylate cyclase activity and decreased cyclic AMP formation. Such effects of GnRHa were shown to be mediated by specific GnRH receptors in the granulosa cell that are coupled to a calcium-dependent mechanism, as in the pituitary gland. However, in contrast to their stimulatory effects on gonadotropin release, GnRH agonists exert predominately inhibitory actions at the ovarian level. These actions were shown to include marked suppression of receptors for FSH as well as for LH and prolactin, with a consequent decrease in FSH-stimulated adenylate cyclase activity. The stimulatory actions of GnRHa on ovarian function become more prominent with increasing maturation of the granulosa cell, and include increases in ovarian weight, basal adenylate cyclase activity and prolactin receptors, and luteinization of mature ovarian follicles. In addition to its effects on granulosa-cell maturation and expression of receptors for LH and prolactin, FSH also promotes the formation of its own receptors and this action has now been shown to be mediated by cyclic AMP, and to be prevented by GnRHa. Also, the expression of gonadotropin receptors by FSH and other cAMP-inducing ligands has been shown to be markedly enhanced by estrogens, indicating the importance of synergistic interactions between FSH and estrogen in the maturation of granulosa cell function. The mechanism of receptor induction by FSH will be further explored by analysis of mRNA and receptor protein synthesis in the cultured granulosa cell system. It is expected that this system will provide a valuable model for studies on the combined actions of steroid and gonadotropic hormones during granulosa cell maturation, and for the investigation of gonadotropin receptor synthesis and turnover during defined in vitro conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00160-09 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Regulation of Adrenal Steroidogenesis

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

PI:	C.A. Strott	Head	ERRB, NICHD
Others:	T. Obara	Visiting Fellow	ERRB, NICHD
	K. Nonomura	Visiting Fellow	ERRB, NICHD
	K. Mikami	Visiting Associate	ERRB, NICHD
	C.D. Lyons	Bio. Lab. Tech.	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, Maryland 20205

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.)

The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone, cholesterol side-chain cleavage (SCC). This enzyme reaction occurs on the matrix side of the inner mitochondrial membrane. Cholesterol must be delivered to and pregnenolone removed from the active site. To study this phenomenon in detail, the guinea pig, a cortisol producer like the human being, has been employed as an animal model.

A. Uptake of radioactive cholesterol and cholesterol linoleate. Both compounds were found to be taken up at different rates in the inner and outer zones of the adrenal cortex, with about 2-fold greater uptake in the outer zone. The time-course and subcellular distribution after uptake were also analyzed.

B. Binding of lipoproteins to membrane receptors. The guinea pig utilizes low-density lipoproteins (LDL) for the bulk of cellular cholesterol uptake. Plasma LDL of the guinea pig was isolated by flotation, iodinated by the iodine monochloride method and the ^{125}I-LDL utilized to examine LDL-receptor function, including the effect of ACTH on LDL binding.

C. Intracellular metabolism of cholesterol. In addition to the large amount of cholesterol present in cellular membranes, the adrenal cortex stores substantial cholesterol (primarily esterified) in large cytoplasmic lipid droplets. The activities of the enzymes responsible for esterifying and de-esterifying cholesterol, viz, acetyl CoA: cholesterol acyl transferase and cholesterol hydrolase were determined.

D. Cytoplasmic SCC stimulator and steroid-binding proteins. Several specific steroid-binding proteins have been detected and analyzed such as cholesterol, pregnenolone (PBP), and pregnenolone sulfate-binding proteins. The PBP is the only one to be purified to date. However, repeated efforts to generate antibodies to the PBP have so far been unsuccessful. The pregnenolone sulfate and cholesterol-binding proteins, as well as, the ACTH-inducible soluble stimulator of cholesterol SCC will be purified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00184-06 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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
	G. Aguilera	Visiting Scientist	ERRB, NICHD
Others:	P. Wynn	Visiting Associate	ERRB, NICHD
	M. Iwashita	Visiting Fellow	ERRB, NICHD
	K. Hirota	Visiting Fellow	ERRB, NICHD
	R. Morgan	Staff Fellow	ERRB, NICHD
	M. Millan	Staff Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)* Gwen Childs, Dept. of Anatomy, University of Texas Medical Branch, Galveston, Texas; Zvi Naor, Dept. of Hormone Research, Weizmann Institute of Science Rebovot Israel. Jack Vanderhoek, Dept. of Biochemistry, George Washington University, Washington, D.C.

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	0.7	1.5

CHECK APPROPRIATE BOX(ES)

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

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

The receptors and actions of the hypothalamic releasing peptides, GnRH and CRF, have been characterized in the pituitary gland, and current studies are directed at the clarification of the mechanisms of action of these peptides upon hormone secretion in cultured pituitary cells. The up-regulation of GnRH receptors by GnRH agonists was formed to be preceded by a phase of receptor loss attributable to internalization of the hormone-receptor complex, to be prevented by inhibition of protein synthesis, and to be independent of receptor recruitment to the cell membrane during the acute phase of gonadotropin release from secretory granules. Stimulation of LH release by high potassium was not preceded by receptor loss prior to up-regulation, and a GnRH antagonist caused no change in receptors, indicating the need for agonist-receptor interaction in the initiation of receptor endocytosis and processing. In studies on the role of phospholipid turnover in GnRH action, the ability of phosphatidic acid (PA) to simulate gonadotroph responses elicited by GnRH suggested that PA may participate in the actions of GnRH agonists upon LH release. The previous finding that arachidonic acid and 5-HETE stimulated LH release and could also be involved in the actions of GnRH was extended by analysis of the metabolism of arachidonic acid, and the demonstration that lipoxygenated derivatives are produced in purified rat gonadotrophs. The potential role of protein kinase C in GnRH action was indicated by its presence in the pituitary gland and the ability of phorbol esters to activate LH secretion. Studies were commenced in the binding of GnRH in the rat brain, to localize the central sites of which GnRH receptors participate in the behavioral and other CNS actions of GnRH-related peptides. The receptors for CRF in the pituitary gland was also further analyzed during the down-regulation that follows adrenalectomy, and studies on brain CRF receptors were initiated by the technique of topical autoradiography with radioiodinated Tyr-oCRF. The properties, regulation, and activation mechanisms of brain CRF receptors will be compared with those of the anterior pituitary gland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00187-05 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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:	T. J. Singh	Visiting Associate	ERRB, NICHD
	R. Dhanireddy	Medical Officer	ERRB, NICHD
	H. Nakabayashi	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Biochemistry, NHLBI, NIH (P.B. Chock)
Human Genetic Branch, NICHD, NIH (J. Chou)
Laboratory of Cellular and Developmental Biology, NIADDK, NIH (M.C. Lin)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Metabolic Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.25	3.0	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)*

Phosphorylation and dephosphorylation of the enzymes controlling rate-limiting metabolic steps is one of the most important mechanisms by which cellular metabolism is controlled by hormones and other regulators. Glycogen synthase and phosphorylase kinase are regulated by this mechanism in response to hormonal actions. These enzymes can be phosphorylated and dephosphorylated, respectively, by multiple forms of protein kinases and phosphatases. It is not entirely understood how hormones affect the various protein kinases and phosphatases. The purposes of this project are: to define the regulation of glycogen synthase and phosphorylase kinase activities by protein kinases and phosphatases; to determine the defects in glycogen metabolism resulting from streptozotocin-induced diabetes in rats; to elucidate the hormonal regulations of glycogen metabolizing enzymes in rat hepatocytes grown in culture; and to use glycogen synthase and phosphorylase kinase as model systems to unfold the regulatory mechanisms for multiple protein kinases and phophatases in response to hormonal actions.

Our investigation into these aspects has revealed: (1) a unique protein kinase, whose regulatory function has yet to be defined, may be a potential target for the action of glucagon; (2) this protein kinase not only phosphorylates glycogen metabolizing enzymes, but also muscle contractile proteins and the components of microtubules; (3) the specificity of this kinase can be clearly distinguished from the other known protein kinases; and (4) a possible alteration in the immuno-properties of rat hepatic glycogen synthase results from streptozotocin-induced diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00190-02 ERRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development and Regulation of Cellular Zonation of the Adrenal Cortex

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

PI:	C.A. Strott	Head	ERRB, NICHD
Others:	T. Obara	Visiting Fellow	ERRB, NICHD
	K. Mikami	Visiting Associate	ERRB, NICHD
	C.D. Lyons	Bio. Lab. Tech.	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 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.25	1.0	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 mammalian adrenal cortex is composed of three concentric zones of differentiated cells which perform distinct functions and which respond to a different set of regulators. The developmental nature of this zonation is not understood. Currently, the major thrust has been to separate the individual zones and examine their constituent cells in terms of structural characteristics and functional activities. The guinea pig, a cortisol producer like the human being, is utilized as an animal model. The following summarizes some of the findings:

1. Cells were examined by light and electron microscopy. Striking differences in neutral fat content and smooth endoplasmic reticulum were noted. Chronic dexamethasone suppression caused atrophy of the zona fasciculata and loss of neutral fat but had no affect on the zona reticularis.
2. The rate-limiting step in steroidogenesis is mitochondrial cholesterol side-chain cleavage (SCC). Acute stress and ACTH administration caused an increase while dexamethasone suppression caused a decrease in cholesterol SCC activity in the fasciculata but not the reticularis.
3. The content of unconjugated and sulfoconjugated steroids was determined in the two zones. In general, steroids with a double bond in ring A of the steroid nucleus were present in a higher concentration in the fasciculata while steroids with a double bond in ring B (unconjugated and sulfoconjugated) were more concentrated in the reticularis.
4. The content of ascorbic acid (AA), measured after HPLC isolation, was twice as high in the reticularis as in the fasciculata. However, chronic dexamethasone suppression caused depletion of AA only from the fasciculata.
5. Cells isolated from the fasciculata increased steroid production in response to ACTH and cAMP while reticularis cells did not respond. Cells from both the fasciculata and reticularis increased cAMP production in response to ACTH.

Conclusion: the ACTH-insensitivity of the reticularis appears to be due to a defect beyond the formation of cAMP.

Z01 HD 00026-09 Fertilization and Activation of Development in Mammals
Bela J. Gulyas, Ph.D.

Z01 HD 00135-07 Pediatric Endocrinology
Barry B. Bercu, M.D.

Z01 HD 00168-08 Ovarian Xenobiotic Metabolism and Oocyte Toxicity
Donald R. Mattison, M.D.

Z01 HD 00907-05 Reproductive Toxicity of Drugs
Donald R. Mattison, M.D.

Z01 HD 00908-05 Genetics of Ovarian Failure
Donald R. Mattison, M.D.

Z01 HD 00921-03 Fetal Diagnosis and Therapeutics
Donald R. Mattison, M.D.

Z01 HD 00922-03 Nuclear Transfer in Mammalian Oocytes
Bela J. Gulyas, Ph.D.

Z01 HD 00923-02 Primate Models for the Study of Human Infertility, Contraception & Fetal Development
Gary D. Hodgen, Ph.D.

NICHD ANNUAL REPORT

Pregnancy Research Branch

October 1, 1983 to September 30, 1984

The research and training activities of the Pregnancy Research Branch have been focused on four principal areas of reproductive biology and medicine: (1) Fertilization in lower mammals, primates and humans; (2) Secretory control of growth hormone releasing hormone and responses to growth hormone treatment; (3) Mechanisms of reproductive toxins; and (4) Fetal, placental and maternal physiology of pregnancy. A total of eleven Fellows were trained in programs linking NICHD to the Uniformed Services University, Georgetown University Medical Center, Fogarty International Center, and the Ford Foundation. The senior investigators presented 17 plenary lectures in the U.S. and abroad, including individual awards for research excellence in reproductive toxicology, in vitro fertilization and contraception. PRB Fellows were awarded "best scientific paper" prizes from the American Fertility Society, Society for Gynecologic Investigation and the International Fertility Society. In addition, 67 research articles were published by PRB investigators during FY '84.

In the area of fertilization, our pursuits have been both entirely basic and pre-clinical. In the most basic studies, Dr. Gulyas sought a model system that would determine whether the paternal genome was required for activation of development. Further, he asked: does the sperm bring to the ooplasm essential factors other than "male" DNA? He selected a mouse model having familiar reproductive patterns and responsive to hormonal treatment to accomplish superovulation. The eggs collected were denuded, including removal of the zona pellucida. Such preparations of eggs allow their chemical fusion. These fused gametes, lacking any sperm products, were incubated to support embryonic cleavage in vitro up to the blastocyst stage. Similarly, Dr. Gulyas accomplished chemical fusion of 2-cell embryos to other 2-cell embryos to form chimeras. These novel techniques brought some surprising and significant findings: 1) the paternal genome is not required for any pre-implantation stages of development; 2) developmental changes soon after implantation do require input from the sperm; and 3) meiosis, per se, can be completed by the pronuclei of two-fused eggs. Dr. Gulyas persisted in his studies of sperm-egg interaction associated with normal fusion of these gametes for fertilization. Principal parameters observed were attachment (sperm to egg), cortical reaction and initiation of cleavage (activation). Among his most interesting findings was that aging eggs loose their capacity for interaction with the sperms. That is, the aged oocyte has the characteristic of lesser solubility of the zona by proteases and reduced fertilizability. The significance of this may be far-reaching in that in vitro fertilization in humans requires human fetal cord serum (why?). More importantly, only certain specimens are acceptable. The main virtue of this fetal cord serum is to facilitate optimal sperm-egg interaction, perhaps by delaying insolubility of zona membranes.

Dr. Bercu's research focused on two themes: 1) growth hormone and 2) gonadotropin releasing hormone. He studied these problems in both children and

non-human primates. Dr. Bercu's studies demonstrated why certain short-statured non-growth hormone deficient children respond to exogenous GH. Using 24-hour pulsatile studies (sampling of GH every 20 minutes), he showed demonstrated neurosecretory abnormalities in the secretion of GH resulting in a decreased output of GH. His studies have also suggested that some ofthe previously held tenets on GH secretion may be wrong. For example: some GH-deficient children may have adequate nocturnal pulses; the largest GH pulse may occasionally occur at other than slow wave sleep in normal children; children with "partial" GH deficiency may require more GH than children with "severe" GH deficiency. As well, Dr. Bercu and his collaborators demonstrated a markedly decreased pulsatile GH secretion in children who have received prophylactic CNS cranial radiation.

Interestingly, Dr. Bercu also showed that time series analysis of pulsatile gonadotropin release uncovers an unexpected short ultradian gonadotropin pulsatile rhythm (11-15 minutes) in adult castrate monkeys. His studies using specific potent GnRH antagonists suggest the value of these drugs in replacing GnRH agonists in certain clinical settings. He has demonstrated rapid and dose-response effects on pulsatile gonadotropin and testosterone secretion in intact animals. The desired effect of these GnRH antagonists was achieved in hours as opposed to delayed actions of the GnRH agonists.

The most significant contribution by Dr. Bercu and his colleagues was the opening of investigative paths into the possible neuropeptide and neurotransmitter dysfunction in short-stature children. These children can potentially benefit from exogenous GH therapy in a manner that can "normalize" their lives.

Dr. Mattison's studies investigated a spectrum of problems associated with reproductive failure due to toxins. These factors may present as metabolic lesions or lead to genetic aberrations. Frequently, the sources of these toxins in women are prescribed medications. For example, cyclophosphamide, an alkylating agent with broad clinical use, produces age and dose-dependent premature ovarian failure. Analysis of the age dependence suggests that changing oocyte number with age due to follicular atresia is completely responsible for this age dependence in women between 20 and 40 years of age.

Dr. Mattison's contributions also include exploitation of a mouse model designed to measure the toxic effects of benzopyrene on oocytes. Benzo(a)-pyrene and synthetic derivatives have been used to explore the responses of oocytes to direct and indirect acting mutagens. Intraovarian injection demonstrates that the ovary contains the enzymes necessary to metabolize this family of xenobiotics to gametotoxic products capable of producing oocyte destruction. Analysis of the dose-response curves suggests that the rate limiting step in activation of benzo(a)pyrene is formation of the 7,8-diol-9,10-epoxide from the 8,8-diol. There are striking similarities, as well as differences, between the enantiomeric forms of the dioloxides in bacterial and mammalian cell mutagenicity, carcinogenicity, and potency for oocyte destruction. At the present time there is no extensively validated, easily conducted assay for female germ cell response to mutagens. The data collected in this program suggests that analysis of oocyte destruction by xenobiotics may be useful for determining female germ cell response to mutagens.

A large portion of the Branch's research and training effort centered on pre-clinical primate studies, where the special human relevance inherent to the reproductive process in monkeys is manifest. Management of patient response to gonadotropin therapy is known to be difficult. Also, long-standing evidence suggested that the pan-hypopituitary woman provided the most predictable response to gonadotropin therapy. Thus, we reasoned that achievement of a "medical hypophysectomy" by administration of a potent GnRH antagonist might reduce individual variation of patient response to gonadotropin therapy. Indeed, we observed that co-administration of the GnRH antagonist with either FSH/LH in combination or "pure" FSH provided markedly reduced differential responses.

The availability of a pure human FSH preparation for studies of the primate ovarian/menstrual cycle permitted novel experiments on gonadotropic stimulation of follicular growth in monkeys. Administration of pure FSH on days 1 through 12 of the menstrual cycle resulted in significant ovarian hyperstimulation, as manifested by the development of multiple (bilateral) ovarian follicles and sustained high serum estradiol levels (400 pg/ml). In spite of overt follicular development and concurrent increases in serum estradiol, timely LH surges were not elicited. Similarly, during FSH-induced ovarian hyperstimulation, GnRH effects on LH secretion were blunted. Equivalent FSH treatments of long-term ovariectomized monkeys had no discernible effects on estrogen-induced LH surges or GnRH responses. Our interpretation is that when supraphysiological FSH levels persist into the late follicular phase, thereby overriding selection of the single dominant follicle of the natural cycle, secretion of an ovarian factor(s) blocks estrogen-induced LH surges. We have termed this activity "gonadotropin surge inhibiting factor."

Another principal area of interest is the mechanism(s) by which endometriosis alters fertility. To elucidate the etiology of infertility due to endometriosis, we autografted endometrial or adipose tissue to the pelvic peritoneum of 21 cynomolgus monkeys. Subsequently, laparotomy was performed to stage the disease and biopsy the implants for routine histologic study. The presence of pelvic adhesions impaired fertility in control monkeys and in animals with moderate or severe endometriosis. The chemical and term pregnancy rates were lower among monkeys with moderate or severe endometriosis, as compared to controls. However, the incidence of term intrauterine pregnancy following a diagnosed chemical pregnancy was not significantly different among groups. These findings suggest that impaired fertility in monkeys with endometriosis is most apparent when associated with moderate or severe disease, and that infertility in monkeys with endometriosis appears to be mediated primarily by failure of follicular rupture and/or pelvic adhesions. These interpretations will now be tested in a group of women whose infertility is associated with confirmed endometriosis.

With regard to the endocrine milieu required for implantation, we have described the collection of <u>in vivo</u> fertilized monkey embryos by lavage of the normal donor's utero-tubal lumens and transfer of these surrogate embryos to the utero-tubal environs of ovariectomized females. The recipients were administered sequential estrogen-progesterone replacement therapy that mimics the natural ovarian/menstrual cycle, thereby developing proliferative and secretory endometrium to accommodate the perinidatory events leading to suc-

cessful placentation and embryogenesis. Eleven surrogate embryos were transferred to steroid treated, ovariectomized recipients; 4 viable pregnancies were detected by measurement of chorionic gonadotropin, all concluding with uneventful deliveries of normal live infants at term. These findings demonstrate the feasibility of establishing and maintaining normal pregnancy by combining surrogate embryo transfer (SET) with an exogenous steroid hormone regimen, even in the complete absence of ovarian function. The clinical implications of these primate studies may be far-reaching in that they indicate new potential for child-bearing by otherwise infertile or sterile women who have a competent uterus, but lack the hormonal milieu provided by ovarian follicular maturation and corpus luteum function in the normal menstrual cycle.

The Pregnancy Research Branch was dissolved on June 30, 1984, coincident with the departure of the Branch Chief, Dr. Gary D. Hodgen and the Head, Section on Reproductive Toxicology, Dr. Donald R. Mattison. Dr. Bela J. Gulyas, Head of the Section on Gamete Physiology, remains in the intramural NICHD program with a reassignment of his laboratory activities. Likewise, the space, equipment, and budgetary resources of the PRB were re-allocated to new or existing program entities. These events close-out several long-standing intramural research activities in infertility, contraception and maternal-fetal medicine studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00026-09 PR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Fertilization and Activation of Development in Mammals

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

P.I.:	B. J. Gulyas	Head	PRB, NICHD
Others:	L. C. Yuan	Chemist	PRB, NICHD
	J. G. Gianfortoni	Guest Scientist	PRB, NICHD

COOPERATING UNITS *(if any)*

J. Dean, LCB, NIADDK

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Gamete Physiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	.50	.50

CHECK APPROPRIATE BOX(ES)

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

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

At fertilization, or upon artificial stimulation, physiological changes occur in the zona pellucida of the murine eggs that render it impermeable to supernumerary sperm. This is the major source of block to polyspermy in the mouse. In addition to becoming impermeable to supernumerary sperm the zona pellucida also becomes resistant (hardened) to dissolution by proteases. Here, we demonstrated for the first time a direct relationship between the age of cumulus-free oocytes and their increased resistance to digestion with α-chymotrypsin, independent of fertilization-associated cortical reaction. Furthermore, concomitant with the decrease in zona solubility there is a decrease in fertilizability of the cumulus-free oocytes. Zygotes from cumulus-free in vitro fertilized oocytes develop at a reduced rate into blastocysts.

We also demonstrated that development of mouse oocytes can be initiated through exposure to alcohol. In these oocytes meiosis is completed, cortical reaction occurs which is then subsequently followed by the hardening of the zona pellucida. In this respect alcohol treatment of mouse oocytes may best resemble the activation of development normally accomplished by the fertilizing sperm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00135-07 PR

PERIOD COVERED
October 1, 1983 to April 14, 1984

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

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

PI:	B. B. Bercu	Investigator	PRB, NICHD
Other:	B. Spiliotis	Medical Staff Fellow	PRB, NICHD

COOPERATING UNITS *(if any)*
Rockefeller University; Salk Institute; Children's Hospital National Medical Center, George Washington University; EBRP, CPRB, HGB, NICHD; LDBA, NIDR; PMB, NIADDK

LAB/BRANCH
Pregnancy Research Branch

SECTION
Unit on Growth Physiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.50	1.50	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 major impact was in the description of GH-neurosecretory dysfunction in a subgroup of short-statured children. This appears to be a sound explanation why certain non-GH deficient children respond to exogenous hGH. Our data suggests that these children have an abnormality in the neurosecretion of GH resulting in a decreased GH output. This observation is an outgrowth of our studies in irradiated children and monkeys. Additional studies draw into question some of the traditional methods and interpretation of GH provocative tests in GH deficiency. Our studies demonstrate that classical provocative testing may give misleading results as to the total output of GH. In addition, biosynthetic hGH will significantly increase linear growth velocity in GH deficient children.

Our laboratory has continued to utilize the non-human primate model to investigate the neuroregulation of GH secretion as well as the mechanisms controlling the onset of puberty in the male. With lesioning studies, we have successfully shown that the arcuate nucleus is important both in the regulation of GH and gonadotropin secretion in the male. In another series of experiments, we have uncovered a short 11-15 minute ultradian rhythm in gonadotropin pulsing. In addition, using a newly synthesized potent GnRH antagonist, we have demonstrated rapid effect and dose-responses in intact male animals. One further study has demonstrated a paradoxical effect, in animals pre-treated with a dopamine hydroxylase inhibitor, of increased doses of human pancreatic tumor growth factor on GH release.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00168-08 PRB

PERIOD COVERED
October 1, 1983 to June 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ovarian Xenobiotic Metabolism and Oocyte Toxicity

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

PI:	D. R. Mattison	Head	PRB, NICHD
Others:	M. S. Nightingale	Chemist	PRB, NICHD
	H. H. Kay	Medical Staff Fellow	PRB, NICHD
	J. T. Chen	Visiting Fellow	PRB, NICHD
	D. R. Kuroda	Guest Researcher	PRB, NICHD
	E. K. Silbergeld	Guest Researcher	PRB, NICHD

COOPERATING UNITS *(if any)*
LBC, NIADDK, NIH

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Reproductive Toxicology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.60	.40	.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.)*

Benzo(a)pyrene and synthetic derivatives have been used to explore the repertoire of responses of oocytes to direct and indirect acting mutagens. Intraovarian injection demonstrates that the ovary contains the enzymes necessary to metabolize this family of xenobiotics to gametotoxic products capable of producing oocyte destruction. Analysis of the dose-response curves suggests that the rate limiting step in activation of benzo(a)pyrene is formation of the 7,8-diol-9,10-epoxide from the 8,8-diol. There are striking similarities, as well as differences between the enantiomeric forms of the diolvepoxides in bacterial and mammalian cell mutagenicity, carcinogenicity, and potency for oocyte destruction. At the present time there is no extensively validated, easily conducted assay for female germ cell response to mutagens. The data collected in this program suggests that analysis of oocyte destruction by xenobiotics may be useful for determining female germ cell response to mutagens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00907-05 PRB

PERIOD COVERED
October 1, 1983 to June 30, 1984

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

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

PI:	D. R. Mattison	Head	PRB, NICHD
Other:	M. S. Nightingale	Chemist	PRB, NICHD
	H. H. Kay	Medical Staff Fellow	PRB, NICHD
	J. T. Chen	Visiting Fellow	PRB, NICHD
	D. R. Kuroda	Guest Researcher	PRB, NICHD
	E. K. Silbergeld	Guest Researcher	PRB, NICHD

COOPERATING UNITS *(if any)*
Clinical Center, NIH
Division of Cancer Cause and Prevention, NCI, NIH

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Reproductive Toxicology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.60	.40	.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.)*

Cyclophosphamide, an alkylating agent with broad clinical use, produces age and dose dependent premature ovarian failure. Analysis of the age dependence suggests that changing oocyte number with age due to atresia is completely responsible for the age dependence in women between 20 and 40 years of age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00908-05 PRB

PERIOD COVERED
October 1, 1983 to June 30, 1984

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

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

PI:	D. R. Mattison	Head	PRB, NICHD
Others:	M. S. Nightingale	Chemist	PRB, NICHD
	H. H. Kay	Medical Staff Fellow	PRB, NICHD
	J. T. Chen	Visiting Fellow	PRB, NICHD
	D. R. Kuroda	Guest Researcher	PRB, NICHD
	E. K. Silbergeld	Guest Researcher	PRB, NICHD

COOPERATING UNITS *(if any)*
Human Genetics Branch, NICHD, NIH
Developmental Endocrinology Branch, NICHD, NIH

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Reproductive Toxicology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.60	.40	.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.)*

Genetic, and metabolic factors associated with premature ovarian failure, or disordered ovarian function have been explored in this project. Over the past year we have explored the effect of galactose on ovarian function in sexually mature rodents. Dietary galactose treatment reversibly inhibits ovulation and corpus luteum formation. Following cessation of the dietary galactose ovarian follicle growth and ovulation resumes promptly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00921-03 PRB

PERIOD COVERED
October 1, 1983 to June 30, 1984

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

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

PI:	D. R. Mattison	Head	PRB, NICHD
Other:	M. S. Nightingale	Chemist	PRB, NICHD
	H. H. Kay	Medical Staff Fellow	PRB, NICHD
	J. T. Chen	Visiting Fellow	PRB, NICHD
	D. R. Kuroda	Guest Researcher	PRB, NICHD
	E. K. Silbergeld	Guest Researcher	PRB, NICHD

COOPERATING UNITS *(if any)*
Clinical Center, NIH

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Reproductive Toxicology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.50	.40	.10

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Nuclear magnetic imaging of pregnant non-human primates has successfully demonstrated detailed fetal and placental anatomy. Paramagnetic ions have been used to enhance placental contrast.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00922-03 PR

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

P.I.:	B. J. Gulyas	Head	PRB, NICHD
Other:	L. C. Yuan	Chemist	PRB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Gamete Physiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.50	.50

CHECK APPROPRIATE BOX(ES)

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

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

Homologous as well as heterologous unfertilized oocytes were fused with a high rate of success utilizing polyethylene glycol. Ultrastructural studies showed that oocyte fusion products have undergone normal cortical reaction and completed second meiotic division. Roughly one-third of the OFPs developed into blastocysts. Chimeras of 2-cell OFPs and 2-cell stages from genetically different mice developed at a high rate into blastocysts. Future studies will focus on utilizing an electrical cell fusion system and reciprocal nuclear transfers between OFPs and fertilized eggs of a genetically different strain of mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00923-02 PR

PERIOD COVERED
October 1, 1983 to June 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Primate Models for the Study of Human Infertility, Contraception & Fetal Development

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

P.I.:	G. D. Hodgen	Head	PRB, NICHD
Others:	R. F. Williams	Senior Staff Fellow	PRB, NICHD
	D. Kenigsberg	Medical Staff Fellow	PRB, NICHD
	M. P. Platia	Medical Staff Fellow	PRB, NICHD
	R. L. Collins	Guest Scientist	PRB, NICHD
	V. M. Sopelak	Guest Scientist	PRB, NICHD
	D. L. Healy	Fogarty Fellow	PRB, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Pregnancy Research Branch

SECTION
Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	3.7	.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 following constitute principal areas of investigation in the 9 months of FY '84:

1) Demonstration that exogenous estrogen and progesterone would provide an adequate endometrium for pregnancy without ovaries by donor egg or surrogate embryo transfer.
2) The fetal thymus provides support for oogenesis. This was shown by depletion of ovarian follicles at birth after fetal thymectomy in utero.
3) Hyperstimulation of the ovaries by gonadotropins is due to a factor we have termed "gonadotropin surge inhibiting factor". It is non-steroidal, highly antigenic and rapidly cleared from circulation and receptors.
4) We have demonstrated the utility of GnRH antagonist therapy combined with gonadotropin administration to reduce individual patient response.
5) Gonadotropin therapy can induce transient hyperprolactinemia that is suppressed by bromocryptine. We suggest it may be a marker for risk of hyperstimulation syndrome.
6) We have demonstrated a practical method to distinguish in diagnosis true ovarian failure from pseudo-ovarian resistence using estrogen-progestin therapy to test suppressibility of FSH levels in circulation and measured by RIA. If antibodies to FSH cause a deceptively high FSH, steroids will not effect FSH levels measured by RIA.

OFFICE OF THE SCIENTIFIC DIRECTOR

Z01 HD 00093-10 Mechanism of Action of Nerve Growth Factor
Gordon Guroff, Ph.D.

Z01 HD 01500-02 Adenovirus(AD) and SV40:Models for Differentiation, Transformation, and Mutagenesis
Arthur S. Levine, M.D.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00093-10 OSD

PERIOD COVERED

October 1, 1983 to September 30, 1984

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:	G. Dickens	Biol. Lab. Tech.	OSD, NICHD
	H. Kuzuya	Visiting Scientist	OSD, NICHD
	V. Zabrenetzky	Staff Fellow	OSD, NICHD
	A. Togari	Visiting Fellow	OSD, NICHD
	N. Nakanishi	Visiting Fellow	OSD, NICHD
	T. Hama	Visiting Fellow	OSD, NICHD

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Growth Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	2.50	1.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This work is designed to provide information on the mechanism of action of nerve growth factor. Nerve growth factor is a polypeptide required for the development and maintenance of the sympathetic and sensory nervous systems. Nerve growth factor controls gene expression in the neurons on which it acts. Gene expression in neurons, and in other cells, determines the course of cellular development. A characterization of the genetic program in these cells may reveal how nerve growth factor induces the synthesis of specific enzymes and the morphological changes necessary for synapse formation. Such information would expand our knowledge of the development of the nervous system, of the tumors which arise from it, of the control of gene expression, and of the role of the growth factors. Our current studies are focused on the intracellular events which follow the binding of nerve growth factor to its membrane receptor and lead to its effect on nuclear events. We have used PC12 cells which differentiate in culture in response to nerve growth factor. Two specific phosphorylations in these cells, one cytoplasmic, the other nuclear, are altered by treatment of the cells with nerve growth factor. Both have now been observed in cell-free preparations making them both amenable to biochemical study. The cytoplasmic system has been resolved into kinase and substrate. It has been determined that the kinase is the component altered by treatment of the cells with nerve growth factor and a complete purification of the kinase is underway in order to define the molecular change responsible for the decreased activity. The phosphorylative changes in the cell are followed by changes in the structure of the DNA which may underlie changes in the transcription of specific genes. The alterations in DNA structure are being probed with specific lytic enzymes in order to correlate them temporally with changes in the expression of specific neuronal properties. The ultimate aim of our studies is to describe the actions of nerve growth factor at the molecular level, and to explore their generality in terms of other growth factors and of the developmental program of other cell types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01500-02 OSD

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adenovirus(Ad) and SV40:Models for Differentiation, Transformation, and Mutagenesis

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

PI:	K. Dixon	Senior Staff Fellow	OSD, NICHD
	A. S. Levine	Head	OSD, NICHD
	C. T. Patch	Senior Investigator	OSD, NICHD
Others:	J. M. Hauser	Microbiologist	OSD, NICHD
	B. J. Mathews	Staff Fellow	OSD, NICHD
	K. Akagi	Visiting Fellow	OSD, NICHD
	M. H. Haddada	Visiting Fellow	OSD, NICHD

COOPERATING UNITS *(if any)* Laboratory of Molecular Microbiology, NIAID (A.M. Lewis, Jr.); Dept. of Medicine, National Jewish Hospital and Research Center, Denver (J. Cook); Laboratory of Molecular Carcinogenesis, NCI (M. Seidman); Depts of Pediatrics and Biomathmatics, UCLA School of Medicine, Los Angeles CA (E. Landaw)

LAB/BRANCH
Office of the Scientific Director

SECTION
Section on Viruses and Cellular Differentiation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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.)*

Differentiation and tumorigenesis: Understanding the mechanisms of regulation of cellular proliferation, migration and differentiation is basic to understanding development of multicellular organisms. One approach to investigating these cellular regulatory mechanisms is to study the behavior of tumor cells that have become abnormal in regulation of these processes as a result of viral transformation. Through the use of cell hybrids formed between Ad2 and SV40 transformed cells, we are beginning to identify the phenotypic characteristics of the transformed cells (e.g., expression of specific viral antigens and cellular fibronectin, and sensitivity to lysis by immune effector cells) that correlate with their ability to form tumors in syngeneic animals. In addition, we find that Ad2-transformed cells appear to be more active than SV40-transformed cells in production of mitogenic factors. We are also developing the SV40 system to study the genetic basis of tumor metastasis. We have found that tumors induced in hamsters by a mutant of SV40 virus develop more slowly than normal and metastasize more frequently. By studying the properties of these abnormal tumor cells we expect to learn more about how cell proliferation and migration are regulated on the genetic level.

Mutagenesis: Chromosomal mutations are the underlying cause of most inherited diseases and many developmental abnormalities. Mutations also appear to play a role in carcinogenesis by a variety of environmental agents. We are using SV40 virus as a probe to investigate the molecular mechanisms by which these agents induce mutations in mammalian cells. Our studies on replication of UV-damaged SV40 DNA have led to a well-defined model of how the mammalian cell replication machinery responds to DNA damage and at what steps in the replication process mutations become irreversibly established. By use of a SV40-derived shuttle vector system we are also beginning to characterize the types of mutations induced by specific agents and to correlate these with the mechanism of mutation induction.

1984 Annual Report
Epidemiology Branch

Project Number	Project Title	Principal Investigator
Z01-HD-00318-04 EB	A Prospective Study of the Frequency and Duration of Infant Feeding Practices.....	G.G. Rhoads
Z01-HD-00323-04 EB	District of Columbia Perinatal Study.....	H.W. Berendes
Z01-HD-00325-03 EB	Neural Tube Defects and Folate.....	J.L. Mills
Z01-HD-00326-03 EB	Premature Thelarche in Puerto Rico.....	J.L. Mills
Z01-HD-00329-02 EB	Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.....	H.W. Berendes
Z01-HD-00331-01 EB	Diabetes in Early Pregnancy Project (DIEP).....	J.L. Mills
Z01-HD-00332-01 EB	The Risk of Adverse Pregnancy Outcome following Cervicitis during Pregnancy.....	G.G. Rhoads
Z01-HD-00333-01 EB	Congenital Anomalies and In Vitro Fertilization (IVF).....	J.L. Mills
Z01-HD-00334-01 EB	Low Birth Weight Across Generations.....	M.A. Klebanoff
Z01-HD-00335-01 EB	Mother's Birth Weight Affects Survival of a Low Birth Weight Infant.....	M.A. Klebanoff
Z01-HD-00336-01 EB	Coitus in Pregnancy: Is It Safe?.....	M.A. Klebanoff
Z01-HD-00337-01 EB	Vomiting during Pregnancy.....	M.A. Klebanoff
Z01-HD-00338-01 EB	Childhood Nutritional Experience and Subsequent Reproductive Performance.....	M.A. Klebanoff
Z01-HD-00339-01 EB	Race, Age, Socioeconomic Status and Low Birth Weight.....	M.A. Klebanoff

Project Number	Project Title	Principal Investigator
Z01-HD-00340-01 EB	Ethnic Differences in Birth Weight and Length of Gestation....	P.H. Shiono
Z01-HD-00341-01 EB	Cesarean Childbirth Rates in the U.S.....	P.H. Shiono
Z01-HD-00342-01 EB	Dietary Intake of Pregnant Women.....	N. Kurinij
Z01-HD-00343-01 EB	The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev, Bedouins.....	H.W. Berendes
Z01-HD-00344-01 EB	Long-Term Effects on Infants of Hypochloremic Metabolic Alkalosis Resulting from Infant Formulas Deficient in Chloride.....	H.W. Berendes

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00318-04 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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)*

George G. Rhoads, M.D., M.P.H., Chief, Epidemiology Branch, EBRP, NICHD

Michele R. Forman	Epidemiologist	CDC
Natalie Kurinij	Research Assistant	EB/EBRP/NICHD
Ernest Harley	Computer Specialist	CS/EBRP/NICHD
Barry Graubard	Biostatistician	BB/EBRP/NICHD
Maureen Edwards	Neonatologist	George Wash. Univ.
Marta L. Axelson	Asst. Professor	University of Maryland

COOPERATING UNITS *(if any)*

Computer Sciences Section, EBRP, NICHD; Biometry Branch, EBRP, NICHD; George Washington Univ. Medical Center, Univ. of Maryland

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.40	.38	.02

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 is a prospective study of maternal characteristics which affect infant feeding behavior in the first year of life. Factors associated with choice and duration of breast feeding are being investigated. The specific objectives of the study are as follows: (1) to provide detailed information on the change in the infant feeding pattern over time; (2) to invesitgate 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00323-04 EB

PERIOD COVERED
October 1, 1983 through September 30, 1984

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)*
Heinz W. Berendes, M.D., M.H.S., Director, EBRP, NICHD

Leslie C. Cooper	Research Nurse	EB, EBRP, NICHD
Daniel W. Denman	Statistician	BB, EBRP, NICHD
Harvey Shifrin	Contracting Officer	CMS, OGC, NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP
Contracts Management Section, OGC

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

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

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" are low birth weight infants (<2500 grams) born in participating hospitals. "Controls" are selected as the next race matched normal weight infant (= > 2500 grams) delivered at the same hospital. The mothers of the cases and controls are being interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information is being verified by using the prenatal information which is attached to the hospital medical record. However, if the hospital medical record does not contain adequate prenatal information arrangements are being made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and will continue until January 31, 1985. The data is being collected by SRA Technologies, Inc. of Arlington, Virginia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00325-03 EB

PERIOD COVERED
October 1, 1983 through September 30, 1984

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)*
James L. Mills, M.D., M.S., Research Medical Officer, EBRP, NICHD

George G. Rhoads Chief, Epidemiology Branch EBRP, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.8	.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

The Epidemiology Branch (EBRP) is designing a case control study to examine the effect of vitamin-folate supplements in the periconceptional period and neural tube defect risk. Drs. Mills and Rhoads are planning a telephone interview study in which mothers who have delivered a child with a neural tube defect (defined as cases), mothers who have delivered a child with another malformation (defined as controls), and mothers who have delivered a normal child (defined as controls) will be compared on the use of vitamins in the period around conception. Prospective study centers and data centers have been selected by reviewers. Negotiations are in progress. Depending on the availability of funds, the study will begin in the fall of 1984 or winter of 1984-85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00326-03 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Premature Thelarche in Puerto Rico

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

James L. Mills, M.D., M.S., Research Medical Officer, EBRP, NICHD

Godfrey Oakley — Chief, Birth Defects Branch — CDC — Atlanta

COOPERATING UNITS *(if any)*

Birth Defects Branch, CDC

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.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 Centers for Disease Control and the Puerto Rico State Health Department report an increase in premature thelarche among Puerto Rican girls. The CDC has requested that Dr. Mills be involved in the investigation of this presumed epidemic.

The CDC has completed the case control portion of their study on premature thelarche. The results to date have not uncovered a specific etiology. However, there is some evidence to suggest that contaminated meat products are involved. Dr. Mills will continue to collaborate with the Birth Defects Branch, Center for Environmental Health, CDC in the analysis of the premature thelarche data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00329-02 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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)*

Heinz W. Berendes, M.D., M.H.S., Director, EBRP, NICHD

Mary Overpeck	Statistician	BB, EBRP, NICHD
Leslie C. Cooper	Research Nurse	EB, EBRP, NICHD
Harvey Shifrin	Contracting Officer	CMS, OGC, NICHD
Joan Maxwell	Coordinator	Greater Wash. Res. Center
Ann Barnet	Project Director	Children's Hosp., Wash.DC

COOPERATING UNITS *(if any)*

Biometry Branch, EBR; Contract Management Section, OGC; Greater Washington Research Center, Washington, DC; Children's Hospital National Medical Center, Washington, DC.

LAB/BRANCH

Epidemiology and Biometry Research Program

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

The Better Babies Project is a three-year research and demonstration effort 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 Project will attempt to identify all pregnant women in the high risk area, help them link up with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

Evaluation of the project will be provided by the National Institute of Child Health and Human Development, Epidemiology and Biometry Research Program (EBRP).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00331-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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)*

James L. Mills, M.D., M.S., Research Medical Officer, EBRP, NICHD

Lois Jovanovic	NY Hospital Cornell University Medical Center
Lewis Holmes	Brigham and Womens Hospital, Harvard Medical School
Joe Leigh Simpson	Northwestern University Medical Center
Jerome Aarons	University of Pittsburgh
Robert Knopp	University of Washington

COOPERATING UNITS *(if any)*

See Above

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
16.5	4.5	12.0

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 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.

This study has reached the last quarter of data collection. Final new pregnancies will be accepted in October, 1984. The last deliveries are anticipated to occur in June, 1985. Data collection should be complete within a few weeks of the termination of the final pregnancies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00332-01 EB

PERIOD COVERED
October 1, 1983 to September 30, 1984

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)*

George G. Rhoads	Chief, Epidemiology Br.	EBRP/NICHD/NIH
B. Frank Polk	Associate Professor	Johns Hopkins Univ.
Linda Berlin	Research Nurse	Johns Hopkins Univ.
Robert P. Nugent	Epidemiologist	EBRP/NICHD/NIH

COOPERATING UNITS *(if any)*
Johns Hopkins University

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.9	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 contract was funded in May, 1983 for two years. Data collection began in November 1983 and is expected to continue until February 1985 with analysis to begin in April 1985, with articles submitted for publication as soon as possible.

All eligible women (age 18 and older) seen in the obstetric clinic at Johns Hopkins University who agree to participate will have their cervix evaluated for signs of inflammation. In addition cultures will be taken for a number of aerobic and anaerobic organisms and a sample of cervical mucus will be evaluated for the presence of inflammatory cells. The women will be interviewed to obtain information on a number of risk factors related to preterm and low birth weight delivery. The women will then be followed to delivery to evaluate the effect of cervicitis on preterm or low birth weight delivery. Approximately 700-800 women are expected to participate in this study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00333-01 EB

PERIOD COVERED
October 1, 1983 through September 30, 1984

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)*

James L. Mills, M.D., M.S.,	Research Medical Officer,	EBRP, NICHD
Heinz W. Berendes	Director	EBRP, NICHD
Ernest E. Harley	Chief, Computer Sciences Section	EBRP, NICHD

COOPERATING UNITS *(if any)*
Computer Sciences Section, EBRP

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	0

CHECK APPROPRIATE BOX(ES)

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

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

A Sources Sought has identified centers which have an adequate number of infants produced by IVF to conduct a study of malformation risk associated with IVF. A Request for Proposals will be issues shortly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00334-01 EB

PERIOD COVERED
October 1, 1983 through September 30, 1984

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)*

Mark A. Klebanoff, M.D., M.P.H.,	Medical Staff Fellow,	EBRP, NICHD
Barry I. Graubard	Mathematical Statistician	BB/EBRP/NICHD
Samuel S. Kessel	Medical Officer	EB/EBRP/NICHD
Heinz W. Berendes	Director	EBRP/NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP, NICHD

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.50	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 demonstration of the association of the birth weight of a mother and birth weight of her children, based on data from the Collaborative Perinatal Project, has been accepted for publication in the Journal of the American Medical Association. Of note is the finding that even after adjustment for multiple covariables (including maternal prepregnancy weight), a mother's birth weight is associated with the birth weight of her offspring. Women weighing 4 to 6 pounds at birth are 3.5 times as likely to have a low birth weight infant as are women weighing 8 or more pounds at birth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00335-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Mother's Birth Weight Affects Survival of a Low Birth Weight Infant

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

Mark A. Klebanoff, M.D., M.P.H., Medical Staff Fellow, EBRP, NICHD

Christine Branche	Summer Student	EB/EBRP/NICHD
George G. Rhoads	Chief, Epidemiology Br.	EBRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

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.)*

It has been previously shown that the birth weight of a mother influences the birth weight of her children. Presumably, small infants of small mothers are normal but small infants of large mothers are not. If such were the case, then small infants of small mothers can be expected to have a greater probability of survival than small infants whose mothers were not small. The maternal birth weight of all low birth weight infants in the Collaborative Perinatal Project will be entered, and analyses of this question will be done.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00336-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Coitus in Pregnancy: Is it Safe?

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

Mark A. Klebanoff, M.D., M.P.H., Medical Staff Fellow, EBRP, NICHD

Robert P. Nugent	Epidemiologist	EB/EBRP/NICHD
George G. Rhoads	Chief, Epidemiology Br.	EBRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.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.)*

This study utilized data from the Collaborative Perinatal Project to prospectively study the role of coitus during pregnancy. Approximately 35,000 women were evaluated for their coital frequency at various points during gestation. Increasing coital frequency was associated with a prolongation of gestation. There was no significant association between coitus at 28-29, 32-33, or 36-37 weeks and perinatal mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00337-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Vomiting During Pregnancy

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

Mark A. Klebanoff, M.D., M.P.H., Medical Staff Fellow, EBRP, NICHD

Patricia A. Koslowe	Epidemiologist	EBS/CESB/MIDP/NIAID
Richard Kaslow	Section Chief	EBS/CESB/MIDP/NIAID
George G. Rhoads	Chief, Epidemiology Br.	EBRP/NICHD

COOPERATING UNITS *(if any)*

Epidemiology and Biometry Section, NIAID

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

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.)*

Vomiting during pregnancy has been described since 2,000 B.C., but few studies have attempted to describe its epidemiology. First trimester registrants in the Collaborative Perinatal Project were screened for the presence of vomiting. Vomiting was more common in blacks, primigravidae, young women, heavy women, non-smokers and women with less education. The absence of vomiting placed a woman at increased risk of fetal loss. There was a modest protective effect on preterm delivery, and no effect on the incidence of low birth weight.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00338-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Childhood Nutritional Experience and Subsequent Reproductive Performance

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

Mark A. Klebanoff, M.D., M.P.H., Medical Staff Fellow, EBRP, NICHD

Zena A. Stein Professor Columbia University

COOPERATING UNITS *(if any)*

Research Foundation for Mental Hygiene, Inc., New York, NY

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

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.)*

Girls born during the Dutch famine of 1944-45 are known to have been growth retarded as a direct result of maternal starvation, however final adult height was not reduced. Girls age 12-14 during the famine were permanently stunted. The subsequent reproductive experience of several cohorts of women who were of different ages during the famine will be determined. These cohorts include women who were born during the famine and women who were pre-pubertal, pubertal and post-pubertal during the famine, as determined by their year of birth. The women born during the famine will be further subdivided into women exposed during their early postnatal life, pre- and postnatal life, and prenatal only.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00339-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Race, Age, Socioeconomic Status and Low Birth Weight

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

Mark A. Klebanoff, M.D., M.P.H., Medical Staff Fellow, EBRP, NICHD

Heinz W. Berendes	Director	EBRP/NICHD
Sarah Brown	Research Assistant	NAS/IOM

COOPERATING UNITS *(if any)*

National Academy of Science/Institute of Medicine

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

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.)*

Race, age and socioeconomic status have been previously shown to be associated with low birth weight, however each of these factors is confounded with other risk factors. This work critically reviews the evidence for an association between low birth weight and the above noted factors, after accounting for other confounders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00340-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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)*

Patricia H. Shiono, Ph.D., Epidemiologist, EB, EBRP, NICHD

Barry Graubard Math. Statistician BB, EBRP, NICHD

COOPERATING UNITS *(if any)*

Biometry Branch, EBRP

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

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.)*

Data from the Kaiser-Permanente Births Defects Study, a large prospective study of pregnancy outcomes, are being used to evaluate differences in the birth weights and gestational ages of babies born to women of different ethnic groups. The ethnic groups included in this study were Whites, Hispanics, Blacks, Asians, and others. Univariate and multivariate analyses of the effects of a large number of variables are being done to determine if the differences in birth weight between the four ethnic groups can be explained. Preliminary analyses show that after controlling for smoking, alcohol, and seven other variables, large differences in birth weight persist between Blacks and Whites and Asians and Whites. The babies of Black women and Asian women are on average about 200 grams lighter than babies of White women. Black women also have about two times the incidence of preterm deliveries compared to White women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00341-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Cesarean Childbirth Rates in the U.S.

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

Patricia H. Shiono, Ph.D., Epidemiologist, EB, EBRP, NICHD

George G. Rhoads Chief, Epidemiology Br. EB, EBRP, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

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.)*

A study is being planned to determine the cesarean childbirth rates in the U.S. and the current hospital policies regarding cesarean childbirth. To obtain current statistics on the rates of primary and total cesarean childbirth, the rates of cesarean childbirth by indication and maternal mortality associated with cesarean and vaginal deliveries, data will be obtained from the National Center for Health Statistics and the Commission on Professional Hospital Activities. A telephone or mail survey will be used to assess current hospital policies on cesarean childbirth, particularly policies relating to the use of trial labor and vaginal deliveries after a previous cesarean delivery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00342-01 EB

PERIOD COVERED

October 1, 1983 through September 30, 1984

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

Dietary Intake of Pregnant Women

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

Natalie Kurinij, M.S., Research Assistant, Epidemiology Branch, EBRP, NICHD

Barry Graubard	Biostatistician	BB/EBRP/NICHD
George G. Rhoads	Chief, Epidemiol. Br.	EBRP/NICHD

COOPERATING UNITS *(if any)*

Biometry Branch, EBRP, NICHD

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

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.)*

Pregnant women are at increased risk of malnutrition due to the increased nutrient demands of pregnancy. Nutrient intake during pregnancy is being assessed using data from the NHANES I survey. The dietary patterns of a national sample of pregnant women is being evaluated to determine differences in nutrient intake and food frequency during each trimester of pregnancy. Nutrient intake during pregnancy is being compared to the nutrient intake of nonpregnant women of childbearing age and to the recommended dietary allowances.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00343-01 EB

PERIOD COVERED: October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: The Effect of Exposure to 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)*

Heinz W. Berendes, M.D., M.H.S.	Director, EBRP	NICHD
Michele R. Forman, Ph.D.		Nutrition Res. Branch CDC, Atlanta, GA
Barry Graubard, M.A.	Senior Statistician	BB, EBRP, NICHD
Lechaim Naggan, M.D., D.P.H.	Dean & Director	Center for Hlth. Sci. Ben Gurion Univ.on the Negev, Beer Sheva, Israel

COOPERATING UNITS *(if any)*

See above

LAB/BRANCH: Office of the Director, EBRP

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethedsa, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

This study intends to document the incidence of breast and bottle feeding among different Bedouin tribes who are changing from a seminomadic to a sedentary life style. Data have been collected on about 2-1/2 thousand women shortly after birth and for a subsample at 5-8 months after birth. This includes background information on perinatal events and delivery complications, prior history of infant feeding practices and infant feeding regarding the current child. Though follow-up data have been collected on changes in infant feeding practices over time and on intercurrent morbidity especially gastroenteritis and respiratory disease resulting in hospitalization.

The data collection is complete and the collected information has been computerized and is undergoing preliminary analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00344-01 EB

PERIOD COVERED: June 1, 1984 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Long-Term Effects on Infants of Hypochloremic Metabolic Alkalosis Resulting from Infant Formulas Deficient in Chloride.

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

Heinz W. Berendes, M.D., M.H.S.	Director, EBRP	NICHD
Barry Graubard, M.A.	Senior Statistician	BB EBRP NICHD
Carol Schultz, Ph.D.		J.R.B. Assoc.
Jose Cordero, M.D., M.P.H.		Birth Defects Branch, CDC, Atlanta, GA

COOPERATING UNITS *(if any)*

See above

LAB/BRANCH: Office of the Director, EBRP

SECTION:

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

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

This is a Congressionally mandated study to determine whether the children exposed to chloride deficient formula in 1979 may have suffered some long-term effects which may be expressed in delayed motor and mental development or decreased school performance. This project is done through a contractional arrangement with JRB Associates and the active collaboration of the Centers for Disease Control. The initial effort will consist of the identification of children exposed to the chloride deficient formula who were sufficiently ill to require hospitalization in 1979 and who had evidence of hypochloremic metabolic alkalosis. Also during the first year a battery of tests will have to be chosen for the evaluation of these children and a group of control children identified.

These children and their control group will be evaluated during the second year. The third year of the study will be devoted to analysis.

NICHD Annual Report

October 1, 1983 through September 30, 1984

Biometry Branch

Project Numbers	Project Title	Principal Investigator
Z01-HD-00801-09 BB	Studies based on the Medical Birth Registries of Norway (1967-1973) and Sweden (1977-1981).....	H. J. Hoffman
Z01-HD-00802-09 BB	Study of Linked Information on Infant Death Certificates and Live Birth Certificates for Selected U.S. States.....	H. J. Hoffman
Z01-HD-00811-05 BB	National Collaborative Cysteamine Study Data Center.....	G. F. Reed
Z01-HD-00813-03 BB	Methodology for Laboratory Animal Research, including Bio-assay, Life Tables, and Dose-Response Studies.....	G. F. Reed
Z01-HD-00818-03 BB	Research in Developing Nonparametric Methods for Biomedical Applications.....	G. F. Reed
Z01-HD-00820-03 BB	Statistical Methods for Epidemiologic Data.....	D. W. Denman
Z01-HD-00821-02 BB	Graphical Display of Statistical Data.....	D. W. Denman
Z01-HD-00830-03 BB	Child Health Supplement to the 1981 NCHS Health Interview Survey..	M. D. Overpeck
Z01-HD-00831-01 BB	Evaluation of Interventions to Prevent Low Birth Weight in the District of Columbia.....	M. D. Overpeck
Z01-HD-00832-01 BB	Changes in Perinatal Mortality by Race in Selected U.S. Cities, 1970-1981.....	M. D. Overpeck
Z01-HD-00840-03 BB	Statistical Discriminate Methods with Applications to Alcoholism Screening.....	B. I. Graubard

Project Numbers	Project Title	Principal Investigator
Z01-HD-00841-03 BB	Methods for Comparing and Analyzing Data from Several Complex Surveys	B. I. Graubard
Z01-HD-00842-02 BB	Development of Statistical Methods to Analyze Cluster Samples	B. I. Graubard
Z01-HD-00843-01 BB	An Investigation of Matched Analysis in Case-Control and Cohort Studies	B. I. Graubard
Z01-HD-00844-01 BB	Analysis of NHANES Anthropometric Measurements on Children	B. I. Graubard
Z01-HD-00850-08 BB	Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia	D. A. Bryla
Z01-HD-00851-03 BB	Trends in Time Relating to Maternal and Child Health and Population Research	D. A. Bryla
Z01-HD-00852-02 BB	1980 National Natality Survey and Fetal Death Survey	D. A. Bryla
Z01-HD-00860-04 BB	Analysis of Biomedical Time Series Data	H. J. Hoffman
Z01-HD-00861-02 BB	Estimation of Fetal Growth Patterns Based on Symphysis-Fundus and Ultrasound Measurements	H. J. Hoffman
Z01-HD-00870-01 BB	Long-Term Effects of Cesarean Section	K. E. Hemminki

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00801-09

PERIOD COVERED

October 1, 1983, to September 30, 1984

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 (1967-1973) and Sweden (1977-1981)

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

PI:	Howard J. Hoffman	Chief	BB EBRP NICHD
Others:	Heinz W. Berendes	Director	EBRP NICHD
	Ernest Harley	Chief	CS EBRP NICHD
	Karen Fetterly	Computer Specialist	CS EBRP NICHD

COOPERATING UNITS *(if any)*

Institute of Hygiene and Social Medicine, Univ. of Bergen, Norway (R. Skjaerven); Dept. of Community Medicine, Univ. of Trondheim, Norway (L. Bakketeig); Depts. of Obstetrics & Gynecology and Social Medicine, Univ. of Uppsala (O. Meirik); Dept. of Social Affairs, Stockholm, Sweden (A. Ericson).

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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-00802-09

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Study of Linked Information on Infant Death Certificates and Live Birth Certificates for Selected 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 EBRP NICHD
Others:	Dolores A. Bryla	Statistician	BB EBRP NICHD
	Mary D. Overpeck	Health Statistician	BB EBRP NICHD
	Ellen Heineman	Statistician (Summer)	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD
	Ernest Harley	Chief	CS EBRP NICHD
	Karen Fetterly	Computer Specialist	CS EBRP NICHD

COOPERATING UNITS *(if any)*

Departments of Health in the following states: California, Minnesota, Missouri, New York State, and North Carolina.

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.3	.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 spece 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. 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.). These studies will be compared with similar studies on a 1950 and 1960 cohort of U.S. births. Additional comparisons will be made to linked data from Canada (1971), Great Britain (1970), Norway (1967-1976), and Sweden (1975-1980).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00811-05

PERIOD COVERED
October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
National Collaborative Cysteamine Study Data Center

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

PI:	George F. Reed	Mathematical Statistician	BB EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Ernest Harley	Chief	CS EBRP NICHD
	Elva Nelson	Statistical Assistant	CS EBRP NICHD
	William Gahl	Senior Staff Fellow	HGB IRP NICHD

COOPERATING UNITS *(if any)*

Univ. California, San Diego School of Medicine (Jerry Schneider)	Uniform Services Univ. of the Health Sciences (James J. Schlesselman)	Univ. of Michigan Medical School (Jess Thoene)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

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

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

This study is a clinical trial to determine the safety and efficacy of cysteamine in the treatment of nephropathic cystinosis, a metabolic disease which usually leads to end-stage renal disease before 10 years of age. All children enrolled in the trial will receive cysteamine. Control information is provided by data collected on 30 patients who were randomized to placebo in a previous trial evaluating the efficacy of Vitamin C for the treatment of this disease. Approximately 60 children will eventually be enrolled in the current trial, which is anticipated to last about three years. Evaluation of the drug's effectiveness will be chiefly determined by the creatinine clearance values of the treated children as compared with those of the historical controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00813-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Methodology for Laboratory Animal Research, Including Bioassay, Life Tables, and Dose-Response Studies

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

PI:	George F. Reed	Mathematical Statistician	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Ellen F. Heineman	Statistician (Summer)	BB EBRP NICHD
	Donald Mattison	Medical Officer	PR IRP NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 animal 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 with animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00818-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Howard J. Hoffman	Chief	BB EBRP NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 sensitive to violations of underlying assumptions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00820-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
	George F. Reed	Mathematical Statistician	BB EBRP NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.8	.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Since many epidemiologic problems cannot be solved by standard techniques, new methods can help 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 the use of generalized linear models and the SAS procedure GLM in regression, analysis of variance, and analysis of covariance. Special interest will be paid to the use of logistic regression and log-linear models. Computing techniques such as the linking together of FORTRAN functions and SAS procedures will also be explored. Useful techniques will be presented to the branch in seminars, and more comprehensive reports will be submitted to the statistical journals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00821-02

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Graphical Display of Statistical 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 EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	George F. Reed	Mathematical Statistician	BB EBRP NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 object of this project is to draw from current literature and computer demonstrations and 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. This may include acquiring new computer hardware and software from outside sources, as well as making full use of support provided by DCRT and developing original methods using existing resources.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00830-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Child Health Supplement to the 1981 NCHS 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	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Dolores A. Bryla	Statistician	BB EBRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Division of Health Interview Statistics (C. Burnham)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 project provides data on a nationwide sample of 17,000 children of indices of child development, childhood morbidity, school performance and behavior. It will establish normative ranges for the U.S. as well as determining the long-term consequences of perinatal and early childhood risks. The survey was conducted by the National Center for Health Statistics in collaboration with NICHD and others.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00831-01

PERIOD COVERED
October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Interventions to Prevent Low Birth Weight in the District of Columbia

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

PI:	Mary D. Overpeck	Health Statistician	BB EBRP NICHD
Others:	Heinz W. Berendes	Director	EBRP NICHD
	Leslie Cooper	Research Nurse	EB EBRP NICHD

COOPERATING UNITS *(if any)*
Better Babies Project, VNA/Family Pace NE, Washington, DC (J. Maxwell)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	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.)*

NICHD staff have worked continuously with sponsors and staff of the D.C. Better Babies Project on study design, training of service coordinators, form development, interventions, and obtaining data for evaluation.

The Epidemiology and Biometry Research Program, NICHD, agreed to provide technical advice on the development of interventions and to evaluate the impact of the D.C. Better Babies Project to improve the use of prenatal care and reduce the incidence of low birth weight in a high risk pregnant population in the District of Columbia. Targeting an area with a low birth rate greater than 15%, the D.C. Better Babies will document the compliance with specific interventions and yield analyses of the effects of the interventions. With the support of a contractor, NICHD will follow the progress and outcome of pregnancies occurring to women participating in the project. Outcome variables will be compared to those available on vital records for nonparticipants in the target area, similar areas of the District of Columbia, and the city as a whole. Evaluation of smoking, alcohol, and nutritional interventions will be done by comparisons to women attending no more than two public health clinics in D.C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00832-01

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Changes in Perinatal Mortality by Race in Selected U.S. Cities, 1970-1981

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

PI:	Mary D. Overpeck	Health Statistician	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD
	Leslie Cooper	Research Nurse	EB EBRP NICHD

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Division of Vital Statistics, Mortality Statistics Branch (H. Rosenberg)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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.)*

This study reviews changes and differences in perinatal mortality for similar populations over a period of rapid change in technology, use of cesarean sections, and medical management of high risk pregnancies.

It will explore whether high rates of neonatal mortality in certain cities reflect phenomena other than shifts in mortality from the late fetal period and to track differences in perinatal experience among biologically similar populations. The approach will be a secondary analysis of data sets provided by the National Center for Health Statistics based on 100 percent reporting of perinatal deaths. Categorical screens of both twenty and twenty-eight weeks gestation to deaths through seven days and one month will be used to eliminate reporting differences among cities and shifting of neonatal deaths into the latter period. These data have not been available publicly for analysis. The analysis should provide new baseline information on the true outcome of pregnancies in biologically similar populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00840-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Statistical Discriminate 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 EBRP NICHD

COOPERATING UNITS *(if any)*

Alcohol, Drug Abuse and Mental Health Administration (R. Rawlings, S. Teper, and M.J. Eckardt); Dept. Obstetrics & Gynecology, Naval Medical Center Bethesda

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 objective is to study the statistical properties of a variety of discriminate functions and to determine how well they differentiate between alcoholic, other diseased, and normal populations using standard batteries of blood chemistries. These populations have been mainly male but populations of pregnant and nonpregnant women are presently being tested in a similar way.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00841-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Mary D. Overpeck	Health Statistician	BB EBRP NICHD
	Philip Rosenberg	Math. Stat. (Summer)	BB EBRP NICHD

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Office for Research and Methodology, Statistical Methods Section (R. Casady)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 objective is to compare available statistical methods for conducting data analysis with complex survey data, using data from the National Natality Follow-back Survey (1981), the Child Health Supplement to the National Health Interview Survey (1981) and Cycle II of the Family Growth Survey. Also, new methods will be theoretically and empirically investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00842-02

PERIOD COVERED

October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD
	Mark Klebanoff	Staff Fellow	EBRP NICHD

COOPERATING UNITS *(if any)*

Centers for Disease Control, Center for Health Promotion and Education, Division of Nutrition (M. Forman)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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 develop new regression models for analyzing clustered observations, as found in familial data, where the individuals in a cluster are correlated and the outcomes are categorical. These models will be studied using data from the Pima Indian and Bedouin Arab infant feeding studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00843-01

PERIOD COVERED

October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	George F. Reed	Mathematical Statistician	BB EBRP NICHD
	Philip Rosenberg	Math. Stat. (Summer)	BB EBRP 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. 20205

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 theoretically and empirically compare matched with unmatched designs of case-control and cohort studies. The Family Growth Cycle III Survey and the NHANES I and II are two potential sources of data upon which the empirical analysis will be based.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00844-01

PERIOD COVERED
October 1, 1983, to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of NHANES Anthropometric Measurements on Children

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

PI:	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
Others:	Philip Rosenberg	Math. Stat. (Summer)	BB EBRP NICHD
	Natalie Kurinij	Research Assistant	EB EBRP NICHD

COOPERATING UNITS *(if any)*
National Center for Health Statistics, Division of Health Examination Statistics, Nutrition Statistics Branch (S. Abraham)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

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

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

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

The objective of this study is to develop an obesity index for children based upon the weight, height and age of the children. This index will be compared to available measures of subcutaneous fat derived from skinfold measurements. This research project will use the anthropometric measurements on children that is contained in the NHANES I and II data sets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00850-08

PERIOD COVERED
October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
	Karen L. Fetterly	Computer Specialist	CS EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD

COOPERATING UNITS *(if any)*
Downstate Medical Center, State Univ., N.Y.; Albert Einstein College of Medicine; Long Island Jewish-Hillside Medical Center; Medical College of Virginia; Univ. of Southern California Medical Center; Univ. of Cincinnati

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.8	.2

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 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 serves as a data center for this study and is 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00851-03

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Trends in Time Relating to Maternal and Child Health and Population Research

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

PI:	Dolores A. Bryla	Statistician	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This objectives of this project are: (1) to develop time trends relating to maternal and child health and population research; (2) illustrate the time trends appropriately; (3) publish the data; and (4) update the data periodically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00852-02

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

1980 National Natality Survey and National Fetal Death Survey

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

PI:	Dolores A. Bryla	Statistician	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Karen L. Fetterly	Computer Specialist	CS EBRP NICHD
	Donald McNellis	Medical Officer (Obstetrics)	CNED CRMC NICHD

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Division of Vital Statistics, Natality Statistics Branch (P. Placek)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The 1980 National Natality Survey and 1980 National Fetal Death Survey conducted by the National Center of Health Statistics (NCHS) contains data on 9,941 live births and 6,386 fetal deaths. For each live birth and fetal death certificate selected, a mother, physician, hospital and radiation questionnaires was obtained by NCHS. This project will provide data on a nationwide sample relating to pregnant women's characteristics, outcome of pregnancy, labor and delivery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00860-04

PERIOD COVERED
October 1, 1983, to September 30, 1984

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 EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Barry Bercu	Medical Officer (Pediatrics)	ES PR IRP NICHD
	Mary Ann Brock	Biologist	CI CP GRC NIA

COOPERATING UNITS *(if any)*
Department of Obstetrics and Gynecology, University of Melbourne, Australia (J. Brown); Univ. of Texas Medical School at Houston (G. Ross); Pediatric Nutrition, Mead Johnson Company (J. Hansen)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.5	.2

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

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

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-00861-02

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Estimation of Fetal Growth Patterns Based on Symphysis-Fundis and Ultrasound Measurements

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

PI:	Howard J. Hoffman	Chief	BB EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD

COOPERATING UNITS *(if any)*

Department of Community Medicine, University of Trondheim, Norway (G. Jacobsen and L. Bakketeig); University Hospital, Trondheim, Norway (C. Brodtkorb); Bell Communications, Murray Hill, NJ (G.W. Reed)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.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 purpose of this study is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyeal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound.

Linear regression models have been fit to the serial symphyseal-fundal height measurements after stratifying the sample mothers according to the outcomes of their pregnancies in terms of small-, appropriate-, or large-for-gestational age births. Using a robust 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HD-00870-01

PERIOD COVERED

October 1, 1983, to September 30, 1984

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

Long-Term Effects of Cesarean Section

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

PI:	Elina Hemminki	Visiting Scientist	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
	Ntinos Myrianthopoulos	Section Chief	DNB NINCDS

COOPERATING UNITS *(if any)*

New York State Department of Health, Division of Community Health and Epidemiology (D. Glebatis, D. Janerich and G. Therriault); National Center for Health Statistics, Division of Vital Statistics, Family Growth Branch (W. Mosher)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of the work is to study long-term adverse effects possibly following a delivery with cesarean section. Effects on subsequent fertility, ectopic pregnancies and on malformations of subsequent children having been studied using U.S. data. Subsequent fertility is studied by comparing women having had a cesarean section to those having had a vaginal delivery in their first pregnancy using data from the 1982 National Survey of Family Growth. Effect on ectopic pregnancies is studied by comparing the past delivery history of women having had ectopic pregnancy to that of women having had a live birth or a spontaneous abortion. The data source is fetal and live birth certificates in Upstate New York. Effects on malformations are studied by comparing the malformation rates of children whose mothers have had a previous cesarean section to that of children whose mothers have had a previous vaginal delivery. The data source is the Collaborative Perinatal Project. Many different types of problems, both for the mother and infant, in the subsequent pregnancies have been studied using the data in the Swedish Birth Register. Future plans include linking this data to hospital discharge register to study problems not related to pregnancies ending in birth.