NICHD ANNUAL REPORT OF INTRAMURAL RESEARCH

October 1, 1984 through September 30, 1985

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
INTRAMURAL RESEARCH PROGRAM

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR
OCTOBER 1, 1984 TO SEPTEMBER 30, 1985

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 twelve 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 comparative ethology.

In November 1984, a new Laboratory was formed within the Intramural Research Program, the Cell Biology and Metabolism Branch. Dr. Richard Klausner, previously associated with the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, was recruited to lead the new Laboratory, and he was joined by two senior colleagues, Drs. Gilbert Ashwell (NIADDK Institute Scholar) and Joseph Harford, as well as a number of post-doctoral fellows. Building 18 was extensively renovated to accommodate these new research interests, and a large addition to Building 18 was constructed as well. The Cell Biology and Metabolism Branch focuses on the developmental aspects of various cellular organelles and receptors, and in particular, the structure and function of these important elements in cell biology. The Branch's laboratory activities take advantage of the extraordinary opportunities in cell biology occasioned by the advent of recombinant DNA and monoclonal antibody techniques. The Branch's clinical research activities reflect the Institute's interest in developmental aspects of cell physiology and metabolism, especially the metabolism of iron.

The Laboratory of Comparative Ethology, established late in 1983, continues to undergo a major building program. An extensive outdoor facility for free-ranging primates, employed in observational studies on the genetics of behavioral development, is now in place, and construction of a three-story indoor facility, as well as breeding quarters and a newborn nursery, has begun. These facilities are located at the NIH's Animal Center in Poolesville, Maryland.

The Institute continues to acquire additional laboratory space. Construction is well underway for a three-floor addition to Building 6. This will provide us with approximately twenty-five new laboratory modules, as well as an extensive animal facility, and will be ready for occupancy in the spring of 1986. This new space will accommodate the Laboratory of Developmental Pharmacology, several sections of the Laboratory of Developmental and Molecular Immunity, and the DNA/protein sequencing and synthesis activity of the Endocrinology and Reproduction Research Branch. During the past year also, three Branches, the Human Genetics Branch, the Developmental Endocrinology Branch, and the Endocrinology and Reproduction Research Branch, moved to

newly renovated laboratory and office space in the Clinical Center. The Laboratory of Theoretical and Physical Biology will move to the Clinical Center quarters vacated by the Laboratory of Developmental Pharmacology when the latter Lab moves to the new addition in Building 6. Finally, plans are being made to add additional space in Buildings 36 and 37 for our two neuroscience Laboratories.

In major personnel actions during the past year, Drs. James Russell, Greti Aguilera, Joseph Harford, and George Chrousos were granted tenure. Dr. Jacob Maizel transferred to the NCI to direct a major new branch concerned with computer applications in molecular biology, and Drs. Wilbert Nixon and Bela Gulyas also vacated tenured positions to take up positions elsewhere in the NIH.

Our staff fellowships for physicians in adult, pediatric, and reproductive-endocrinology, as well as the fellowship in human genetics, continue to thrive, and in the past year, we have initiated a new program which offers three years of full-time training in laboratory research for physicians whose previous laboratory experience has been minimal. These physicians are offered stipends through the new NIH Intramural National Research Scholarship Award Grant Program (NRSA).

The NICHD has developed still other mechanisms for the provision of post-doctoral stipends that do not utilize regular government positions, as a response to the decreasing number of such positions in virtually all Federal agencies. These new mechanisms include, in addition to the Intramural NRSA, a contractual program for the support of intramural training administered by the National Research Council of the National Science Foundation, primarily intended for Ph.D.s who are within five years of receipt of the doctoral degree. We have also been extremely successful in identifying new donors of stipends, including endowments by private industry and foundations. Contractual funds administered by the NIH's Fogarty International Center have been employed for the support of sabbatical visits by junior and senior scientists from abroad. Moreover, a number of foreign post-doctoral fellows have been awarded stipends for training in our Laboratories under the terms of formal bilateral agreements generated between the NIH and several countries in Europe, Asia, and the Middle East. Finally, three medical students supported by the new Howard Hughes Foundation program at the NIH are working in our laboratories during an elective year.

Peer review of intramural research, conducted by the Institute's Board of Scientific Counselors and ad hoc experts, continues to receive great emphasis, with rigorous site visits to each Lab at three and one-half year intervals. During the past year, visits were made to the Laboratory of Developmental Pharmacology and the Laboratory of Comparative Ethology, with detailed critiques prepared as a consequence of these visits. The membership of the Board of Scientific Counselors reflects the increasing diversity of research interests within this Intramural Program. The current Board membership includes:

Aron Moscona, Ph.D., Louis Block Professor of Biological Sciences, University of Chicago (Chairman)
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
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 strengthening of all aspects of the management of animals employed in our research, under the supervision of the Institute's full-time veterinarian, Dr. John Donovan. The Institute's Animal Research Committee, chaired by Dr. Charles Strott, works closely with Dr. Donovan in this regard.

Our Summer Student Program was very successful this year, with more than fifty undergraduate, graduate, and medical students working in our Laboratories, despite the fact that most were here on a volunteer basis.

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.

Seminars sponsored by the twelve Laboratories and Branches in this Program were numerous and well attended throughout the year, such that this Institute organized a relatively large fraction of the NIH's overall offering of intramural seminars and workshops. During the past year also, three major conferences with participants from throughout the world were hosted by Laboratories of the Intramural Research Program, including:

Cytochrome P-450 Genes and Their Regulation (Laboratory of Developmental Pharmacology, Airlie, Va.)
Pineal and Retinal Relationships (Laboratory of Developmental Neurobiology, Sarasota, Fl.)
Molecular and Developmental Neurobiology (Laboratory of Neurochemistry and Neuroimmunology and Laboratory of Developmental Neurobiology, Bethesda, Md.)

During the past year, we were especially honored by the award to Dr. Igor Dawid (Chief, Laboratory of Molecular Genetics) of the Department of Health and Human Service's highest honor, the Distinguished Service Award (Scientific), for his landmark contributions to developmental biology. Dr. John Robbins was awarded the Public Health Service Meritorious Service Medal for his work on the development of new bacterial vaccines, and Dr. Lynn Loriaux was the first recipient of the NIH's new Annual Award for "Clinical Teacher of the Year." Dr. Andreas Chrambach received the NIH Director's Award, for his numerous innovations in molecular separation techniques, and Drs. Bruce Nisula and Gordon Cutler received the PHS Commendation Medal for their clinical research on thyroid and pituitary disorders. Additionally, many of the

Institute's senior investigators held honorary lectureships and visiting professorships during the year; major prizes for scientific accomplishment were awarded to a number of our scientists by various universities and societies.

Finally, during the coming year, we shall be sponsoring two distinguished senior scientists in the Fogarty Scholars Program, Professor Donald Brown of the Carnegie Institute (Baltimore) and Professor Itzhak Parnas of the Hebrew University (Jerusalem), who will work in our Laboratories during their sabbaticals.

In December of 1982, this Institute embarked upon an ambitious program designed to improve the quality and quantity of its intramural scientific productivity, even as the rate of growth in research support declined. As one reflection of our success in this regard during the past three years, NICHD intramural scientists this year published twice the number of peer-reviewed original scientific reports, appearing in journals of stature, than had been the case prior to the beginning of this new direction. Moreover, one out of every two of our post-doctoral scientists is now fully supported by a stipend awarded from a non-NIH source--reflecting, we believe, the current quality and productivity of this Intramural Program.

CELL BIOLOGY AND METABOLISM BRANCH

Z01 HD 01600-01 Structure and Function of the Murine T Cell Antigen Receptor
Richard D. Klausner, M.D.

Z01 HD 01601-01 Molecular Aspects of the Regulation of the Human Transferrin Receptor
Joe B. Harford,Ph.

Z01 HD 01602-01 Regulation of Intracellular Iron Metabolism
Jos van Renswoude, M.D.

Z01 HD 01603-01 Membrane Traffic in Secreting Cells: A Model of Multiple Protein Sorting
Ignacio Sandoval, Ph.D.

NICHD Annual Report
October 1, 1984 to September 30, 1985

Cell Biology and Metabolism Branch

On October 1, 1984 the Cell Biology and Metabolism Branch was formed. This year has witnessed the establishment of this new branch headed by Richard Klausner. The goal of this new branch was to provide a setting for the study of the cell biology of receptors and organelles. This includes a wide array of approaches from electron microscopy to monoclonal antibodies to protein biochemistry to molecular biology. Receptors are a complex set of molecules that mediate virtually all aspects of interactions between the cell and its environment. They mediate cell-cell communication via hormones and neurotransmitters, invasion by viruses, uptake of nutrients, regulation of growth and differentiation and recognition of specific molecules. In addition the branch is studying the development and differentiation of intracellular organelles. This is aimed at understanding the structure and function of secretory organelles, lysosomes and organelles involved in the movement of receptors and other membrane proteins. Finally the studies in the branch on the human transferrin receptor have been extended to an effort to define the details of cellular iron metabolism. Abnormalities in the regulation of normal iron metabolism characterize certain diseases including hereditary hemochromatosis, one of the most common genetic diseases in man. The branch runs a clinic for hereditary hemochromatosis and is studying the molecular basis of this disease in the laboratory.

The establishment of the new branch required a large amount of construction. Building 18 was completely redesigned and its interior rebuilt. The building contains four major laboratory areas, a walk-in cold room, an isolated tissue culture room, a dark room, a microscopy room, an isolated radioiodination room and equipment rooms. A temporary building, 18T, was constructed adjoining building 18 to house offices, storerooms, library, conference space and equipment rooms.

Construction continues with an addition to building 18 which will contain more laboratory space for molecular biology, equipment space, a small animal holding room for rodents and autoclave/washer facility. This space should be completed in October 1985.

Description of research program of the Cell Biology and Metabolism Branch

The research program of the branch is divided into three major areas containing five separate research groups. All groups are currently in the Receptor and Organelle Structure and Function section headed by Richard Klausner.

Area One: Biology of Receptors of the Immune Response. This area contains two groups. One, headed by Richard Klausner is engaged in studying the T cell antigen receptor.

A complete description of the components of the murine T cell antigen receptor complex has been developed based upon the ability to immunoprecipitate the complex using a clonotypic anti-receptor monoclonal antibody developed by L. Samelson. The receptor recognized by this antibody is expressed on a continuously growing hybridoma, 2B4 which recognizes the carboxy terminal fragment of pigeon cytochrome C.

When 2B4 cells are labeled with the hydrophobic radioiodinated covalent probe trifluoromethyl iodophenyl diazarine (TID), four distinct proteins other than the α, β receptor are specifically immunoprecipitated by the A2B4 antibody which recognizes the α-β heterodimer. A variety of other antibodies against T cell surface proteins fail to precipitate this complex. When another T cell hybridoma was labeled with TID and its receptor immunoprecipitated, the same set of associated proteins was observed. The specific co-immunoprecipitation of these proteins from two different cell lines only by the appropriate monoclonal antibodies that recognize the receptor on each of these lines ruled out any interpretation other than that the four proteins are specifically associated with the clonotypic α-β chains of the antigen receptor.

After identifying the four proteins, studies aimed at characterizing them in terms of biochemical properties were undertaken. The four chains have been given Greek letters. The gamma (γ) chain has a molecular weight of 25,000 daltons. Asparagine-linked carbohydrate chains can be removed by treatment with endoglycosaminidase F leaving a peptide of molecular weight 14,000. The delta (δ)chain is also a glycoprotein whose molecular weight drops from 21,000 to 16,000 upon removal of the carbohydrate. The δ chain is a phosphoprotein whose isoelectric point becomes more alkaline after treatment with alkaline phosphatase. Removal of both sialic acid and phosphate reveals a very basic protein with an apparent isoelectric point of greater than 8.5. The epsilon (ε) chain is not a glycoprotein and its molecular weight is 26,000. Interestingly it contains intrachain disulfide bonds and in the absence of reduction its apparent molecular weight is 22,000. The zeta (ζ) chain is also not glycosylated and its molecular weight is 16,000. In the membrane the ζ chain exists as a disulfide linked homodimer. Thus the complete T cell antigen receptor can be described as a seven chain membrane complex composed of six distinct polypeptides.

The addition of specific antigen to the 2B4 T cells results in the rapid phosphorylation of the δ chain of the receptor. This phosphorylation only occurs if the antigen is presented by an appropriate Ia molecule-bearing cell. Furthermore the phosphorylation is inhibited by anti-Ia antibodies which block the activation of the T cell. These T cells can be activated by the mitogen concanavalin A and this also leads to phosphorylation of the δ chain. However, in contrast to antigen this does not require presenting cells and is not blocked by either anti-receptor antibodies or anti-Ia antibodies. The δ chain can also be phosphorylated by the addition of calcium ionophores in the presence of calcium. The antigen-induced phosphorylation of the δ chain occurs on serine residues. Furthermore at least two distinct sites are phosphorylated in response to antigen stimulation.

The δ chain can also be phosphorylated in cells by the addition of phorbol esters such as PMA. The level of phosphorylation in a population of cells is higher than can be achieved by antigen and presenting cells are not required. There is no evidence for additional sites on the δ chain being phorphorylated by PMA and in fact only one of the two sites phosphorylated by antigen is affected by phorbol esters. Also in contrast to the antigen induced phosphorylation, PMA leads to phosphorylation of the ε chain to nearly the same extent as the δ chain. The residue phosphorylated in response to PMA is serine.

Because PMA leads to the phosphorylation of the δ chain the activation of protein kinase C by endogenous diacylglycerol in response to antigen stimulation was examined. Antigen, only in the presence of an appropriate presenting cell, leads to the breakdown of phosphatidyl inositol in the T cells as measured by the production of water soluble inositol phosphates. The production of these products must arise from the action of a phospholipase C which liberates diacylglycerol along with the lipid base. Furthermore the stimulated metabolism of phosphatidyl inositol can be specifically inhibited by dibutyryl cyclic AMP. Consistent with the role of lipid metabolism in the phosphorylation is the inhibition of the antigen induced δ chain phosphorylation by cyclic AMP. Cyclic AMP fails to inhibit the δ chain phosphorylation induced by PMA or calcium ionophore.

The coupling of antigen-activated receptor to phospholipid metabolism is being analyzed to determine which enzyme or enzymes are directly activated in this process. Data have been obtained which support the activation of a phospholipid kinase as the proximal step in T cell activation. An in vitro cell free assay for this kinase has been developed. It is a magnesium requiring integral membrane protein that utilizes ATP and phosphatidyl inositol to produce monophosphophosphatidyl inositol.

The second group studying receptors of the immune response is headed by Warren Leonard. Warren Leonard recently jouned the branch. He is establishing his own research group studying the mechanism of action and regulation of T cell growth factor (IL-2) receptors. This group adds to the branch's program on the molecular basis of receptor function in immune activation.

While in the National Cancer Institute, Dr. Leonard cloned, sequenced, and expressed cDNAs encoding the human interleukin-2 (IL-2) receptor. More recently, he identified genomic phage clones spanning most of the IL-2 receptor gene, and has determined the map of the gene, the sequence of the exons and exon/intron splice junctions, and the location of two of the three IL-2 receptor promoters. Dr. Leonard's initial investigations are examining the following:

1. Determining the difference between high and low affinity IL-2 receptors. Only the high affinity IL-2 receptor appears to be biologically active. When the IL-2 receptor cDNA is transfected into mouse L cells, only low affinity receptors are expressed. The leading hypothesis for the

difference is that associated subunit(s) must be present to form a high affinity receptor, although the possibility of a second gene cannot be excluded. Studies will focus on (a) expressing the IL-2 receptor cDNA in T cells to determine in hibh affinity binding is achieved, and (b) performing chemical cross-linking experiments with ^{125}I-IL-2 and precipitations with both anti-IL-2 and anti-IL-2 receptor antibodies to identify other subunits. Preliminary experiments are consistent with either the existence of associated subunits or with the ability of more than one IL-2 molecule to bind to a receptor molecule.

2. Making use of the promoter regions of the gene to search for DNA binding proteins. Pilot studies will be performed using the methods of Wu (exonuclease III) and of Emerson and Felsenfeld (essentially a Western blotting methodology).

3. In a collaborative study, the promoter regions are also being studied by Dr. Uli Siebenlist, who is evaluating DNaseI hypersensitivity.

4. Looking for potential enhancer elements. Two different DNA fragments that have their 3' termini near the 5' end of the cDNA and extend different distances 5' have been ligated to the chloramphenicol acetyltransferase gene. These constructs differ in their apparent promoter activity in CAT assays, suggesting that the longer construct includes critical DNA regions for optimal promoter function. Attempts will be made to identify the critical regions.

Area Two: The second area of research focuses on the cell and molecular biology of iron metabolism. Two groups work in this area. One, directed by Joe Harford, studies the human transferrin receptor.

Treatment of K562 cells, a human erythroleukemia cell line with the iron chelator desferrioxamine raised the levels of the transferrin receptor approximately 3-fold over that of control cells. In contrast, the levels of the receptor were reduced significantly by provision of exogenous iron to cells in the form of diferric human transferrin, ferric ammonium citrate, or hemin. Using metabolic pulse labeling of cells with [^{35}S]-methionine, receptor-specific immunoprecipitation, and SDS-polyacrylamide gel electrophoresis the rate of biosynthesis of the transferrin receptor was found to be elevated by desferrioxamine and reduced by iron provision. None of these treatments had any effect on the rate of transferrin receptor degradation. The alteration in receptor biosynthesis were correlated with corresponding changes in the level of receptor-specific mRNA. This was determined utilizing a cDNA probe for the receptor to probe Northern blots of RNA preparations from cells under various states of iron availability.

Hemin was found to be a particularly potent modulator of receptor biosynthesis. It had been suggested by others that hemin directly affected receptor expression by a mechanism other than supplying iron to the regulatory iron pool. By performing crossed concentration dependence experiments with hemin and desferrioxamine, this model was shown to be

erroneous. The effect of hemin on receptor biosynthesis proved to be fully attributable to the ability of hemin to supply chelatable iron.

To determine whether receptor mRNA levels were altered via changes in the transcription rate, nuclei were isolated and in vitro transcription experiments performed. Nuclei from cells treated with hemin were compared with those from desferrioxamine-treated cells. Transcribed RNA was labeled with [α-^{32}P] uridine and the mRNA encoding the transferrin receptor was selected by hybridization to an immobilized receptor cDNA clone. These experiments indicated a significantly higher rate of receptor mRNA synthesis in desferrioxamine-treated cells than in cells treated with hemin. Manipulations of intracellular iron result in greater than 100 fold differences in receptor mRNA. The half-life of this specific mRNA is quite short, being less than 30 minutes in K562 cells.

Promotor activity is present in an isolated region of the gene containing approximately 120 base pairs upstream of the transcription initiation site. This is demonstrated by expression of the human receptor after transfection of mouse L cells using genomic DNA for the receptor that contains the 5' upstream region. However, this expressed gene fails to demonstrate regulation by iron. Furthermore, when this promotor is attached to the bacterial enzyme chloramphenicol acetyl transferase it will support the transcription of the enzyme in short term transfection assays. Again no regulation of this expression by iron is seen.

Treatment of K562 cells with a monoclonal antibody (OKT9) against the transferrin receptor has marked effects on receptor dynamics and the survival of the receptor. The antibody employed recognizes a receptor determinant distinct from the ligand binding site. OKT9 treatment leads to a rapid ($T\tau$ < 10 min) redistribution of receptor such that a lower percentage of the receptors involved in iron uptake are displayed on the cell surface. On a somewhat longer time scale the number of receptors in the "cycling pool" is reduced. Radiolabeling of K562 cells with either [^{35}S]-methionine or by lactoperoxidase-catalyzed surface iodination allowed the half-life for the transferrin receptor to be assessed. Treatment with OKT9 resulted in a decrease in this parameter from 8 hr to 3 hr. Although transferrin receptor turnover is unaffected by ligand the antibody treatment results in a "down-regulation" of the receptor analogous to ligand-mediated down regulation observed in other receptor systems such as those for insulin or epidermal growth factor. Based on continued iron uptake from [^{59}Fe] transferrin, it appears that receptors with bound OKT9 continue to traverse the transferrin cycle. The profound effect on receptor half-life is apparently accomplished by a relatively subtle decrease in recycling efficiency per cycle from 98% to 95%.

The second group studying iron metabolism has been headed by Jos van Renswoude, a visiting scientist in CBMB. This group has focused on the intracellular aspects of iron metabolism.

A substantial fraction of iron, delivered to the cell via the transferrin cycle, ends up in ferritin. The remainder is distributed over a variety of intracellular targets, e.g. heme proteins, iron-sulfur centers, etc. The regulation of the distribution of newly delivered iron between storage/detoxication (ferritin) and utilization (e.g. heme synthesis) compartments has been studied using various perturbants of cellular iron homeostasis. These include: diferric transferrin at saturating concentrations, ferric protoporphyrin IX (hemin), the cell-permeant iron chelator, desferrioxamine, and the monoclonal anti-transferrin receptor antibody, OKT9. It was found that the fractional distribution of ^{59}Fe to ferritin ranges from 0.05 to 0.7 and that it is strictly correlated with the size of the total intracellular ferritin pool. When the cellular ferritin levels are high a relatively high percentage of the newly delivered iron will go to ferritin. When ferritin levels are low, a relatively small fraction will become ferritin-associated. This correlation between ferritin level and fractional delivery of iron to ferritin is formulated as a nomogram, characteristic of the type of cell studied. The distribution of iron to ferritin proved to be independent of the amount of iron taken up by the cell, per unit time. The distribution function therefore has the characteristics of a partition. The first implication of this finding is that the fractional delivery of iron to ferritin in K562 cells (growing exponentially in culture) can simply be predicted from the ferritin level at the time of iron entry. Second, this partition drives the distribution of intracellular iron that may serve to maintain cellular iron homeostasis vis-a-vis utilization processes such as the biosynthesis of iron-containing proteins. Ferritin levels reflect the iron status of the cell. Sustained iron supply (diferric transferrin, hemin) leads to an increase in cellular ferritin through enhanced biosynthesis whereas iron deprivation via chelation of intracellular iron (desferrioxamine) or via reduction of iron input via down-regulation of the transferrin receptor (with OKT9) results in a fall of the intracellular ferritin concentration as a result of decreased biosynthesis. The effects of perturbation of cellular iron homeostasis on ferritin levels are rapid in these cells. The ferritin concentration can vary over a 50-fold range within 36 hours. The half life of K562 cell ferritin is relatively short: 4-5 hours. The degradation of this ferritin is complex and under continuing study. Ferritin degradation is characterized by an obligatory lag period before degradation is started, and there are preliminary data suggesting that the light and the heavy subunit turnover at different rates. The lag period may represent a set of chemical/physical changes within the ferritin molecule, necessary to allow complete proteolytic breakdown of the molecule. Regulation of the degradation ferritin by different drugs has been observed and these act at the level of the lag phase.

The above mentioned relationship between fractional delivery of iron to ferritin and existing ferritin levels in K562 cells bears a striking similarity to the "rule" which governs intestinal iron absorption: The percentage of an oral iron load that is absorbed, i.e., goes into the circulation, is qualitatively inversely proportional to the total body iron stores and hence to cellular ferritin levels. The implication of this

similarity is that intestinal iron absorption defects might be studied using model systems, such as K562.

Area Three: Ignacio Sandoval, a visiting scientist in CBMB, directs a group studying the origin, dynamics and function of intracellular organelles. They are specifically interested in proteins that make up the membranes of the Golgi, lysosomes and secretory vesicles.

We have obtained specific monoclonal antibodies against integral membrane proteins from secretory granules to study the traffic of these organelles in secreting cells. The traffic of secretory granules most likely results from the sorting of their membrane proteins in different sites of the cell. One of these monoclonal antibodies, called 5G10, has been extensively used for this purpose in studies carried on in RBL cells. The antibody recognizes an 80 kdalton membrane glycoprotein in RBL cells metabolically labeled with [^{35}S] methionine. In non-secreting cells the protein is exclusively located in the membrane of secretory granules, as shown by their reaction with granules stained for the secretory product heparin with Wright's reagent. Furthermore, the exposure of the 5G10 epitope on the cell surface during secretion has confirmed the localization of the 5G10 antigen in the membrane of secretory granules, and has allowed us to study the insertion of these membranes into the plasma membrane during the release of secretory products by exocytosis. The correlation between membrane insertion and secretion has been demonstrated by the increase in the levels of 5G10 antigen expressed on the surface of cells stimulated to secrete with either DNP-BSA (i.e. allergen) activated IgE or the Ca^{++} ionophore A23187, as well as by the failure of irrelevant IgE to stimulate the surface expression of the antigen and the interruption of that expression following the dissociation of the DNP-BSA-anti DNP IgE with DNP lysine. Furthermore, the insertion of the membranes from secretory granules into the plasma membrane is a very fast process, as shown by the expression of maximal levels of 5G10 antigen on the cell surface only 5 minutes after the stimulation of secretion.

The regulated secretion displayed by RBL cells has also allowed us to study the retrieval of the inserted membranes independently from insertion. We have calculated from the rate of surface disappearance of the antigen, measured in cells whose secretion was stopped, that its half-life in the plasma membrane is about 10 minutes. That the disappearance of surface 5G10 antigen is due to internalization has been directly shown by measuring the internalization of iodinated 5G10 antibody by secreting RBL cells. Interestingly this rate is comparable to that of surface ligands internalized by receptor-mediated endocytosis. Monitoring of cells continuously stimulated to secrete has revealed that the expression of the 5G10 antigen on the cell surface is sustained for 45 min after which it declines slowly. This sustained expression probably results from the continuous insertion of the membranes from secretory granules into the plasma membrane and their rapid removal by endocytosis. This membrane retrieval is most likely carried on by the cell to maintain the constancy of the chemical composition of the plasma membrane.

Immunoelectron microscopy studies, using antibody 5G10, have revealed that the internalization of the membranes of secretory granules is initiated via coated-pits. Furthermore, immunofluorescence microscopy studies show that the internalized antigen stays briefly in vesicular organelles located in the cell periphery, probably in endosomes, before moving to the area containing the Golgi apparatus and is finally found in secretory granules. Study of the transit of the antigen through the Golgi apparatus, a step that could be required to repair and reprogram the protein, is being performed by analyzing the glycosylation of deglycosylated antibody internalized by endocytosis. The recycling of the 5G10 antigen back to secretory granules seems to be a very effective process, as no degradation of the radioiodinated 5G10 antibody bound and internalized with the antigen has been detected. Electron microscopy experiments are being carried out to characterize each one of the organelles traversed by the 5G10 antigen in its route from the plasma membrane to secretory granules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01600-01 CBMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Structure and Function of the Murine T Cell Antigen Receptor

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

PI:	R. D. Klausner	Head	CBMB, NICHD
Others:	L. E. Samelson	Senior Staff Fellow	CBMB, NICHD
	A. M. Weissman	Medical Staff Fellow	CBMB, NICHD
	J. J. O'Shea	Senior Staff Fellow	CBMB, NICHD
	J. B. Harford	Senior Investigator	CBMB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.99	3.99	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 receptor on murine T cells that recognizes antigen presented by a specific Ia-bearing presenting cell is being studied in order to understand the mechanism of specific immune activation. The approaches used are based on the isolation, identification, characterization and purification of the components of this receptor. In addition biochemical studies are aimed at identifying the mechanisms whereby occupancy of this receptor transduces a signal to activate the T cell.

Using an antigen specific T cell hybridoma that recognizes a fragment of pigeon cytochrome C we have demonstrated that occupancy of the receptor in a manner that leads to cell activation results in the phosphorylation of a protein specifically associated with the T cell receptor. Furthermore, the interaction of T cell, antigen and presenting cells leads to increased levels of phosphorylated phosphatidylinositol. In addition enhanced breakdown of these phosphorylated lipids leads to the production of water soluble inositol phosphates via a phosphodiesterase.

By immunoprecipitating the antigen receptor with a monoclonal antibody directed against the unique binding site of the receptor, we have identified a specific set of proteins which are non-covalently associated with the clonotypic α and β chains, that alone previously defined the receptor. Four unique proteins, termed γ, δ, ε, & ζ comprise this complex. They are all expressed on the surface of the T cell and are most likely all transmembrane proteins and one is a homodimer. Thus the minimum complex that defines the T cell antigen receptor contains seven chains including the α-β dimer that recognizes specific antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01601-01 CBMB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Aspects of the Regulation of the Human Transferrin Receptor

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

PI:	J. B. Harford	Senior Investigator	CBMB, NICHD
Others:	R. D. Klausner	Head	CBMB, NICHD
	K.K. Rao	Visiting Associate	CBMB, NICHD
	A. M. Weissman	Medical Staff Fellow	CBMB, NICHD
	T.A. Rouault	Medical Staff Fellow	CBMB, NICHD
	J. Casey	Guest Worker	CBMB, NICHD
	B. Di Jeso	Visiting Fellow	CBMB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	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.)*

Iron is taken into proliferating cells via a high affinity receptor for the serum iron carrying protein transferrin. The transferrin receptor in a human erythroleukemia cell line is being studied as an integral membrane protein whose synthesis, degradation, and dynamics are highly regulated. The expression of the transferrin receptor has been shown to be modulated by the availability of iron in that provision of iron to the cells results in decreased receptor expression whereas chelation of intracellular iron causes augmented expression. The mechanism underlying these phenomena has been investigated by comparing directly the rates of biosynthesis and degradation of the transferrin receptor under conditions of varied iron availability. These studies indicated that receptor expression is modulated by alterations in the rate of biosynthesis by the receptor. Using a cDNA probe for the receptor, it was found that changes in biosynthetic rate are a reflection of altered levels of receptor mRNA. In vitro transcription experiments with isolated nuclei showed that the receptor mRNA is more actively transcribed in nuclei from iron-depleted cells than in those from cells grown in an efficient iron source.

Monoclonal antibodies to the transferrin receptor also modulate receptor expression. The effect of the antibodies on receptor degradation biosynthesis and dynamics have been investigated. It was found that exposure of cells to anti-receptor monoclonal antibodies results in a rapid redistribution of cellular transferrin receptors such that a lower percentage are expressed on the cell surface. This treatment also leads to enhanced degradation of the receptor and a resultant alteration in receptor biosynthesis that is due to iron deprivation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01602-01 CBMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Regulation of Intracellular Iron Metabolism

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

PI:	J. van Renswoude	Visiting Associate	CBMB,NICHD
Others:	E. Mattia	Visiting Associate	CBMB, NICHD
	R. D. Klausner	Head	CBMB, NICHD

COOPERATING UNITS *(if any)*

G. G. Ashwell	Institute Scientist	LBM, NIADDK
Dj. Josic	Guest Researcher (Oct. 1, 1984 - Apr. 1, 1985)	LBM, NIADDK

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	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 project is aimed at: 1. elucidating the pathways of intracellular iron traffic and the molecular mechanisms involved in the regulation of intracellular iron metabolism, and 2. identifying and characterizing the molecular defect(s) underlying the expression of hereditary hemochromatosis. As a logical sequel to previous work in which the physiologic process by which iron is delivered to cells, the transferrin cycle, was characterized in K562 human erythroleukemia cells, a study was undertaken to gain insight into the intracellular distribution of iron between storage and utilization pools and to understand how this distribution is regulated. It was found, in K562 cells, that the fractional delivery of iron to ferritin (storage compartment) is strictly and directly correlated with the intracellular ferritin level. The relationship between ferritin levels and fractional delivery of iron to ferritin has been formulated as a nomogram, and has the characteristics of a partition. It is assumed, as a working hypothesis, that this partition serves to maintain cellular iron homeostasis with regard to iron utilization processes. It has further been found that, in K562 cells, the regulation of ferritin levels is highly dynamic in response to changes in iron supply to the cells, with respect to both biosynthesis and degradation. These findings are used to study whether cells from patients with hereditary hemochromatosis are different from normal cells with respect to the way they regulate their intracellular iron distribution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01603-01 CBMB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Membrane Traffic in Secreting Cells: a Model of Multiple Protein Sorting

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

PI:	I. V. Sandoval	Visiting Scientist	CBMB, NICHD
Others:	R. D. Klausner	Head	CBMB, NICHD
	J. S. Bonifacino	Visiting Associate	CBMB, NICHD
	C. Suarez-Quian	Guest Worker	CBMB, NICHD
	J. G. Barriocanal	Guest Worker	CBMB, NICHD
	L. C. Yuan	Chemist	CBMB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, 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.)*

The membranes of secretory granules are inserted into the plasma membrane following the release of secretory products by exocytosis. A monoclonal antibody, 5G10, has been raised which recognizes the membranes of secretory and Golgi-derived vesicles. Fusion of secretory granules with the plasma membrane can be followed by the appearance of the 5G10 antigen on the cell surface. The process is fast and maximal levels of the 5G10 antigen, a 80 kdalton glycoprotein component of the membrane of secretory granules, are measured in the cell surface by five minutes after stimulation of secretion in rat basophilic leukemia cells. The 5G10 antigen is rapidly and efficiently internalized after insertion in the cell surface by endocytosis. The half-life of this internalization is about 10 minutes. The endocytosis of the membrane components of secretory granules allows the cell to maintain a constant chemical composition of the plasma membrane. Endocytosis of the 5G10 antigen inserted into the membrane is initiated via coated pits. The internalized antigen after transiting through the Golgi is found again in secretory granules. The antigen remains there until specific secretion is stimulated.

Z01 HD 00610-05 Puberty and its Disorders: Physiology, Pathophysiology and Therapy
Gordon B. Cutler, Jr., M.D.

Z01 HD 00613-05 Clinical and Basic Studies of Male Reproduction
Richard J. Sherins, M.D.

Z01 HD 00614-05 Biology of Hormone Binding Proteins
Bruce C. Nisula, M.D.

Z01 HD 00615-05 Steroid Antagonists
George P. Chrousos, M.D.

Z01 HD 00616-05 Structure, Function, and Physiology of Glycoprotein Hormones
Bruce C. Nisula, M.D.

Z01 HD 00618-04 Physiology and Clinical Applications of Corticotropin Releasing Hormone
George B. Chrousos, M.D.

Z01 HD 00619-04 Hypothalamic-Pituitary-Gonadal Interaction
D. Lynn Loriaux, M.D.

Z01 HD 00621-03 Mechanism of Linear Growth
Fernando Cassorla, M.D.

Z01 HD 00622-03 Diagnostic and Therapeutic Applications of Growth Hormone Releasing Factors
George R. Merriam, M.D.

Z01 HD 00623-02 Adrenal Physiology and Pathophysiology
Gordon B. Cutler, Jr., M.D.

NICHD Annual Report
October 1, 1984 to September 30, 1985

Developmental Endocrinology Branch

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, development and reproduction. The endocrine system is being studied in fetal and neonatal life, childhood, young adulthood and old age. Most of our current research is focused on the pubertal transition. Systems under study include the hypothalamic pituitary-gonadal axis, the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-thyroid axis, the systems regulating growth, and placental-fetal interactions.

The following summary will highlight the past years research accomplishments. It is not intended to be a complete compendium of the year's research activities. This can be obtained from the individual project reports.

1 - Studies on Growth -

The primary focus of our current studies on growth is to understand the relative roles of the steroid hormones and the various growth factors in regulating skeletal growth and epiphysial maturation.

The role of the somatomedins in linear growth and epiphyseal maturation is controversial. Precocious puberty, with its early growth acceleration, provides an experiment of nature in which the endocrine concommittants of growth can be examined. We compared somatomedin C (SmC) levels in 41 children with precocious puberty, 87 age-matched controls, and 110 normal pubertal children. SmC levels were significantly greater in patients with precocious puberty when compared to age-matched controls. SmC levels correlated with pubertal stage in both normal children and children with precocious puberty. The treatment of children with precocious puberty with the LHRH analog, D-Trp^{6}-Pro^{9}-Net-LHRH (4 ug/kg/d), decreased both the growth rate and the SmC levels. The decrease in growth rate was about 50%, while the decrease in SmC was only about 5%. Changes in growth correlated strongly with changes in plasma sex steroid concentrations, but weakly with changes in SmC. These findings suggest that SmC is not the principle modulator of pubertal growth. Our current working hypothesis is that SmC plays a permissive role in this process.

The role of the steroid hormones in skeletal growth has been examined in several studies. We have previously demonstrated that dose of ethinyl estradiol giving optimal growth in girls with Turner's syndrome was 100 ng/kg/d. This dose is considerably lower than the currently recommended dose. Our studies, however, were of short duration. We have now examined whether or not this growth stimulation is sustained during a 6 month treatment period and whether or not the bone age is advanced at an accelerated rate. We randomly assigned 16 girls with Turner's syndrome, age 5 to 15 years, to a 6 month treatment period with 100 ng/kg/d of ethinyl estradiol or placebo. We measured lower leg growth rate, height, and bone age. Ethinyl estradiol at the dose of 100 ng/kg/d for 6 months produced sustained growth without undue bone age advancement. This finding suggests that ultimate stature in patients with turners syndrome may be improved with low dose estrogen treatment and that the currently popular regimen of almost 10 times the dose we employed may actually be harmful and reduce ultimate height. If so, this represents an important advance in the treatment of this disorder.

In the last year it was found that human growth hormone may be contaminated with the infectious agent for Jacob-Kreutzfeld disease. As a result, human growth hormone is no longer available for clinical use. This lends urgency to studies attempting to identify treatment alternatives for children with short stature.

Many children with growth hormone deficiency respond to a single dose of growth hormone-releasing hormone (GHRH) with an acceleration in growth. This suggests that repeated doses of GHRH might be used to treat growth hormone deficiency. To examine the effects of GHRH on growth, the response rate, and what factors might predict response, we administered GHRH, 1 {g/kg every 3 h for 10 days, to 8 children with growth hormone deficiency. Five of these patients were also studied during a placebo injection period. GHRH stimulated growth in 4 children. These four had elevations of growth hormone in response to GHRH and a variable rise in somatomedin-C. In these four, GHRH accelerated growth more than human growth hormone given at the standard recommended dose. The growth response to GHRH seemed to correlate inversely with bone age and directly with the growth hormone rise after GHRH. We conclude that GHRH may be an effective alternative to growth hormone in selected patients with
growth hormone deficiency.

Studies on Reproduction:

New insights relevant to the reproductive process center about findings in infertile men and the demonstration of a potential new contraceptive for women.

The commonest cause of male infertility is idiopathic. The sperm appear normal in number, morphology, and activity. They are, however, unable to fertilize an ovum. Recent work suggests that proteins coating the head of sperm serve as the recogni tion site for the sperm-egg interaction. Several of these proteins, of epididymal origin, have been isolated by Dr. Jorge Blaquier (a guest worker from Buenos Aires, Argentina). He has made polyclonal antibodies against these proteins and can now map the distribution of several of these proteins on spermatozoa. We have found that, in a high proportion of men with idiopathic infertility, the distribution of these proteins over the sperm head is abnormal. The abnormality can be qualititive or quantitative. This finding may be important in the effort to clarify the mechanism underlying idiopathic male infertility and suggests an approach to rational therapy.

Effective contraception continues to be a problem of world wide importance. Current methods are either incompletely effective or carry a significant health risk. Theoretically, an antagonist of progesterone should be effective as a contraceptive agent. We have examined such a compound for safety and efficacy.

RU486, a newly developed antiprogestin supplied by Roussel-UCLAF, was examined for toxicity in a man with adrenal cancer. 20 mg/kg/day for 12 weeks yielded no evidence of toxicity. 5 mg/kg is completely effective as an antiprogestin. When given as a single 5 mg dose, menses was induced in all women in the luteal phase of the cycle. This finding strongly supports the antiprogestational action of this drug. A single monthly dose of RU 486, 5 mg/kg, prevented pregnancy in a group of 20 fertile rhesus monkeys. A placebo treated group had 15 pregnancies in the same period. Thus, this compound holds promise as a once a month contraceptive agent that appears to be of very low toxicity. These findings also suggest that this agent may serve as the long sought after 'day after' contraceptive agent.

Studies on the Hypothalamic-Pituitary-Adrenal Axis.

Studies in the past year have centered about the use of CRF in the differential diagnosis of Cushing's syndrome. A prospective comparison of CRF with the standard dexamethasone suppression test showed the CRF test to be quicker, simpler and less expensive than the dexamethasone suppression test. The sensitivity and specificity of the test was as good as or better than the standard dexamethasone suppression test. It was effecient in differentiating ACTH dependent Cushing's syndrome from ACTH independent Cushing's syndrome as well as being able to reliably separate patients with the ectopic ACTH syndrome from those with ACTH secreting pituitary microadenomas. CRH also has proven useful in clarifying the pathophysiology of the hypercortisolism associated with psychiatric disorders. The response to CRH administration in these disorders is blunted compared to the response in both normal subjects and patients with Cushing's disease. These findings support the notion that the hypercortisolism in Cushing's syndrome is CRF independent while that associated with psychiatric disorders is CRF dependent. This finding suggests that a test might be devised that will differentiate the hypercortisolism of psychiatric disorders from that of ACTH dependent Cushing's syndrome early in the course of either disorder.

The role of glucocorticoids in allowing man to tolerate stress has been reexamined. Current dogma has it that man requires increased plasma concentrations of cortisol to sustain severe stress such as surgery or systemic infection. No biochemical basis for this need, however, has been identified. We performed an experiment in which adrenalectomized cynomolgus macaque monkeys were subjected to surgical stress at varying levels of glucocorticoid replacement. It was found that no advantage was gained by replacement at doses greater than the basal secretory rate of cortisol in this species. This suggests that the increased cortisol levels may be an epiphemenon related to the stress response. The increased cortisol production may reflect increased endogenous opiate production, for example, as the primary event.

Studies of the Hypothalamic-Pituitary-Gonadal Axis.

Studies over the past year have centered on developing rational therapies for the less common causes of isosexual precocious puberty. Both the McCune Albright syndrome and familial male isosexual precocious puberty have been found to be independent of gonadotropin secretion. Hence, LHRH analogue therapy is ineffective in these patients. We have shown that the aromatase inhibitor, testolactone, diminishes plasma estrogen concentrations in patients with the McCune Albright syndrome and slows, as a consequence, the progression of secondary sexual characteristics in these subjects.

The antiandrogen spironolactone has been used to treat boys with familial male precocious puberty. this approach has been effective in halting the progression of male sexual characteristics, but has been ineffective in slowing the accelerated rate of growth characteristic of the condition.

Interestingly, the addition of the aromatase inhibitor testolactone slowed the accelerated growth. These findings point to the importance of estradiol for the pubertal growth spurt in both girls and boys.

Studies of fetal-placental physiology.

The objective of this study has been to understand the basic endocrinology of the glycoprotein hormones. Hormones under study include hCG, LH, FSH, and TSH. Studies in the last year have emphasized work on the hCG molecule. Abnormal forms of hCG and their metabolites are of clinical interest as molecular markers of the malignant transformation of the placenta (trophoblast). Previous work conducted under this project demonstrated that patients with gestational trophoblastic neoplasms (cancer arising from the placenta) frequently excrete into their urine forms of hCG deficient in sialic acid as well as carboxyterminal fragments of the hCG β-subunit. Neither of these unusual molecules is excreted in the urine of health pregnant women. The occurrence of caboxyterminal peptide fragments in association with hCG forms bearing an altered carbohydrate structure suggested a precursor-product relationship. To address this hypothesis, we gave healthy human subjects infusions of either native hCG or desialylated hCG and analyzed their urines for carboxyterminal fragments. Subjects given desialylated hCG, but not those given native hCG, excreted carboxyterminal fragments in urine. These results indicate the existence of a peripheral metabolic pathway that cleaves carboxyterminal fragments from desialylated hCG and allows their excretion in urine. Thus, the frequent presence of carboxyterminal peptide fragments in the urine of patients with gestational trophoblastic neoplasia can be accounted for in part, if not entirely, by the peripheral metabolism of forms of hCG bearing an abnormal carbohydrate structure.

As indicated above, forms of hCG deficient in sialic acid are prevalent in the urine of patients with gestational trophoblastic neoplasia, but not in subjects infused with native hCG or in healthy pregnant women. It has been widely believed that serum glycoproteins, like hCG, are principally metabolized via a pathway in which desialylation is the initial step. Once desialylated, such glycoproteins would be taken up by hepatic receptors for galactoseterminated glycoproteins. Ashwell and Morell were the first to show that removal of sialic acid from the carbohydrate chains of glycoproteins drastically reduces their survival in the circulation and showed that this was due to accelerated uptake by receptors located on hepatocytes. Our observation suggested that desialylation may not play a significant role in the normal metabolism of sialylated serum glycoproteins in vivo. To examine this question, we studied the metabolism of hCG in rats. Rats were given infusions of either native hCG or desialylated hCG with or without desialylated fetuin which blocks hepatic receptors for galactose-terminated glycoproteins. Blockade of the hepatic receptors did not impede hCG turnover in the circulation, impair hepatic uptake or catabolism of hCG, or lead to the accumulation of desialylated products of hCG in plasma. These findings demonstrate that there is negligible catabolism of glycoproteins, such as hCG, via a pathway that involves peripheral desialylation and subsequent uptake by hepatic receptors for galactose-terminated glycoproteins. These results have impact in two areas. First, the role of the hepatic receptors for galactose-terminated glycoproteins needs to be reexamined. Their role, in the rat at least does not appear to be the disposal of sialylated circulating glycoproteins of the CG-type in the disposal of secreted forms of glycoproteins that are incompletely sialylated or desialylated intracellularly prior to secretion. This construct gives new insight into the meaning of the prevalence of desialylated hCG in gestational trophoblastic neoplasia. They imply that the abnormal carbohydrate structure of hCG reflects abnormal glycosylation mechanisms in malignant trophoblastic tissue, a notion previously suggested by studies of choriocarcinoma cells in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00610-05 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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	6.0	.5

CHECK APPROPRIATE BOX(ES)

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

X ☐ (a1) Minors

X ☐ (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, studies of the mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the recognition and treatment of secondary causes of central precocious puberty, the treatment of the McCune-Albright syndrome with an aromatase inhibitor, and the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor.

Others:	D. L. Loriaux	Head, 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
	P. Feuillan	Med. Staff Fellow	DEB, NICHD
	L. Laue	Med. Staff Fellow	DEB, NICHD
	J. Levine Ross	Guest Worker	DEB, NICHD
	O. Pescovitz	Med. Staff Fellow	DEB, NICHD
	S. Rose	Med. Staff Fellow	DEB, NICHD
	M. Uriarte	Guest Worker	DEB, NICHD
	J. Winterer	Clinical Associate	DEB, NICHD

Cooperating Units

LDP, National Institute of Mental Health (E. Susman, E. Nottelmann, G. Inoff, L. Dorn, 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 (R. Klein); Clin Center, NIH (M. Skerda, A. McNemar, K. Hench, A. Dwyer, T. Shawker; Department of Pediatrics, Yale Univ. (F. Comite); Department of Obstetrics and Gynecology, SUNY at Buffalo (A. Munabi); Department of Child Psychiatry, University of Minnesota (W. Sonis); Department of Pediatrics, University of Michigan (C. Foster); Department of Obstetrics and Gynecology, SUNY at Stony Brook (D. Kenigsberg); Department of Radiology, Fairfax Hospital (K. Rieth); National Institute of Dental Research (M. Roberts); Department of Pediatrics, Johns Hopkins University (C. Van Dop); Birth Defects Branch, Center for Disease Control (G. Oakley).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00613-05 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Clinical and Basic Studies of Male Reproduction

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

PI: R. J. Sherins Head DEB, NICHD

Others: (see attached list)

COOPERATING UNITS *(if any)*

(see attached list)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Reproductive Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 stimulation of androgen production alone in men with partial panhypopituitarism or pituitary tumors may be sufficient to induce fertility 2) that enyme deficiency within sperm correlates highly with deficient motility of sperm from infertile men 3) that the sperm of some infertile men show absence of specific epididymal proteins required for sperm-egg fusion 4) that cerebral glucose metabolism is altered in hypogonadal subjects 5) that sex steroids regulate gondotropin secretion from the pituitary primarily by modulating recruitment of new gonadotropes 6) that GnRH modultes glycosylation of LH subunits and 7) that prolactin secretion is markedly enhanced and parallels the high FSH levels induced by decreased androgen and increased estrogen.

Others:	D.L. Loriaux	Head, SSH	DEB, NICHD
	G.B. Cutler	Senior Investigator	DEB, NICHD
	B.C. Nisula	Senior Investigator	DEB, NICHD
	G.R. Merriam	Junior Investigator	DEB, NICHD
	D.L. Vogel	Guest Worker	DEB, NICHD
	L. Liu	Medical Staff Fellow	DEB, NICHD
	G. Daniel	Medical Staff Fellow	DEB, NICHD
	J. Winterer	Medical Staff Fellow	DEB, NICHD
	S. Rose	Medical Staff Fellow	DEB, NICHD
	J. Blaquier	Serono Fellow	DEB, NICHD
	J.D. Booth	Guest Researcher	DEB, NICHD
	Y.F. Shi	Guest Researcher	DEB, NICHD
	C. Gagnon	Guest Researcher	DEB, NICHD
	E. de Lamirande	Guest Researcher	DEB, NICHD

Cooperating Units:

B.D. Weintraub	CEB, NIADDK
C. Eil	Div. Endo., Nat'l Naval Med. Ctr.
T. Kinsella	ROB, NCI
D. Poplack	POB, NCI
M. Lippman	DCT, NCI
R. Makuch	DMB, NCI
C. Gagnon	Dept. Urol., McGill Univ., CANADA
R. Fischell	A.P.L., Johns Hopkins Univ.
D. Jimerson	LCS, NIMH

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00614-05 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
	A. Lynch	Bio. Lab. Tech.	DEB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Marylad 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The broad objective of this project is to understand the biology of serum hormone transport proteins and the role that they play in human disease. Recent research findings include: (1) The elucidation of the relative roles of plasma testosterone bound to albumin and that bound to sex-hormone binding globulin in determining testosterone bioavailability to the tissues, and (2) the application of sex-hormone binding globulin as a biochemical index of thyroid hormone action to demonstrate the maintenance of a euthyroid state during physical conditioning in humans. Future investigations under this project will delineate the role of human adrenal function in modulating the plasma level of corticosteroid-binding globulin and explore pharmacological and pathological factors that modulate serum hormone transport proteins. Effort will also be placed on development of an in vitro system for elucidation of the mechanisms of modulation of intracellular hormone levels by plasma constituents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00615-05 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	D. D. Brandon	Chemist	DEB, NICHD
	G. B. Cutler, Jr	Head, DES	DEB, NICHD
	S. Kawai	Visiting Fellow	DEB, NICHD
	D. L. Loriaux	Head, SSH	DEB, NICHD
	G. Merriam	Investigator	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	R. Udelsman	Medical Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

Division of Veterinary Resources (M. Morin)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Hypothalamic Releasing Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	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.)*

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

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

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

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00616-05 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
	L. Liu	Med. Staff Fellow	DEB, NICHD
	A. Lynch	Bio. Lab. Tech.	DEB, NICHD
	S. Rose	Med. Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

Epidemiology Branch, NIEHS, NIH (A. Wilcox)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.8	.9

CHECK APPROPRIATE BOX(ES)

X ☐ (a) 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 goal of this project is to understand the structure, function, and physiology of the human glycoprotein hormones--chorionic gonadotropin, thyrotropin, luteinizing hormone, and follicle-stimulating hormone--and from that knowledge to develop diagnostic and therapeutic clinical applications. Recent research progress includes the following: demonstration of the existence in humans of a peripheral metabolic pathway that cleaves carboxyterminal peptide fragments from desialylated chorionic gonadotropin; quantitation of the kinetic parameters governing the metabolism of desialylated chorionic gonadotropin in humans; characterization of hepatic pathways for catabolism of variably sialylated serum glycoproteins in rats; and establishment of the feasibility of large-scale epidemiologic studies of early pregnancy loss based on measurement of chorionic gonadotropin in urine specimens. Future emphasis of the project will be on the development of clinical applications for ultrasensitive assays for glycoprotein hormones, elucidation of renal mechanisms for catabolism of chorionic gonadotropin and related molecules, assessment of the role of the carbohydrate moiety of thyrotropin in adenylate cyclase activation, and characterization of a naturally occurring inhibitor of the ovarian response to follicle-stimulating hormone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00618-04 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Physiology of Stress 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, BPB, NIMH (P. Gold); Clinical Neurosurgery Section, SNB, NINCDS (E. Oldfield); Laboratory of Developmental Psychology, NIMH (E. Sussman, E. Nottelman, G. Inoff).

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Hypothalamic Releasing Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	3.6	0.2

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

In this project we seek to advance understanding of the endocrine mechanisms of stress and 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 relationships 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 pulselike 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. Administration of hCRH in a pulsatile fashion restores the secretory pattern of cortisol in patients with hypothalamic adrenal insufficiency.

Others:	P. C. Avgerinos	Guest Researcher	
	G. B. Cutler, Jr.	Head, DES	DEB, NICHD
	W. T. Galluci	Guest Researcher	DEB, NICHD
	S. Kawai	Visiting Fellow	DEB, NICHD
	D. L. Loriaux	Head, SSH	DEB, NICHD
	A. Luger	Visiting Fellow	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	R. Rittmaster	Medical Staff Fellow	DEB, NICHD
	T. Schuermeyer	Visiting Fellow	DEB, NICHD
	T. Tomai	Guest Researcher	DEB, NICHD
	R. Udelsman	Medical Staff Fellow	DEB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00619-04 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Hypothalamic-pituitary-gonadal interactions

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

P.I.	D.L. Loriaux	Head	DEB, NICHD
Others:	G.R. Merriam	Junior Investigator	DEB, NICHD
	L. Nieman	Medical Staff Fellow	DEB, NICHD
	B. Albertson	Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

P. Platia, Medical Staff Fellow, CC, NICHD
Roussel-UCLAF (Dr. E.E. Baulieu), Paris, France

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Steroid Hormones

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.63	1.63	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 the use of a newly developed antiprogestational compound, RU 486, as a potential contraceptive agent in women. Available contraceptive agents for women are either incompletely effective or have hazardous side effects associated with their use. Thus, new agents are constantly being sought. RU 486 is a potent antiprogestational drug that should theoretically interrupt the effect of progesterone on the endometrium and thus block implantation. The feasability of using this drug for this purpose has been examined in the past year. We have established the dose that reliably blocks luteal phase progesterone effects (5 mg/kg) and have shown that, when given on the 26th day of the cycle, the next reproductive cycle is normal. We have used this material prospectively as a contraceptive in a breeding colony of 50 rhesus monkeys. It was completely effective in preventing pregnancy in these animals. Hence, the stage is set for human studies with this promising new agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-03 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
	G. R. Merriam	Investigator	DEB, NICHD
	S. Rose	Medical Staff Fellow	DEB, NICHD
	S. Malozowski	Visiting Fellow	DEB, NICHD
	M. Nicoletti	Visiting Fellow	DEB, NICHD
	D. L. Loriaux	Head, SSH	DEB, NICHD

COOPERATING UNITS *(if any)*

Clinical Center, NIH (M. Skerda); Metabolism Branch, NCI (M. Gelato) Catholic University of Nijmegen, The Netherlands (I. M. Valk); Hahnemann Medical School, Philadelphia, Pennsylvania (J. L. Ross);

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	0

CHECK APPROPRIATE BOX(ES)

☒ (a) 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. We are also investigating the effects of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature through a randomized, double blind, placebo-controlled clinical trial. Finally, we are studying the effects of growth hormone-releasing factor on linear growth in growth hormone deficient children by using different treatment regimens in order to optimize growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00622-03 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormone

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

PI:	George R. Merriam	Junior Investigator	DEB, NICHD
Others:	F. Cassorla	Visiting Associate	DEB, NICHD
	M. Gelato	Medical Staff Fellow	DEB, NICHD
	D. Loriaux	Head	DEB, NICHD
	S. Malozowski	Visiting Fellow	DEB, NICHD
	M. Nicoletti	Guest Worker	DEB, NICHD
	W. Nixon	Senior Investigator	DEB, NICHD
	O. Pescovitz	Medical Staff Fellow	DEB, NICHD

COOPERATING UNITS *(if any)*

University of Catania; University of Cincinnati; INTA, Chile; Johns Hopkins University; Tufts University; University of Poznan; University of Virginia

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Steroid Hormones

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.5	0.25

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Growth hormone-releasing hormone (GHRH) is a 44-amino acid peptide which stimulates release and synthesis of growth hormone (GH). The aims of this project are a) to define the role of GHRH in the regulation of GH secretion b) to study the modulation of GHRH responses in altered physiologic states and the possible diagnostic utility of GHRH testing; and c) and to explore the potential utility of GHRH for the treatment of GH deficiency. (a) During this year we have demonstrated that the GH response to continuous GHRH exposure declines after an acute rise, with a period of desensitization to subsequent GHRH exposure. This response occurs even when release of GH is prevented, indicating that the response is mediated at least in part by receptor occupancy and not only through depletion of releasable GH stores. (b) We have studied the normal range of GHRH responses in healthy aging men, and in boys and girls throughout pubertal development. Despite a decrease in spontaneous GH secretion, GHRH responses are maintained in older adults. Responses also vary little during puberty. This permits adult normative data to be used as a reference for studies in children. In obesity, where spontaneous GH secretion is decreased, responsivity to GHRH is also reduced. By contrast, when GH secretion is increased, as in malnutrition, anorexia nervosa, and Laron type dwarfism, responses to GHRH are not increased over normal. This suggests that responses to GHRH are near-maximal under normal conditions. GH deficient children have lower mean responses than normal children, but the majority do respond to GHRH, and many responses overlap the normal range. Thus, GHRH testing is not reliable for diagnosing GHD; but demonstrates that most GHD is due to a deficiency of GHRH, and not to a pituitary lesion. Thus GHRH could be used to treat these GHD children if given repeatedly. (c) In an initial test of this hypothesis, a group of GHD children were treated with GHRH, placebo, and GH. In the majority, GHRH restored GH secretion, elevated somatomedin C, and accelerated linear growth. The response was comparable to the effects of GH. Currently patients are being treated with a range of GHRH doses and frequencies to determine the optimum treatment regimen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00623-02 DEB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:
3.0	3.0	0

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.)*

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.

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
	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
	R. Rittmaster	Medical Staff Fellow	DEB, NICHD
	J. Levine Ross	Guest Worker	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)

ENDOCRINOLOGY AND REPRODUCTION RESEARCH BRANCH

Z01 HD 00022-12 Renin-Angiotensin System and Aldosterone Regulation
Greti Aguilera, M.D.

Z01 HD 00035-13 The Structure and Function of Biologically Active Molecules
John L. Morell

Z01 HD 00146-10 Structure and Function of Chorionic Gonadotropins
Hao-Chia Chen, Ph.D.

Z01 HD 00147-10 Mechanism of Action of Peptide Hormones in Steroidogenic Cells
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00149-10 Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00150-10 Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase
Maria L. Dufau, M.D., Ph.D.

Z01 HD 00151-10 Receptor-mediated Regulation of Gonadal Function
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00160-10 Regulation of Adrenal Steroidogenesis
Charles A. Strott, M.D.

Z01 HD 00184-07 Regulation of Pituitary Hormone Secretion
Kevin J. Catt, M.D., Ph.D.

Z01 HD 00187-06 Hormonal Regulation of Cellular Metabolism
Kuo Ping Huang, Ph.D.

Z01 HD 00190-03 Development and Regulation of Cellular Zonation of the Adrenal Cortex
Charles A. Strott, M.D.

Z01 HD 00191-01 Mechanisms of Neuroendocrine Regulation
Greti Aguilera, M.D.

NICHD ANNUAL REPORT
Endocrinology and Reproduction Research Branch
October 1, 1984 to September 30, 1985

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 in endocrine target cells. Of particular interest are the analysis of pituitary-gonadal and pituitary-adrenal regulation, the control of ovarian activity during the reproductive cycle and pregnancy, and the participation of hormone receptors in the regulation of pituitary, gonadal, and adrenal function. In the current year, research has been pursued 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 role of neuropeptides in hypothalamic-pituitary regulation, the control of gonadal and adrenal function by pituitary hormones, the renin-angiotensin system and aldosterone secretion, 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) 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). The receptor-mediated actions of gonadotropin-releasing hormone (GnRH) and other regulators of pituitary hormone secretion are studied in cultured anterior pituitary cells. In the gonadotroph, occupancy of GnRH receptors and activation of gonadotropin secretion was followed by receptor turnover and subsequent up-regulation of receptor number. In contrast, potent GnRH antagonists dissociated slowly from the GnRH receptor and did not cause down-regulation of receptors and desensitization of the gonadotroph. The bound antagonist remained localized at the cell surface for much longer periods than bound agonist analogs, suggesting that receptor activation is necessary to initiate internalization of the hormone-receptor complex. GnRH was found enhance phosphatidylinositol (PI) turnover with increased formation of phosphatidic acid (PA) and arachidonic acid (AA). In studies on the actions of these compounds on LH release, PA stimulated cGMP formation and LH release in a calcium-dependent manner, suggesting that endogenous PA may function as a calcium ionophore in the gonadotropic action of GnRH and its agonist analogs. Protein kinase C was shown to be abundant in purified gonadotrophs and to be activated by phorbol esters and diacylglycerols, which also stimulated LH release. GnRH-stimulated release of luteinizing hormone (LH) from gonadotroph-enriched cells was accompanied by a rapid and dose-dependent decrease in

cytosolic protein kinase C and by a corresponding increase in the particulate enzyme. Retinal directly inhibited the activity of cytosolic protein kinase C and also attenuated the release of LH from GnRH-stimulated gonadotrophs. These findings, and the ability of GnRH to cause rapid translocation of cytosolic protein kinase C to a membrane-associated form, suggest that hormonal activation of protein kinase C is an intermediate step in the stimulation of pituitary LH secretion by GnRH.

The hormonal control of gonadal endocrine function is studied in ovarian and testicular target cells. In the ovarian granulosa cell, which is a model of hormone-induced cellular differentiation, FSH causes early and delayed increases in cAMP production, with induction of protein kinase, peptide hormone receptors (LH, Prl) and steroidogenic enzymes from 18-24 hr of culture. The RNA and protein required for differentiation are synthesized largely during the second day of culture, and endogenously produced estrogen is essential for the full expression of LH receptors. Granulosa cell maturation is also induced by cAMP generators such as choleragen and forskolin, and is modified by GnRH, EGF, PDGF, adenosine, calcium agents, and estrogens. Most of these agents act rapidly to influence granulosa-cell differentiation, but their effects are largely manifested during the second day of culture. Inhibition of granulosa cell maturation by GnRH is highly calcium-dependent, similar to its stimulatory action in the pituitary, and is reproduced by elevation of cytosolic calcium. GnRH also attenuates the FSH-induced rise in Type II protein kinase, initially decreasing enzyme activity and later reducing the synthesis of the RII cAMP-binding subunit. In the adult testis, GnRH receptor blockade decreased Prl receptors and testosterone production but did not alter the desensitization response to exogenous gonadotropin, showing that testicular GnRH does not mediate the down-regulating effects of elevated gonadotropin on testicular function. The neonatal rat testis was found to be relatively immune to the inhibitory effects of estrogen, and to show largely positive responses to elevations in plasma gonadotropins and PRL, instead of the receptor loss and desensitization seen in the adult. These features of the fetal-neonatal population of Leydig cells may serve to ensure optimal androgen production during the critical period of sexual differentiation.

The Unit on Endocrine Physiology (Dr. Greti Aguilera) investigates the physiological and pathological aspects of the renin-angiotensin system in rodent and primate models, with emphasis in the role of angiotensin II (AII) in the regulation of aldosterone secretion and circulatory homeostasis. AII mediates the increases in aldosterone secretion during sodium restriction, but the adrenal response to the peptide also depends on the sensitivity of the glomerulosa cell to AII. Adrenal sensitivity to AII is increased during sodium restriction, and previous studies in the rat have indicated that up-regulation of adrenal AII receptors contributes to the enhanced adrenal sensitivity to AII. However, in the primate the increased adrenal sensitivity to AII has been found to depend on increased 18-hydroxylase activity rather than AII receptor regulation. The recently characterized cardiac peptide, atrial natriuretic factor (ANF) is a potent inhibitor of aldosterone production *in vivo* and *in vitro*, being more effective in inhibiting AII- than ACTH-stimulated steroidogenesis. Studies are in progress to determine the possible role of ANF in the regulation of adrenal sensitivity to AII in several physiological conditions. The action of AII is highly calcium-dependent, and studies with the dihydropyridine calcium channel agonist, Bay K 8644, have indicated that voltage-dependent calcium channels are involved in the mechanism of action of AII. The participation of calcium-dependent protein kinases in the control of adrenal glomerulosa function was

suggested by the preferential location of calcium-calmodulin and calcium-phospholipid dependent protein kinases in the zona glomerulosa of the adrenal cortex, and by the ability of AII to decrease cytosolic calcium-calmodulin dependent protein kinase activity in isolated adrenal glomerulosa cells. The central receptors and actions of AII are being further studied in the brain, to characterize the interactions of ANF with the neural effects of AII, and to determine the localization of AII receptors in the primate brain.

The neuroendocrine components of homeostatic regulation are also studied, with emphasis on the mechanisms of stress responses and the hypothalamic control of corticotropin (ACTH) release. The receptors and actions of corticotropin releasing factor (CRF) have been characterized in the pituitary gland and nervous system. The increases in ACTH secretion that follow adrenalectomy are accompanied by marked increases in basal ACTH release in isolated pituitary cells, and by decreases in pituitary CRF receptors and CRF-stimulated adenylate cyclase activity. Studies are in progress to determine the mechanisms of receptor desensitization and maintenance of elevated ACTH secretion after adrenalectomy and stress. Analysis of neuropeptide release from median eminences in vitro showed a decrease in CRF release and an increase in vasopressin release after adrenalectomy, suggesting that VP is a major factor in the control of ACTH release. Autoradiographic analysis of frozen brain sections has shown that CRF receptors are prominently located in the cerebral cortex and limbic system-related areas. Brain CRF receptors are coupled to adenylate cyclase, and in contrast to the pituitary receptors are not down-regulated after adrenalectomy. Functional CRF receptors were also found in sympathetic ganglia and in the adrenal medulla, indicating that CRF is involved in the peripheral response to stress. These findings have revealed that CRF exerts receptor-mediated actions within the nervous system at sites involved in the behavioral and autonomic responses to stress, as well as on the pituitary-adrenal secretion of stress hormones.

(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. Recent studies on the properties and purification of solubilized prolactin receptors have given new information on receptor structure and have led to the isolation of small amounts of purified receptor. Cross-linking studies with ^{125}I-hGH have shown the existence of subsets of high (81 and 91K) and low (31 and 37K) binding subunits in the gonads. The high and low Mr species were also found in mammary gland, liver, and kidney. The presence of subspecies of similar Mr in the gonads reflect differences in glycosylaction or phosphorylation within the subunit population. The smaller (37K) species appears to represent a disulfide-linked portion of the larger (91K) component. The ovarian receptor was purified by affinity chromotography to a specific activity of 20 pmol/µg, close to that expected from its apparent Mr, with retention of hormone binding activity. The purified ovarian receptor contained 88K and 95K variants of the high Mr subunit, and a single 41K subunit. Both of the purified receptor subunits were biologically active as shown by hormone binding to the free subunits after trans-blotting to nitrocellulose. This approach has permitted the isolation of active lactogen receptors from the ovary in microgram amounts, and has provided the first evidence for the presence of a high MW binding component in the purified free receptor.

In studies on gonadotropin action, the previously observed effect of LH on guanyl nucleotide binding and protein phosphorylation in Leydig cell plasma membranes was extended by further analysis of the GTP-dependence of the calcium-sensitive phosphorylation of a 44K protein that may be related to the guanyl nucleotide regulatory protein family. The mechanisms of gonadotropin-induced desensitization of Leydig cell steroidogenesis were further analyzed to clarify the nature of the 'early' and 'late' biosynthetic lesions that are responsible for the post-stimulatory depression of androgen production, and the role of endogenous estrogens in this process. Such steroidogenic lesions, which are independent of changes in LH receptors or protein kinase activation, are prominent in the adult testis but minor in the fetal-neonatal population of Leydig cells. Recent studies have shown that as a result of gonadotropin action, estrogen-mediated desensitization is initiated by an early cyclic AMP-dependent activation of aromatase, which is followed by a significant rise in estradiol formation due to increased substrate availability. Leydig cells are the major site of estradiol synthesis in the adult rat testis, and the low aromatase activity observed in immature rat Leydig cells could explain the lack of desensitization observed in fetal and early life. Estrogen action that precedes desensitization includes increased synthesis of a 27K estradiol-regulated protein. This protein was found to be immunologically similar to a major estradiol-regulated 27K protein of MCF-7 tumor cells, and provides a sensitive probe for detection of estrogen action. Antibodies against the MCF-7 protein will be employed for functional and structural characterization of the 27K protein and for studies of the nuclear actions of estrogen in the Leydig cell. There is a continuous basal supply of steroidogenic cholesterol in the mitochondrion regardless of the presence of gonadotropins, a process probably regulated by the levels of endogenous steroids. The early lesion is not due to an inappropriate concentration of precursors, since the levels of cholesterol in the inner mitochondrial membrane are increased. A heat-labile inhibitory protein factor was identified in mitochondria and shown to be markedly increased by hCG treatment. This factor, which competitively inhibits cholesterol side-chain cleavage activity, could contribute to the early steroidogenic lesion and may also serve as an endogenous modulator of steroid hormone biosynthesis.

Further studies on LH bioactivity have provided new insights into the regulation of pituitary-gonadal function of man, rhesus monkey and rat. Modulation of the frequency and bio:immuno ratio (B:I) of plasma LH pulses provides an important physiological mechanism for regulating the concentrations of bioactive LH available to the gonads. The bioactivity of circulating LH is modulated by gonadal steroids (normal men have higher B:I than cycling females; castration in rats decrease the B:I), possibly via changes in glycosylation. The decrease in B:I of LH in castrated animals could also be related to increased LH secretion rate. Older men with prostatic cancer have low B:I ratios, indicating the potential importance of changes in B:I with age and sickness. With advancing age, B:I and plasma testosterone are inversely related, and ill men over age 40 have lower B:I ratios. The qualitative nature of LH varies as a function of aging and illness in men, and the secretion of LH with low B:I, may be etiologically relevant in patients with impotence and normal immunoreactive pituitary hormones and LH periodicity, and low normal testosterone levels. During GnRH or clomiphene therapy in a patient with impotence and pituitary calcification, a rise in B:I indicated a relationship between pituitary GnRH action and the potency of secreted LH. The endocrine consequences of reversible endogenous estrogen excess on the pituitary-gonadal axis in man were analyzed in a patient with an estrogen-

producing adrenal tumor, in whom hypogonadism was attributable to selective reduction in bioactive LH and low B:I ratio. The latter changes could result from estradiol action at the hypothalamic level to reduce GnRH secretion, and from direct effects on pituitary LH processing. It was also demonstrated that the adenine analog (4-Aminopyrazolo-(3,4-d)-Pyrimidine) has an inhibitory action on GnRH release from the hypothalamus, and the relation of this effect to the mechanism of neurohormone secretion is being further analyzed.

(c). The Section on 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. The guinea pig, a cortisol-producing species, is employed as a model for studies on the regulation of steroidogenesis in the adrenal cortex, and the development and regulation of cellular zonation in the adrenal cortex. The guinea pig adrenal is relatively large, with a cortico-medullary ratio of 80:1, and is readily separated into glomerulosa-fasciculata and reticularis zones. The latter zone is of particular interest since little is known of its functional nature, steroidogenic capability, and specific regulation. The role of ACTH in regulating the zona fasciculata is well characterized; however its role in regulating the zona reticularis is unclear. When dexamethasone was administered to animals to suppress secretion of endogenous ACTH, the zona fasciculata showed the expected atrophy and decrease in steroid production, but the zona reticularis was unaffected. In contrast to the zona fasciculata, lipoprotein receptor activity and steroid synthesis in the reticularis are not stimulated by ACTH. On the other hand, adenylate cyclase activity, ascorbic acid depletion, and hydrolysis of cholesteryl ester stores are promoted by ACTH in the zona reticularis as they are in the zona fasciculata. Thus, the zona reticularis has retained certain specific responses to ACTH while losing others.

The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone, a process whereby cholesterol must be made available and delivered to and pregnenolone removed from the active site located in the inner mitochondrial membrane. To study this complex process in detail, adenylate cyclase activity and cyclic AMP production has been determined using isolated cells and membrane particles. Also, cyclic AMP-dependent protein kinase activity was found mostly in the soluble fraction (80-90%), composed of the type II isozyme, and 40% more active in the outer cortex. A study of soluble and particulate phosphoproteins is in progress. Other studies include analyses of the binding of lipoproteins to membrane receptors, cellular uptake of cholesterol and cholesteryl esters, and the de novo synthesis and metabolism of cholesterol. A specific pregnenolone-binding protein is being further purified by high-performance liquid chromatography. Preliminary data suggest that the binding protein is phosphorylated by a cAMP-dependent protein kinase.

(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).

In studies on the human CRF molecule, ten peptides corresponding to sequences 9-41 to 17-41 of hCRF were synthesized and purified in order to test the role of Arg^{16} on the face of the postulated α helical form of the hormone in the expression of agonist or antagonist activity. A 2-3 fold increase in binding to rat pituitary membrane receptor was observed on addition of Arg, with a general increase in binding on elongation. However, the shorter fragments 11- and 12-41 binds more strongly than the longer 9- and 10-41 peptides. Agonist activity is expressed in fragments of length 4-41, with antagonist activity peaking at 11- and 12-41, and re-expression of agonist activity in the still shorter fragments 14- and 15-41. These results indicate the importance of Arg^{16} as a critical residue for expression of the receptor binding and bioactivity of CRF.

In pursuit of more highly potent gonadotropin releasing hormone agonists, dimeric des-Gly^{10}-[$D\text{-}Lys^{6}$]-GnRH-NHEt analogs cross-linked at the ε-amino group of $D\text{-}Lys^{6}$ with -Gly-$COCH_2$-CO-Gly_n- (Ia, n=0; Ib, n=1; Ic, n=2) were synthesized, purified and evaluated for their biological activities. Such cross-linking of the agonist into dimers was found to enhance both <u>in vivo</u> and <u>in vitro</u> biological activities, and dimer Ib exhibited the highest anti-fertility effect ever reported.

In preparation for projected structure-function studies, two peptides of 14 and 18 amino acid residues corresponding to α helical and helix turn regions of the cytoplasmic portion of the human transferrin receptor were synthesized for use as immunogens. The antibodies are to be used in a study of the function of these domains in receptor dynamics and regulation. Also, a peptide of 22 amino acid residues in a protein expressed during gastrulation of <u>Xenopus laevis</u> was synthesized for use as a immunogen to be employed in developmental studies.

Recent studies on the biology of human chorionic gonadotropic (hCG) included the development of a simple procedure for collection, concentration, and a specific urinary hCG radioimmunoassay were applied successfully in a pilot study for the detection of early fetal loss in a population of health women. In 86 menstrual cycles studied, 19 samples (one sample from each cycle) showed positive hCG level. Among those hCG-positive women, eleven had no detectable level of hCG in the subsequent month indicating early fetal loss. This 58% fetal loss, which is derived from a general population of normal subjects, may be more representative than those of other studies which were carried out in selected groups of women.

Further structure-function studies were performed on a chemically deglycosylated hCG derivative (HF-hCG) previously shown to possess enhanced receptor binding activity but reduced ability to stimulate cyclic AMP and steroid production in gonadal cells. HF-hCG also antagonized cAMP production in hCG- and FSH-stimulated granulosa cells as well as in hCG-stimulated Leydig cells. Addition of bivalent anti-hCG antibodies, but not their monovalent Fab fragments, to the inactive HF-hCG:LH-receptor complex in differentiated granulosa cells, significantly increased both cAMP and progesterone production. Since both conformation-specific and sequence-specific hCG-antibodies were equally effective, and antibody bivalency was required to induce the stimulatory effect, cross-linking and microaggregation of the hormone:receptor complex is the most likely cause of target-cell activation by anti-hCG antibodies after initial binding of the antagonist derivative.

(e). <u>The Section on Metabolic Regulation</u> (Dr. K.-P. Huang) studies the regulation and hormonal control of glycogen metabolism in normal and diabetic tissues, and the activities of glycogen synthase and phosphorylase kinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00022-12 ERRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Renin-Angiotensin System and Aldosterone Regulation

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

PI:	G. Aguilera	Research Biologist	ERRB, NICHD
	K. J. Catt	Head	ERRB, NICHD
Others:	T. Kigoshi	Visiting Fellow	ERRB, NICHD
	W. P. Hausdorff	Guest Researcher	ERRB, NICHD
	M. A. Millan	Sr. Staff Fellow	ERRB, NICHD
	M. P. Platia	Med. Staff Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Dept. of Medicine, University of Melbourne (F.A.O. Mendelsohn)
Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation (Endocrine Physiology Unit)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.98	1.48	0.5

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to analyze physiological and pathological aspects of the renin-angiotensin system, 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. Previous studies in the rat have indicated the importance of adrenal AII receptor regulation in the changes in adrenal sensitivity to AII. However, in the primate the adrenal sensitivity to AII depends on regulatory changes in 18-hydroxylase activity rather than AII receptor regulation. The recently characterized cardiac peptide, atrial natriuretic factor (ANF) is a potent inhibitor of aldosterone production in vivo and in vitro, being markedly more effective in inhibiting AII- than ACTH-stimulated steroidogenesis. Studies are in progress to determine the possible role of ANF in the regulation of adrenal sensitivity to AII in several physiological conditions. The action of AII is highly calcium-dependent, and studies in the rat using the dihydropyridine calcium channel agonist Bay K 8644 provide further evidence for the involvement of voltage-dependent calcium channels in the mechanism of action of AII, but not of that of ACTH. The participation of calcium-dependent protein kinases in the control of adrenal glomerulosa function was suggested by the preferential location of calcium-calmodulin and calcium-phospholipid dependent protein kinases in the zona glomerulosa of the adrenal cortex, and by the ability of AII to decrease cytosolic calcium-calmodulin dependent protein kinase activity in isolated adrenal glomerulosa cells. Studies are in progress to further characterize the central actions of AII, including the interaction of AII with ANF and localization of AII receptors in the primate brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00035-13 ERRB

PERIOD COVERED: October 1, 1984 to September 30, 1985

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
Others:	G. Aguilera	Research Biologist	ERRB, NICHD
	J.L. Morell	Research Chemist	ERRB, NICHD
	J.H. Brown	Research Chemist	ERRB, NICHD
	Y. Kitajima	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Endocrinology and Reproduction Research Branch

SECTION: Section on Molecular Structure & Protein Chemistry

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

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

CHECK APPROPRIATE BOX(ES)

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

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

This project focuses on structural design, chemical synthesis and modification of polypeptide important to reproductive and developmental biology.

A. Human corticotropin releasing factor. Ten peptides corresponding to the sequences 9-41 to 17-41 of hCRF were synthesized and purified in order to test the role of Arg^{16} on the face of the postulated α helical form of the hormone in the expression of agonist or antagonist activity. Results indicate a 2-3 order increase in binding to rat pituitary membrane receptor on addition of Arg^{16} with a general increase in binding on elongation. However, the shorter fragments 11- and 12-41 binds more strongly than the longer 9- and 10-41 peptides. Agonist activity is expressed in fragments of length 4-41 with antagonist activity peaking at 11- and 12-41, and re-expression of agonist activity in the still shorter fragments 14- and 15-41. These results indicate the importance of Arg^{16} as a critical residues for expression of activity in hormone.

B. Dimeric gonadotropin releasing hormone agonists. Three dimeric des-Gly^{10}-[D-Lys^{6}]-GnRH-NHEt cross-linked at the ε-amino group of D-Lys^{6} with $-Gly_n-COCH_2-CO-Gly_n-$ (Ia, n=0; Ib, n=1; Ic, n=2) were synthesized, purified and evaluated for their biological activities. Results indicate that cross-linking of an agonist into dimers enhances both in vivo and in vitro biological activities. The dimer Ib exhibits the highest anti-fertility effect ever reported.

C. Human transferrin receptor peptides. Two peptides of 14 and 18 amino acid residues corresponding to an α helical and helix turn region of the cytoplasmic portion of the human transferrin receptor were synthesized for use as immunogens. The antibodies are to be used in a study of the function of these domains in receptor dynamics and regulation.

D. A peptide of 22 amino acid residues in a protein expressed during gastrulation of Xenopus laevis has been synthesized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00146-10 ERRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	K. J. Catt	Head, SMR	ERRB, NICHD
	M. Knecht	Staff Fellow	ERRB, NICHD
	P. Feng	Visiting Fellow	ERRB, NCIHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Structure & Protein Chemistry

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☒ (a) 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) in body fluids of cycling women was investigated for monitoring pregnancy and spontaneous fetal loss. The role of carbohydrate structures in hCG for the gonadotropic action was also the focus of this study.

A. A simple procedure for collection, concentration, and a specific urinary hCG radioimmunoassay were applied successfully in a pilot study for the detection of early fetal loss in a population of health women. In 86 menstrual cycles studied, 19 samples (one sample from each cycle) showed positive hCG level. Among those hCG-positive women, eleven had no detectable level of hCG in the subsequent month indicating early fetal loss. This 58% fetal loss, which is derived from a general population of normal subjects, can be more representative than those of other studies which were carried out in selected groups of women.

B. A chemically deglycosylated hCG derivative (HF-hCG) was shown to possess enhanced receptor binding activity but reduced ability to stimulate cyclic AMP and steroid production in gonadal cells. It also antagonized cAMP production in hCG- and FSH-stimulated granulosa cells as well as in hCG-stimulated Leydig cells. Addition of bivalent anti-hCG antibodies, but not their monovalent Fab fragments, to the inactive HF-hCG:LH-receptor complex in differentiated granulosa cells, significantly increased the levels of both cAMP and progesterone production. Since both conformation-specific and sequence-specific hCG-antibodies were equally effective, and antibody bivalency was required to induce the stimulatory effect, cross-linking and microaggregation of the hormone:receptor complex is the most likely cause of target-cell activation by anti-hCG antibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00147-10 ERRB

PERIOD COVERED: October 1, 1984 to September 30, 1985

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

Mechanism of Action of Peptide Hormones in Steroidogenic Cells

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

PI:	M.L. Dufau	Head	ERRB, NICHD
Others:	C-H. Tsai-Morris	Staff Fellow	ERRB, NICHD
	S. Luna	NRSA Fellow	ERRB, NICHD
	D.R. Aquilano	Visiting Fellow	ERRB, NICHD
	C.A. Winters	Chemist	ERRB, NICHD
	D. Ciocca	Guest Researcher	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:
2.25	2.00	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 control of androgen production by the Leydig cell is directly regulated by luteinizing hormone via specific receptors. Treatment with gonadotropin causes initial LH receptor up-regulation followed by down-regulation, and desensitization of steroidogenic enzymes: "early lesion" (prior to pregnenolone) and "late lesion" E_2-dependent (17α-hydroxylase 17-20 desmolase). These are independent of receptor loss or protein kinase activation. The negative control of receptors and lesions is not observed in the immature or fetal Leydig cell. The goal of this project is to understand the steps involved in the hormonal control of testicular function. We have demonstrated that as a result of gonadotropin action, E_2-mediated desensitization is initiated by a cyclic AMP-dependent early activation of aromatase, which is followed by a significant rise in E_2 formation due to an increased substrate availability. Leydig cells are the major site of E_2 synthesis in the adult rat testis. The low aromatase activity observed in immature rat Leydig cells could explain the lack of desensitization observed in fetal and early life. E_2 action that precedes desensitization includes increased synthesis of a 27K E_2-regulated protein. This protein was found to be immunologically similar to a major E_2-regulated 27K protein of MCF-7 cells. This provides a sensitive probe for detection of E_2 action and should prove useful for further functional and structural characterization of the protein and for studies of the nuclear actions of E_2 in the Leydig cell. There is a continuous basal supply of steroidogenic cholesterol in the mitochondrion regardless of the presence of gonadotropins, a process probably regulated by the levels of endogenous steroids. The early lesion is not due to an inappropiate concentration of precursors, since the levels of cholesterol in the inner mitochondrial membrane are increased. A heat-labile inhibiting protein factor was identified in mitochondria and shown to be markedly increased by hCG treatment. This factor, which competitively inhibits cholesterol side-chain cleavage activity, could contribute to the early steroidogenic lesion and may also serve as an endogenous modulator of steroid hormone biosynthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00149-10 ERRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	M.L. Dufau	Head	ERRB, NICHD
Others:	K.J. Catt	Head, SMR	ERRB, NICHD
	A. Khanum	Visiting Fellow	ERRB, NICHD
	M. Blank	Expert	ERRB, NICHD
	M. Ching	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*

Departments 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:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies on LH bioactivity and gonadal receptors have provided insights into the regulation of pituitary-gonadal function of man, rhesus monkey and rat. Modulation of the frequency and bio:immuno ratios (B:I) of plasma LH pulses also provides an important physiological mechanism for regulating the concentrations of effective LH available to the ovary and testis. The bioactivity of circulating LH appears to be rapidly modulated by gonadal steroids (i.e. normal men have higher B:I than cycling females; castration in rats decrease the B:I), possibly via changes in glycosylation. Also, the decrease in B:I of LH in castrated animals could be related to increased LH secretion rate. The B:I in older men with prostatic cancer as found to be low, indicating the potential importance of changes in B:I with age and sickness. Regression analysis showed an inverse relationship of B:I and plasma testosterone with age, and ill men over age 40 had lower B:I. These findings indicate that the qualitative nature of LH varies as a function of aging and illness in men. The abnormal nature of LH secreted with low B:I may be etiologically relevant in patients with impotence and normal immunoreactive pituitary hormones and LH periodicity, and low normal testosterone levels. The increased B:I during GnRH or clomiphene therapy in a patient with this type of disorder, and known to have pituitary calcification, indicated a functional relationship between pituitary GnRH exposure and the greater potency of secreted LH. The endocrine consequences of reversible endogenous estrogen excess on the pituitary-gonadal axis in man were analyzed in a detailed study of a patient with an estrogen-producing adrenal tumor. In this case, hypogonadism was attributable to selective reduction in bioactive LH and low B:I ratio. The LH reduction could result from E_2 action at the hypothalamic level to reduce GnRH secretion, and from direct effects on pituitary LH processing. We have also demonstrated that the adenine analog (4-Aminopyrazolo-(3,4-d)-Pyrimidine) has an inhibitory action on GnRH release from the hypothalamus, and are analyzing the relation of this effect to the mechanism of neurohormone secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00150-10 ERRB

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase

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

PI:	M.L. Dufau	Head	ERRB, NICHD
Others:	C. Winters	Chemist	ERRB, NICHD
	S. Kusuda	Visiting Associate	ERRB, NICHD
	M. 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:
1.25	0.50	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.)*

This aspect of the program is concerned with a) characterization of gonadotropin and prolactin receptors of the testis and ovary; b) the physical and functional relationships of the LH receptor site and adenylate cyclase; and c) cAMP-dependent and independent phosphorylation during gonadotropin action. In studies on the properties of testicular lactogen receptors, detergent-solubilized preparations had higher binding capacity than the membrane fraction, due to unmasking of a receptor pool. The crosslinked hormone-receptor complexes were distributed between two subunit sets with Mr of 113 & 103K or 59 & 53K, equivalent to 91 & 81K and 37 & 31K (calculated) for the free receptor. The existence of bands with close Mrs could be attributed to differences in glycosylation or phosphorylation. Parallel crosslinking experiments with other tissues (i.e. ovary, mammary gland, liver and kidney) showed similar resolution. As in the ovary, the 37K species appears to be contained within the 80K species through disulfide linkage. Variants of low and high affinity subunits were usually indistinguishable in cross-linking studies with ovarian soluble preparations. In contrast, silver staining profiles of purified ovarian receptors (20.4 nmol/mg protein) showed 88K and 95K variants of the high Mr subunits, while the low Mr subunit was observed as a single band of 41K. The purified receptor subunits were biologically active as demonstrated by hormone binding to free receptors transblotted to nitrocellulose. We have devised a procedure for purification of microgram quantities of active lactogen receptors from rat ovaries. Also, we have shown that extensive internalization and degradation of bound hCG is not required for the early phase of gonadotropic desensitization in the Leydig cell. Studies of the hormone and G-nucleotide effect on membrane phosphorylation have shown that in the presence of submaximal levels of GTP, oLH induces significant increase in phosphorylation of a 44K protein. These findings and our previous observations, suggest that hormone binding to the LH receptor induces a G-nucleotide/Ca^{2+} dependent phosphorylation of the 44K protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00151-10 ERRB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	M. Knecht	Sr. Staff Fellow	ERRB, NICHD
Others:	M. L. Dufau	Head, SME	ERRB, NICHD
	H.-C. Chen	Head, SMSPC	ERRB, NICHD
	O. Shinohara	Visiting Fellow	ERRB, NICHD
	P. Feng	Visiting Fellow	ERRB, NICHD
	A. J. Baukal	Biomedical Engineer	ERRB, NICHD

COOPERATING UNITS *(if any)*

Dept. of Clinical Chemistry, University of Helsinki (I. Huhtaniemi);
Dept. of Physiology and Biophysics, USC School of Medicine (D.W. Warren).

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:
2.75	1.5	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 hormonal control of gonadal endocrine function is studied in ovarian and testicular target cells. In the ovary, the FSH-stimulated granulosa cell is a model of hormone-induced cellular differentiation. In cell cultures, FSH causes early and delayed increases in cAMP production, with induction of peptide hormone receptors (LH, Prl) and steroidogenic activity from 18-24 hr of culture. During FSH action, the RNA and protein required for differentiation were synthesized largely during the second day of culture, and endogenously produced estrogen was found to be essential for the full expression of LH receptors. Granulosa cell maturation is induced by cAMP generators such as choleragen and forskolin, and is modified by GnRH, EGF, PDGF, adenosine, calcium agents, and estrogens. Most of these agents act relatively rapidly to influence granulosa-cell differentiation, but their effects are largely manifested during the second day of culture. Inhibition of granulosa cell maturation by GnRH is highly calcium-dependent, similar to its stimulatory action in the pituitary, and is related to elevation of cytosolic calcium. GnRH also attenuates the FSH-induced rise in Type II protein kinase, initially decreasing enzyme activity and later reducing the synthesis of the RII cAMP-binding subunit. Protein kinase C was identified in granulosa cells and shown to phosphorylate several cytosolic proteins. In the adult testis, GnRH receptor blockade decreased Prl receptors and testosterone production but did not alter the desensitization response to exogenous gonadotropin, showing that testicular GnRH does not mediate the down-regulating effects of elevated gonadotropin on testicular function. The neonatal rat testis was found to be relatively immune to the inhibitory effects of estrogen, and to show largely positive responses to elevations in plasma gonadotropins and PRL, instead of the receptor loss and desensitization seen in the adult. These features of the fetal- neonatal population of Leydig cells may serve to ensure optimal androgen production during the critical period of sexual differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00160-10 ERRB

PERIOD COVERED

October 1, 1984, to September 30, 1985

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:	K. Nonomura	Visiting Fellow	ERRB, NICHD
	K. Mikami	Visiting Associate	ERRB, NICHD
	C.D. Lyons	Bio. Lab. Tech.	ERRB, NICHD
	Y. Lee	Sr. Staff Fellow	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:
2.75	2.0	0.75

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The regulation of steroidogenesis by the adrenal cortex is a complex process involving the transduction of signals from the plasma membrane to specific intracellular sites, protein phosphorylation, protein activators and/or inhibitors, cellular uptake and disposal of cholesterol, cholesterol synthesis and metabolism, intracellular steroid transport mechanisms, and steroid secretory processes. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone, a process whereby cholesterol must be made available and delivered to and pregnenolone removed from the active site located in the inner mitochondrial membrane. To study this complex process in detail, the guinea pig, a cortisol producer like the human being, has been employed as an animal model.

A. Plasma membrane adenylate cyclase. Adenylate cyclase activity and cyclic AMP production has been determined using isolated cells and membrane particles.

B. Cytoplasmic cAMP-dependent protein kinase. Cyclic AMP-dependent protein kinase activity was found mostly in the soluble fraction (80-90%), composed of the type II isozyme, and 40% more active in the outer cortex. A study of soluble and particulate phosphoproteins is in progress.

C. Binding of lipoproteins to membrane receptors. Low density lipoprotein receptor activity and response to hormonal and drug manipulations was measured.

D. Cellular uptake of cholesterol and cholesteryl esters. Labeled compounds injected intravenously incorporate into circulating lipoproteins and are taken up by various tissues of which the most avid is the adrenal cortex.

E. De novo synthesis and metabolism of cholesterol. The activities of enzymes HMG-CoA reductase (rate-limiting in cholesterol synthesis), acetyl CoA: cholesteryl acyl transferase, and cholesteryl esterase are being investigated.

F. Specific steroid-binding proteins. A specific pregnenolone-binding protein (PBP) is being further purified using high performance liquid chromatography. Preliminary data suggest that the PBP is phosphorylated by a cAMP-dependent kinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 ED 00184-07 ERRB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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	Research Biologist	ERRB, NICHD
Others:	R. O. Morgan	Staff Fellow	ERRB, NICHD
	J. P. Chang	Guest Researcher	ERRB, NICHD
	E. E. McCoy	Guest Researcher	ERRB, NICHD

COOPERATING UNITS *(if any)*
Dept. of Anatomy, University of Texas Medical Branch, Galveston, Texas (G. Childs); Dept. of Hormone Research, Weizmann Institute of Science, Rehovot, Israel (Z. Naor); Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.5	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 receptor-mediated actions of gonadotropin-releasing hormone (GnRH) and other regulators of pituitary hormone secretion are studied in cultured anterior pituitary cells. Occupancy of pituitary receptors and activation of gonadotropin secretion was followed by receptor turnover and subsequent up-regulation of receptor number. In contrast, potent GnRH antagonists dissociated slowly following receptor binding and did not cause down-regulation of receptors and desensitization of the gonadotroph. The bound antagonist remained localized at the cell surface for much longer periods than bound agonist analogs, suggesting that receptor activation is necessary to initiate internalization of the hormone-receptor complex. The ability of GnRH to enhance phosphatidylinositol (PI) turnover with increased formation of phosphatidic acid (PA) and arachidonic acid (AA) led to studies on the actions of these compounds on LH release. PA stimulated cGMP formation and LH release in a calcium-dependent manner, suggesting that endogenous PA may function as a calcium ionophore in the gonadotropic action of GnRH and its agonist analogs. Protein kinase C was shown to be abundant in purified gonadotrophs and to be activated by phorbol esters and diacylglycerols, which also stimulated LH release. The distribution of protein kinase C between cytosol and membrane fractions was analyzed in cultured pituitary gonadotrophs during treatment with GnRH. GnRH-stimulated release of luteinizing hormone (LH) from gonadotroph-enriched cells was accompanied by a rapid and dose-dependent decrease in cytosolic protein kinase C and by a corresponding increase in the particulate enzyme. Retinal directly inhibited the activity of cytosolic protein kinase C and also attenuated the release of LH from GnRH-stimulated gonadotrophs. These findings, and the ability of GnRH to cause rapid translocation of cytosolic protein kinase C to a membrane-associated form, suggest that hormonal activation of protein kinase C is an intermediate step in the stimulation of pituitary LH secretion by GnRH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00187-06 ERRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	K.-F. J. Chan	Staff Fellow	ERRB, NICHD
	T.J. Singh	Visiting Associate	ERRB, NICHD
	H. Nakabayashi	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Biochemistry, NHLBI, NIH (P.B. Chock), Human Genetics 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	3.25	-

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-dephosphorylation of enzymes controlling rate-limiting 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 two of such enzymes 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 activities of the various protein kinases and phosphatases. Previous studies have defined the action of glucagon and β-adrenergic agonists via the pathway involving cAMP and cAMP-dependent protein kinase. However, the actions of other hormone-mediated phosphorylation systems have yet to be correlated directly with the action of certain kinases. Tumor-promoting phorbol esters mimic the action of some hormones which regulate glycogen synthase activity in isolated hepatocytes. The pleiotropic responses elicited by these phorbol esters are presumably through binding and activation of their receptor, which has been tentatively identified as a phospholipid-dependent and calcium-activated protein kinase (protein kinase C). This protein kinase is ubiquitous in eukaryotes and seems to play a pivotal role in mediating the actions of signal-induced breakdown of inositol phospholipids. Protein kinase C from rat brain has been purified to near homogeneity in high yield. This enzyme phosphorylates glycogen synthase without causing its inactivation, in contrast to the effect of exposing intact hepatocytes to tumor-promoting phorbol esters. These findings indicate that the actions of other mediators in addition to protein kinase C must be necessary to express the effect of phorbol esters. Polyclonal and monoclonal antibodies against protein kinase C have been prepared for immunocytochemical studies, and the regulation of protein kinase C activity by autophosphorylation is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00190-03 ERRB

PERIOD COVERED: October 1, 1984 to September 30, 1985

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:	K. Mikami	Visiting Associate	ERRB, NICHD
	C.D. Lyons	Bio. Lab. Tech.	ERRB, NICHD

COOPERATING UNITS *(if any)*

Unit on Research Animal Resources, NICHD (John Donovan);
Laboratory of Cell Biology and Genetics, NIADDK (Mark Levine)

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:
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

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

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

The mammalian adrenal cortex is a heterogeneous collection of cells which are arranged in specific regions or concentric zones. Each zone is thought to carry out a specific function (produce a particular steroid hormone) and respond to a specific regulator. There appears to be considerable overlap, however, in that the same stimulatory factor may influence and some steroids can be produced and secreted by more than one zone. The developmental nature of adrenocortical zonation is not well understood. Whether the cells in each zone arise as distinct and unique entities or are derived in some fashion from a common precursor is not at all clear. The guinea pig, a cortisol producer like the human being, is utilized as an animal model because of the relatively large size of the adrenal, a corticomedullary ratio of approximately 80:1, and the relative ease by which outer and inner zones can be separated. The outer zone is composed of the zona glomerulosa and zona fasciculata, while the inner zone consists of the zona reticularis. It is the latter zone which is of particular interest since little is known of its functional nature, steroidogenic capability, and specific regulation.

A. Role of ACTH in regulating the zona reticularis. The role of ACTH in regulating the zona fasciculata is well characterized; however its role in regulating the zona reticularis is unclear. When dexametasone was administered to animals to suppress secretion of endogenous ACTH, the zona fasciculata reduced steroid production and atrophied, as expected, but the zona reticularis was unaffected.

B. Role of ACTH in regulating specific activities in the zona reticularis. In contrast to the zona fasciculata, lipoprotein receptor activity and steroid synthesis are not stimulated by ACTH. On the other hand adenylate cyclase activity, ascorbic acid (vitamin C) depletion, and hydrolysis of cholesteryl ester stores are promoted by ACTH in the zona reticularis as they are in the zona fasciculata. Thus, the zona reticularis has retained certain specific responses to ACTH while losing others.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00191-01 ERRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Mechanisms of Neuroendocrine Regulation

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

PI:	G. Aguilera	Research Biologist	ERRB, NICHD
	K.J. Catt	Head	ERRB, NICHD
Others:	A.B. Abou-Samra	Visiting Fellow	ERRB, NICHD
	M.A. Millan	Sr. Staff Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Surgery Branch, NCI (R. Udelsman)
Lab. Clin. Science, NIMH (D. Jacobowitz)
Nuclear Medicine, CC (M. Holmes)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation (Endocrine Physiology Unit)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 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 purpose of this project is to analyze the neuroendocrine components of homeostatic regulation, with emphasis on the mechanisms of adaptation to stress. In studies on the hypothalamic control of corticotropin (ACTH) release, the receptors and actions of corticotropin releasing factor (CRF) have been characterized in the pituitary gland and nervous system. Increases in ACTH secretion following adrenalectomy were accompanied by marked increases in basal ACTH release in isolated cells, and by decreases in pituitary CRF receptors and CRF-stimulated adenylate cyclase activity. Studies are in progress to determine the mechanisms of receptor desensitization and maintenance of elevated ACTH secretion after adrenalectomy and stress. Analysis of the secretion of CRF and vasopressin (VP) from median eminences in vitro showed a decrease in CRF release and an increase in VP release after adrenalectomy, suggesting that VP is a major factor in the control of ACTH release. Autoradiographic analysis of frozen brain sections has shown that CRF receptors are prominently located in the cerebral cortex and limbic system related areas. Brain CRF receptors were also coupled to adenylate cyclase, and in contrast to the pituitary receptors were not down-regulated following adrenalectomy. In addition, functional CRF receptors were found in sympathetic ganglia, and in the adrenal medulla, indicating that CRF is involved in the peripheral response to stress. Studies are also being conducted in the pituitary corticotroph to elucidate the mechanisms of action of the several factors involved in the regulation of ACTH secretion. These findings have revealed that CRF exerts receptor-mediated actions within the nervous system at sites involved in the behavioral and autonomic responses to stress, as well as on the pituitary-adrenal secretion of stress hormones.

HUMAN GENETICS BRANCH

Z01 HD 00131-11	Human Biochemical Genetics William A. Gahl, M.D., Ph.D.
Z01 HD 00133-08	Study of Glycogen Storage Disease James B. Sidbury, Jr., M.D.
Z01 HD 00403-04	Magnesium Metabolism in Mothers and Neonates Joan L. Caddell, M.D.
Z01 HD 00404-03	Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases Jean DeB. Butler, Ph.D.
Z01 HD 00405-07	Structure of the Methionine Initiator tRNA Genes in the Human Genome Michael Z. Zasloff, M.D., Ph.D.
Z01 HD 00408-02	Pathophysiology and Treatment of Human Genetic Diseases Michael A. Zasloff, M.D., Ph.D.
Z01 HD 00409-02	Kinetics of Calcium Metabolism in Childhood James B. Sidbury, Jr., M.D.
Z01 HD 00909-06	Effects of Ethanol on the Mother and the Fetus Anil B. Mukherjee, M.D., Ph.D.
Z01 HD 00910-06	Uteroglobin Anil B. Mukherjee, M.D., Ph.D.
Z01 HD 00912-06	Gene Regulation and Cellular Differentiation Janice Y. Chou, Ph.D.

NICHD ANNUAL REPORT

HUMAN GENETICS BRANCH

October 1, 1984 to September 30, 1985

The Human Genetics Branch conducts research which attempts to elucidate the pathophysiology of human genetic 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 mechanism of processing of eukaryotic RNAs; the basic cellular mechanisms involved in transport of biological information fromthe cell nucleus to cytoplasm; the expression of Alu sequences and other natural anti-sense RNAs; the treatment and pathophysiology of hereditary heterotopic ossification disorders in man; the pathophysiology, treatment and molecular basis of Osteogenesis Imperfecta; and the pathophysiology and treatment of the mucopolysaccharidoses.

Over the past several years we have been studying the expression of a human $tRNA_i^{met}$ gene, a species cloned by this laboratory from the human genome. One of the several genes cloned contained a mutation within the T loop of the tRNA coding region. Since such a mutation would have generated a tRNA with a pyrimidine in a highly conserved site occupied only by a purine in bacterial and eukaryotic tRNA species, we suspected that this variant human tRNA might be defective. In a series of experiments utilizing both in vitro and in vivo systems we demonstrated that this mutation had profound effects on the processing reactions which mature the primary gene transcript to tRNA and the transport mechanism which delivers the tRNA from the nucleus to the cytoplasm. As a result of these findings the laboratory was directed to an exploration of the processing enzymes actually utilized in eukaryotic tRNA biosynthesis as well as the mechanism of nuclear tRNA transport.

We have isolated the nuclear enzymes which are responsible for processing the $pre\text{-}tRNA_i^{met}$ primary transcript from X. laevis oocytes. The enzyme which processes the 3' terminus is a single polypeptide of about 97,400 in size. It generates a mature end and releases the 3' trailer intact. Its most striking feature is that it will only cut the primary gene transcript after the short 5'leaer has first been removed. This substrate restriction of the 3' processing enzyme thus imposes a cutting order on the maturation pathway and demands that the 5' processing enzyme act initially.

The 5' processing enzyme is a very complex structure consisting of some 15 polypeptides with an aggregate molecular weight of about 400,000. It poses a ring-like structure on electron microscopic examination and may undergo transition to more complex physical forms on activation in the oocyte nucleus. Our current interest is to define the cell biology of these enzymes, the structural requirements of each, and their role in tRNA transport.

In the area of tRNA transport we have determined the portions of the $tRNA_i^{met}$ molecule recognized by the mechanism in X. laevis oocytes. We constructed 30 different point mutants by in vitro methods and determined the transport phenotype by micro-injection and micro-dissection methods developed in our laboratory. The data demonstrate very clearly that the two highly conserved loops of the tRNA, the T and D loops, are critical for transport. In addition, every mutant defective in transport was also inefficiently processed. This result tells us that both the processing and transport mechanisms see the same portions of the tRNA molecule and open the possibility that one of the processing enzymes functions as a component in tRNA transport.

Studies began in the area of mRNA transport this year. The system we have utilized is the thymidine kinase gene of Herpes simplex I in the X. laevis oocyte. Injectio nof the gene into the X. laevis oocyte nucleus results in the appearance of active enzyme several hours after injection. When kinetics of mRNA synthesis are followed we have found that RNA is generated in two "phases": Initially, TK RNA accumulates in the oocyte nucleus, and most is degraded. By 2 hours, however, newly made RNA appears to be effectively transported to the cytoplasm. The details of this system are under study.

We have explored the molecular biology of a particular ALU sequence in mouse this past year and have discovered a new pathway by which this sequence generates a small RNA in the cytoplasm of mouse cells. The RNA studied is contained in the anti-sense orientation to the first intervening sequence in the mouse alpha-fetoprotein gene. We find that the primary transcript of this Alu sequence is processed by an endonuclease, transported from nucleus to cytoplasm, and packaged ribonucleoprotein. The levels of this processed RNA vary between different mouse tissues, present in greatest abundance in fetal liver. We believe that this conversion pathway represents one means by which a natural anti-sense RNA is handled by a cell.

Studies on the treatment and pathophysiology of fibrodysplasia ossificans progressiva (FOP) continue. We are in the midst of a treatment protocol evaluating the efficacy of the synthetic retinoid 13-cis retinoic acid in this disease. Current data suggest that the Vitamin A derivative is effective in reducing the appearance of new ossification centers. Other clinical studies focused on diagnostic modalities of use in the condition and we have shown that both Tc^{99} bone scanning and CT scan procedures effectively delineate the progress of the disease. The CT scan, furthermore, demonstrated the presence of new bone within fascia surrounding muscle, substantiating our view that the disorder expresses itself in tissues of that location rather than within muscle itself. Because of an early report that certain prostanoids were found to be elevated in tissue culture media bathing fibroblasts grown from active lesions of patients with FOP, plasma levels of PGE2 were assayed on some 25 patients this past year. From this RIA-based measurement all patients were found to have markedly elevated levels of this compound. We are currently attempting to define the structure and identity of this prostanoid by direct methods.

Studies were begun in the area of collagen molecular biology as it relates to human bone disease, principally,osteogenesis imperfecta. In the clinical sphere, many pedigrees were collected and appropriate tissues and blood samples were gathered to provide sources of DNA and RNA. The basic purpose of the effort is to define the molecular lesion in osteogenesis imperfecta to learn

more about the normal mechanisms involved in bone development and growth in man. Initial efforts are directed toward an analysis of the collagen polypeptides and their genes since several studies from other laboratories have pointed to these loci as fundamental to the defect in some cases. Related to this area, we have attempted to define whether the same splicing patterns are utilized in production of collagen a-2 Type I polypeptides from bone and skin fibroblasts, an issue that we view to be important in the study of connective tissue diseases in man. Initial studies from our laboratory suggest that the 5' ends of a-2 differ in these two tissues. We are in the process of determining the mRNA structure by cloning tissue-specific mRNA species from these two sources. Two clinical studies in osteogenesis imperfecta were initiated this year. One asks whether bracing the lower extremities of an infant with O.I. will accelerate walking and reduce bowing deformity. The other, an endocrine study, asks whether the striking differences in growth velocity exhibited by some but not all patients with O.I. might reflect the activity of the growth hormone - somatomedin axis.

Efforts in the treatment of the mucopolysaccharidoses were continued. Initial reports that implantation of human amnion epithelium could effectively correct the biochemical defect in several of the MPS disorders spurred our own clinical study in this area. After analysis of almost 20 children over the past year, we have been unable to detect any increase incirculating lysosomal enzyme or any obvious clinical response. One value of the study, however, was our establishment of clinical criteria which should provide a clinical framework on which to gauge any future attempts at treatment. In addition, several clinical features of the MPS conditions not fully appreciated previously were uncovered. It was discovered that a surprisingly large number of children with MPS I and II develop hydrocephalus prior to or coincident with deterioration of CNS function. This suggests that early shunting may be of some use in these conditions. Other studies using electroretinography to assess the state of activity of the retinal neuronal population demonstrated that patients with the MPS disorders with CNS disease generally have rather severe rod-cone degeneration. This data will hopefully be used in the management of visual problems of children with these conditions and provide objective measures of the neuronal health of the CNS in individuals with MPS. Basic studies on the purification of iduronate sulfatase continue with considerble difficulty. Efforts have been underway for some time to purify this enzyme in order to initiate studies on the defect in the Hunter Syndrome. A new procedure utilizing preparative polyacrylamide gel electrophoresis under non-denaturing conditions was developed this past year substantially improving purity and yield and it is hoped that homogeneity (or something close to it) will be achieved in the coming year. With this protein, attempts at beginning serious molecular biology can be considered.

II. Section on Developmental Genetics

The Section on Developmental Genetics has been studying: (i) the function and genetic regulation of a steriod-dependent small molecular weight (15K) protein called uteroglobin, and (ii) the genetic predisposing factors in Fetal Alcohol Syndrome (FAS).

During the past few years several proteins have been characterized as endogenous antiinflammatory substances. These proteins are potent phospholipase inhibitors and exert their antiinflammatory effects by reducing the level of tissue

prostaglandins. Some of these proteins are lipomodulin, macrocortin and renocortin. All these proteins are induced by glucocorticoids and thus it has been suggested that the antiinflammatory effects of glucocorticoids are mediated by the above mentioned proteins. Uteroglobin is a progesterone-induced antiinflammatory protein in the uterus and corticosteroid-induced protein in the tracheobronchial epithelium of the rabbit. During the past year we have investigated whether uterogobin is also an inhibitor of phospholipases and thus its similarity to the above mentioned proteins. We found that uteroglobin inhibits PLA2 derived from porcine pancreas as well as from cultured mouse macrophages. A dose dependent inhibition of PLA2 by uteroglobin was observed with a maximum inhibition at a concentration of 20 μM of this protein. We speculate that previously observed effects of uteroglobin, including its immunomodulatory, platelet aggregation inhibitory and uterine antimotility effects, could be explained by one central effect, i.e., PLA-2 inhibition.

In a related study, it has recently been found that in humans a protein similar to uteroglobin could be identified by radioimmunoassay, Western blot and HPLC. The human protein has so far been identified in human tracheobroncheal, uterine and prostatic epithelium by immunofluorescence. Further characterization of this protein, including its function, is now being investigated.

Involvement of genetic factors in the pathogenesis of fetal alcohol syndrome (FAS) is strongly implicated from the facts that a) only 33% of alcoholic mothers give birth to FAS babies, and b) there are instances of fraternal twins born of chronic alcoholic mothers where only one of the twins is afflicted with FAS. During the past year, we have shown that precipitating thiamine deficiency in the mother can lead to intrauterine growth retarded (IUGR) babies in the rat. These IUGR babies have been followed-up for their postnatal growth and for their learning ability. We found that the IUGR babies born of thiamine deficient mothers remained significantly lower in their body weight and size, and they were significantly deficient in their learning ability as judged from their exploratory behavior studies compared to their control counterparts. These data suggest that postnatal growth retardation and deficient learning ability, characteristic features of FAS, may be related to thiamine deficiency in utero. Since there are some strains of rats which are more susceptible to thiamine deficiency than others, these sensitive strains are now being studied to see if they have a transketolase with a high K_m for thiamine pyrophosphate (TPP). If so, these animals will be used as models to study predisposing genetic factors in FAS in more detail. To characterize these animals biochemically, we have developed a method for purifying the apotransketolase from various tissues to determine the K_m for TPP. Since the effects of thiamine deficiency is most pronounced in the brain and since most of the studies on transketolase are done on skin fibroblasts or RBC's, it will be important to show whether or not the K_m for TPP of the fibroblast or RBC enzyme is similar to that of the brain enzyme.

III. Section on Cellular Differentiation

Our studies have concerned regulation of gene expression during normal and abnormal differentiation processes. Studies on the expression of human placental alkaline phosphatase (PAP) gene were continued. This heat-stable phosphatase has been considered to be a marker for malignant transformation and is under developmental control. Tunicamycin, an inhibitor of protein glyco-

sylation was found to suppress PAP biosynthesis in addition to inhibiting protein glycosylation in general. The unglycosylated PAP monomer, formed in the presence of tunicamycin, had a degradation rate similar to that of the glycosylated monomer. Tunicamycin suppressed PAP mRNA activity leading to the observed decrease in biosynthesis. Our studies of the expression of PAP gene at the molecular level have been extended over the past year. Genes encoding PAP subunits were cloned. Efficient isolation of these genes was accomplished by probing a phage λgt 11 recombinant DNA expression library with polyvalent antibodies directed against highly purified PAP made in our laboratory.

Studies on α-fetoprotein (AFP) gene expression in the temperature-sensitive (ts) fetal liver cells were continued. We have demonstrated that AFP synthesized in cells exhibiting a transformed phenotype is a glycoprotein of 65,000 daltons. An unglycosylated AFP-specific polypeptide of 48,000 dalton was the precursor of a glycosylated AFP-specific polypeptide of 59,000 dalton which existed only intracellularly. The 59,000 dalton polypeptide was processed to the 65,000 dalton AFP found in both cells and medium. We have shown previously that the unglycosylated form of mature AFP is a polypeptide of 66,000 daltons. We now have found that these two AFP variants arise by translation of two mRNAs of 20S and 16S which are produced from a single AFP gene. Preliminary experiments indicate that the 16S RNA differed from the 20S AFP mRNA at the 5' end.

We have established adult hepatocyte lines by transforming adult liver cells with SV40 tsA mutants that are ts in the gene required for maintenance of transformation. The adult hepatocytes should complement the fetal hepatocytes in delineating the mechanisms controlling differentiation, transformation, and maturation. The SV40 tsA mutant-transformed adult hepatocytes are ts for growth and differentiation. They synthesized greatly increased levels of albumin and transferrin at 40°C, the temperature which the cells exhibiting a normal differentiated phenotype. We have now found that the levels of mRNAs for albumin and transferrin were greatly increased at 40°C. Our data indicate that control of differentiation is regulated at the transcriptional level. In addition to albumin and transferrin, we have found that these adult hepatocyte lines expressed two other liver-specific functions, tyrosine aminotransferase (TAT) and fibrinogen. The expression of genes for TAT and fibrinogen was also temperature-sensitive and dependent upon the presence of glucocorticoid hormone. Our studies thus show that differentiation in these hepatocytes is programmed. The simultaneous activation or inactivation of a spectrum of highly specialized functions in these cells might aid in the discovery of factors that regulate the differentiation processes.

In studies on the biosynthesis of human transferrin, an iron-binding glycoprotein and a growth factor for most cultured cells, we found that HepG2 hepatoma cells produced two species of anti-transferrin precipitable polypeptides with apparent molecular weights of 74,500 daltons, predominantly extracellular, and of 72,000 daltons, predominantly intracellar. Secretion of the polypeptides into the media occurred 30-60 minutes after translation. In vitro translation studies of RNA isolated from HepG2 cells suggests post-translational processing perhaps at the membrane level. Human transferrin from normal liver or serum has been shown to be a single polypeptide with apparent molecular weight in the range of 73,200-79,500 daltons. Production of a second transferrin-specific polypeptide by human hepatoma cells may indicate an altered mechanism in transferrin gene regulation. In the presence of glycosylation

inhibitor tunicamycin, a single polypeptide of 71,000 daltons was produced by HepG2 cells. This suggests that the two transferrin-specific polypeptides may have different extents of glycosylation. When cells are grown in the presence of sodium butyrate, the 71,000-72,000 molecular weight species is induced. This may provide a useful system in which transferrin gene regulation in liver cells following transformation may be studied.

IV. Section Biochemical Genetics

The Section on Human Biochemical Genetics continues to pursue the biochemical bases for genetic disorders in man. This involves bench research on cultured cells and physiological specimens and clinical investigation of pathological manifestations and therapeutic interventions.

A primary area of interest remains the lysosomal storage disease, cystinosis. After having shown that the primary defect in cystinosis lies in the deficiency of a lysosomal carrier system for cystine, we further demonstrated how cysteamine (β-mercaptoethylamine) depletes cystinotic lysosomes of cystine. The drug, which passes through both the plasma and lysosomal membranes and participates with intralysosomal cystine in a disulfide interchange reaction, produces cysteine and cysteine-cysteamine mixed disulfide, both of which readily exit the cystinotic lysosomes. This provides the basis for our treatment of cystinotic children with oral cysteamine. After seven years of a clinical trial, we are finding that long-term cysteamine therapy improves growth if begun before 3 years of age, and slows the inexorable glomerular deterioration that characterizes the disease. Only the future will reveal whether cysteamine therapy initiated very early in life can protect fully against kidney failure. We are preparing for the time when that goal becomes reality by extending our knowledge of which other organ systems are clinically involved in cystinosis. We have found cerebral calcifications in a 24-year-old cystinotic man, as well as progressive visual impairment, posterior synecchiae, lens and retinal crystals, severe blepharospasm, and other ophthalmologic complications in several post-transplant cystinotic patients. We now recognize insulin-dependent diabetes mellitus, restrictive lung disease, and decreased salivary gland function as other late manifestations of cystinosis. All these findings will allow us to chart the course of clinical intervention after the issue of renal deterioration is resolved.

Two other clinically relevant discoveries have been made in the past year. The more important, that individuals with renal tubular Fanconi syndrome have a marked plasma deficiency of free carnitine, derives from the fact that 97% of free carnitine is normally reabsorbed by the renal tubules, which are dysfunctional in Fanconi syndrome. Over 20 children with the reabsorption defect, including 19 cystinotics, had mean ($\pm$ SD) plasma free carnitine levels of 11.7 $\pm$ 4.0 nmol/ml, compared with 42.0 $\pm$ 9.0 nmol/ml in normal individuals. The deficiency was shown to result from an increased fractional excretion of free and acyl carnitine, which averaged 33 and 26%, respectively, in Fanconi syndrome subjects compared with 3 and 5%, respectively, in normals. Hepatic oxidation of free fatty acids proceeded normally despite the plasma carnitine deficiency, as evidenced by normal production of β-hydroxybutyrate and acetoacetate after a 24-hour fast in two cystinotic individuals. However, 10 muscle biopsies revealed a mean 60% depletion of tissue free carnitine and lipid droplet accumulation typical of muscle carnitine deficiency. An ongoing protocol involves supplementation with oral L-carnitine (100 mg/kg/day), and

has already revealed that plasma carnitine levels are restored to normal within 24 hours of initiation of therapy. We are hopeful that repeat muscle biopsies after 8 months of carnitine replacement will show resolution of the pathological features of muscle carnitine deficiency.

A second finding relates to the administration of cysteamine, whose chronic (19-59 months) use has been found associated with a blunting of prolactin release upon TRH stimulation in 5 cystinotic children. No abnormality of TSH release was observed, and there appeared no clinical concomitants of the phenomenon.

In one other intriguing disorder, mucolipidosis II or I-cell disease, cystine accumulates within lysosomes. The disulfide is the only amino acid stored, and fibroblasts provide the model system for the study of this occurrence. We measured egress of cystine from lysosome-rich granular fractions of normal, cystinotic, and I-cell fibroblasts. The half-times for such cystine egress were 40 min, infinity, and over 100 min, respectively. This suggested that the cystine carrier was impaired in I-cells, perhaps because it required processing by lysosomal enzymes which are deficient in this disease due to the well-known absence of mannose-6-phosphate recognition markers.

We are also studying two variants of a storage disorder characterized by the intralysosomal accumulation of free sialic acid, namely, free sialic acid storage disease and its Finnish counterpart, Salla disease. Patients afflicted with these disorders manifest mental retardation and poor development in infancy. Cultured cells have lysosomal inclusions and contain thirty times normal amounts of free sialic acid. We have found that the mutant cell granular fractions display negligible egress of free sialic acid, while normals loaded with sialic acid exhibit a brisk egress of sialic acid. This suggests that the disorders represent lysosomal membrane transport defects.

A 31-year-old man came to our attention with a 20-fold increase in plasma methionine. We diagnosed his disorder as methionine adenosyltransferase deficiency by liver biopsy, and have extensively characterized him as clinically normal. This carrier substantial importance for the five young children (each less than 6 years of age) previously reported with the extremely rare disorder. The subject also represents a unique opportunity to determine sulfur flux from methionine to inorganic sulfate in an individual with a major block in the transsulfuration pathway.

In a clinical protocol, we are treating six pyridoxine-nonresponsive homocystinurics with betaine, a source of methyl groups which stimulates the remethylation of homocysteine to methionine. The double-blind, placebo-controlled crossover study is designed to determine if betaine therapy improves generalized osteoporosis, a documented complication of homocystinuria. Computerized tomography is employed as a sensitive indicator of vertebral body bone density, which is low in homocystinuric patients.

Finally, we have shown that cysteamine can react *in vivo* with apolipoprotein E. Isoforms of this protein, which have cysteine replacing arginine at one of two substitution sites, are charge-shifted by cysteamine treatment both *in vitro* and *in vivo*. The demonstration of protein modification by circulating cysteamine makes feasible the treatment by oral cysteamine of diseases with cysteine for arginine substitutions which result in nonfunction proteins. A

prime example would be antithrombin III Toyama, in which a single cysteine for arginine mutation results in an antithrombin III which cannot inhibit the clotting cascade, giving rise to life-threatening thromboemboli. Oral cysteamine therapy may correct the functional defect.

There are several other metabolic disorders whose etiology, clinical characteristics, and treatment we are only beginning to pursue. The Section will continue to attack intriguing cellular mysteries when their solution bears directly on the human condition.

V. Section on Disorders of Carbohydrate Metabolism

This section continues studies on the treatment of the glycogen storage diseases, the kinetics of calcium metabolism in man, and the pathophysiology and treatment of various forms of childhood obesity. We have shown that glycogen storage disorders can be effectively treated by oral administration of cornstarch, replacing the complex and often fatal treatments previously attempted in these diseases. We continue to study means of optimizing therapy, including a study of the therapeutic efficacy of several different naturally occurring vegetable starches. Studies of the metabolic pathways utilized for glucose production in individuals with the glycogenoses has been initiated employing stable isotope methodology to trace metabolic routing. Stable isotope methodology has also been applied to a study of calcium kinetics in man providing the first picture of available pools during pregnancy and in several human disorders of calcium metabolism.

A study of the consequences of magnesium deficiency in the human neonate, with parallel studies in an animal model, the weanling rat fed varying levels of dietary magnesium has been undertaken. This included a retrospective analysis of data from 200 premature neonates with apnea neonatorum to learn whether magnesium supplementation was beneficial. Using the 35 g weanling rat as a model, studies have been concerned with catechlamine release in magnesium deficiency, the interaction of magnesium and calcium in furosemide-treated rats, and light and electron studies of the heart and lung in acute magnesium deficiency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00131-11 HGB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	William A. Gahl, M.D., Ph.D.	Senior Staff Fellow	HGB, NICHD
Others:	Isa Bernardini	Technician	HGB, NICHD
	Juan Bernar, M.D.	Medical Staff Fellow	HGB, NICHD
	Gregory Harper, Ph.D.	Visiting Fellow	HGB, NICHD
	Martin Renlund, M.D., Ph.D.	Guest Researcher	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, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

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

(1) Cystine movement out of lysosomes is enhanced by permeant potassium ions. Cysteamine depletes cystinotic lysosomes of cystine by forming cysteine-cysteamine mixed disulfide, which leaves the lysosome by a process not requiring the cystine carrier, which is defective in cystinosis. (2) Children with nephropathic cystinosis manifest improved growth and slowed renal deterioration if treated with cysteamine before 3 years of age. Children with renal Fanconi syndrome exhibited a marked deficiency of plasma and muscle free carnitine due to failure of the kidney to reaborb carnitine. Carnitine supplementation restored plasma free carnitine levels to normal. Cystinotic children receiving cysteamine chronically displayed a blunted prolactin response to TRH. Heterozygote testing verified that the occurrence of Fabry disease and cystinosis in 2 siblings represented a rare manifestation of the two classical mutations in a single family. Late complications of cystinosis were shown to include involvement of the CNS, eyes, pancreas, lungs, and salivary glands. (3) Mucolipidosis II, or I-cell, fibroblasts were shown to store cystine due to impaired egress of cystine out of isolated granular fractions. The half-times for such egress were over 100 min for I-cell lysosomes and 40 min for normals. (4) Free sialic acid storage disease fibroblasts store free sialic acid in their lysosomes. Sialic acid egress from these lysosomes was negligible compared with normals, suggesting that the disorder represents a defect in lysosomal transport of free sialic acid. (5) A normal 31-year-old man with methionine adenosyltransferase deficiency offers 25 years of additional natural history to the disorder, previously described only in 5 children age 6 or below. (6) Six patients with homocystinuria are receiving betaine therapy in a double-blind, placebo-controlled study to determine if the drug improves vertebral body bone density, measured by CT scan. (7) Cysteamine charge-shifted apolipoprotein E molecules *in vitro* and *in vivo*, making feasible the treatment by oral cysteamine of diseases with cysteine-for-arginine substitutions which result in nonfunctional proteins.

COOPERATING UNITS

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C. Porter, George Washington University Medical Center
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G. Ruley, George Washington University Medical Center

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00133-08 HGB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

PI:	James B. Sidbury, Jr, M.D.	Head	HGB, NICHD
Others:	Alfred Yergey, M.D.		HGB, NICHD
	Joseph Muenzer, M.D., Ph.D.	Medical Staff Fellow	HGB, NICHD
	Abraham Karkowsky, M.D.	Medical Staff Fellow	HGB, NICHD
	Nora Estaban, M.D.		

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, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.0	.05

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The study seeks to investigate the various glucose interactions in the glycogen storage diseases. The approach includes the evaluation of hydrolysis and absorption of cooked and uncooked starches, determination of hepatic glucose production and determination of the flux of glucose metabolites.

The investigation of the responses of children with Type I glycogen storage disease to the administration of different raw starches suggests interfamily differences in the rate of hydrolysis, suggesting polymorphism of pancreatic amylase.

The study of the rate of glucose production by the liver of patients with Type I GSD using ^{13}C-glucose has been initiated. In addition to determining the rate of glucose production by the liver, we will follow the pattern ^{13}C into the Kreb cycle intermediates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00403-04 HGB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)*

PI:	Joan L. Caddell, M.D.	Guest Researcher	HGB, NICHD
Others:	James B. Sidbury, Jr., M.D.	Head	HGB, NICHD

COOPERATING UNITS *(if any)*

Joan Blanchette-Mackie, Ph.D.	Anatomist, Senior Investigator	NIADDK
Kate Snowden, Nate Jackson, Ph.D.	Small Animal Section	
Barry Graubard	Math Statistician	BB, NICHD

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Disorders of Carbohydrate Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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

Research concerned magnesium (Mg) metabolism in the young infant and animal. Two hundred premature neonates with apnea and bradycardia, 93% of whom also had the respiratory distress syndrome, were studied retrospectively to learn whether Mg supplementation was beneficial. Group A = 61 infants who received 5 or more days of Mg Rx (11±1); Group B: 5 with 3-4 days of Mg (3.6±0); and Group C: 134 with 0-2 days of Mg (0.5±.1). Compared with Group A, more Group C infants developed tachycardia ($p<0.001$), had apnea ($p<0.001$), bradycardia ($p<0.001$) and cardiorespiratory crises ($p<0.001$) over a longer period of time; had more prolonged chest retraction and postural drainage and more blood gas derangement. One C died following apnea. After going home, all A infants survived and none were readmitted for apnea; one B was readmitted for apnea and died; and 28 C infants were readmitted for apnea. There were four more C deaths, all diagnosed as the Sudden Infant Death syndrome. Prospective clinical trials of Mg supplementation are planned. Three studies in animal models are reported. 1) Renal calcinosis has been reported in furosemide-treated premature infants. Weanling rats were fed adequate or suboptimal Mg. Furosemide aggravated the Mg deficiency syndrome and was associated with severely disordered Ca metabolism only in rats fed suboptimal Mg. Mg preserved Ca homostasis in furosemide-treated animals. 2). In studies of plasma catecholamines in weanling Mg deficient and Mg-fed rats, stimuli that had no effect on catecholamines in Mg-fed rats provoked precipitous increases in Mg deficient rats. Similar levels were found in Mg-fed rats only after inducing strychnine seizures. 3) Weanling rats were raised and studied aseptically to learn the effect of acute Mg deficiency on the lungs following the sudden seizure episode of that syndrome. Pulmonary findings included edema, hemorrhage and congestion. Mg-fed controls with strychnine-induced tetany had relatively mild changes.

COOPERATING UNITS

George F. Reed, Ph.D.	Math Statistician	BB, NICHD
Howard Hoffman	Chief	BB, NICHD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00404-03 HGB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

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

PI:	Jean DeBrohun Butler, Ph.D.	Senior Investigator	HGB, NICHD
Others:	Dr. William Gahl	Senior Staff Fellow	HGB, NICHD
	Dr. Greg Harper	Visiting Fellow	HGB, NICHD
	Dr. Sondra Levin	Medical Staff Fellow	HGB, NICHD
	Dr. Anil Mukherjee	Senior Scientist	HGB, NICHD

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Dr. Peter Pentchev, NINCDS
Dr. Stephanie Padilla, EPA
Dr. Martin Zatz, NIMH
Dr. Frank Tietze, NIADDK

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, Bldg. 10, Rm. 10N313, 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.)*

1. Study of the treatment of cystinotic fibroblasts with cysteamine, pantethine and WR-1065. All lower cellular cystine levels. Pantethine and WR-1065 are less toxic than cysteamine. Pantethine was used in a clinical trial.
2. Efforts are being made to detect a cystine transport system in lysosomes by isolation of pure lysosomes on pecol gradients.
3. Continued study of metallothionein as a source of cystine in cystinosis. This protein contains 1/3 cysteine residues and is present in a twofold excess in cystinotic fibroblasts.
4. Continued study of a mutant mouse which stores cystine in lysosomes as do cystinotic patients. Possible anomalies in cholesterol and fatty acid metabolism are being investigated.
5. Continued study of the glutathione cycle which, when manipulated with inhibitors or stimulators, will directly vary the cystine levels in cystinotic cells.
6. Availability of diagnostic service for detection of cystinosis in new cases.
7. Inhibition of phospholipase A_2 by uteroglobin was detected.
8. Acylation of membrane proteins is being investigated.
9. Study of cellagen synthesis in osteogenesis imperfecta is being initiated.
10. Teaching of tissue culture techniques and proper use of equipment to new personnel was continued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00405-07 HGB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)*

PI:	Michael A. Zasloff, M.D., Ph.D.	Head	HGB, NICHD
Others:	Samuel Adeniyi-Jones, M.D., PhD.	Visiting Associate	HGB, NICHD
	Janet A. Tobian, Ph.D.	Staff Fellow	HGB, NICHD
	Jose G. Castano, M.D., Ph.D.	Guest Research	HGB, NICHD
	Pilar de la Pena, Ph.D.	Visiting Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Molecular Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	.5

CHECK APPROPRIATE BOX(ES)

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

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

Studies were conducted in the areas of tRNA transport and processing, the molecular biology of the Alu sequence, and the mechanism of mRNA transport. Based on an analysis of some 30 in vitro-generated point mutants in the human $tRNA_i^{met}$ gene, we have shown that mutations which perturb nuclear tRNA transport are clustered in the T and D loops of the molecule, the most conserved portion of the tRNA molecule. All tRNA species defective in transport were also found to be inefficiently processed, suggesting functional linkage of the two systems. The 5' and 3' processing enzymes, the enzyme involved in tRNA maturation in eukaryotes were purified to homogeneity. The 3' processing enzyme is a single polypeptide which cuts the 3' trailer off the pre-tRNA species only after prior removal of the 5' leader. The 5' processing enzyme is a very complex species of about 400,000 in weight consisting of some 15 polypeptides. Recent electron microscopic analysis shows the enzyme to be a ring particle. Studies on the biology of the Alu sequence in mouse have yielded a new conversion pathway for a small cellular RNA of 135 nt. This B1 RNA lies complementary to an intron in the mouse alpha-fetoprotein gene. We have shown that this new RNA is transcribed, processed specifically, transported, and packaged in the cytoplasm of cells into a novel ribonucleoprotein particle. The levels of this species vary between different mouse tissues suggesting a role in tissue-specific gene expression. It may serve as the first example of the way a cell handles a natural anti-sense RNA.

Studies have been initiated on defining the mechanism of mRNA transport. We have made the unexpected observation that mRNA transcription and transport can be uncoupled in the X. laevis oocyte, providing a fruitful system for the study of this critical process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00408-02 HGB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

PI:	Michael A. Zasloff, M.D., Ph.D.	Head	HGB, NICHD
Others:	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 Neufeld, Ph.D., GBB, NIADDK
Suvimol Hill, M.D., Diagnostic Radiology Department, CC, NIH
Muriel Kupfer-Kaiser, M.D., NEI

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

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

Studies at the basic and clinical level continue in several heritable bone disorders and the mucopolysaccaridoses. Purification of the enzyme iduronate sulfatase, the enzyme deficient in the Hunter Syndrome, continued. A clinical study of the use of human amnion membrane implantation into children with the MPS disorders has been completed with the conclusion that the procedure is not effective in either clinical or biochemical correction in several of the MPS conditions. Several clinical findings emerged from the study, including an awareness of the extent of hydrocephalus in the MPS population and the existence of retinal degeneration. The procedures used in patient evaluation should provide appropriate clinical guidelines for subsequent attempts at therapy. Studies of the use of 13-cis retinoic acid in the treatment of fibrodysplasia ossificans progressiva continue with some 14 patients now being treated. Preliminary studies have revealed the presence of markedly elevated blood prostanoids (PGE2) in all patients with FOP. The nature of this compound is under study. Studies in osteogenesis imperfecta continue. Fibroblasts and blood have been collected on several pedigrees to provide material to begin molecular genetic analysis of Type I collagen polypeptides. Studies were continued to determine the structure of the transcriptional unit in bone and fibroblasts for one of the Type I polypeptide genes. Clinical studies on O.I. underway include the use of bracing in infants with O.I. and regulation of the growth hormone axis in the condition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00409-02 HGB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Kinetics of Calcium Metabolism in Childhood

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

PI: James B. Sidbury, M.D.	Head	HGB, NICHD

COOPERATING UNITS *(if any)*

Joseph Muenzer, M.D., Ph.D.	Medical Staff Fellow	LTPB, NICHD
Nancy Vieira	Biologist	LTPB, NICHD
Alfred Z. Yergey, Ph.D.	Research Chemist	LTPB, NICHD

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Disorders of Carbohydrate Metabolism

INSTITUTE AND LOCATION
NICHD, 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.)*

The study utilizes 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.

The study has been amended to include the study of pregnant and lactating women. Our subject has been studied in the third trimester, during lactation and the study will be repeated 6 months after lactation has concluded.

The Prader-Willi Syndrome continues in relation to the genetic components, evidense for altered metabolism and in the future calcium balance studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00909-06 HGB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

PI:	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
	Uwe K. Schumacher, M.S.	Chemist	HGB, NICHD

COOPERATING UNITS *(if any)*

Peter Martin, M.D. Institute of Alcohol Abuse and Alcoholism

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

NICHD, Bldg. 10, Rm. 10N314, Bethesda, Maryland 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.)*

Investigation on the role of genetic predisposing factors in developing fetal toxicity of ethanol is continued. Involvement of genetic factors in the pathogenesis of fetal alcohol syndrome (FAS) is strongly implicated from the facts that (a) only 33% of alcoholic mothers give birth to FAS babies, and (b) there are instances of fraternal twins born of chronic alcoholic mothers where only one of the twins is afflicted with FAS. During the past year, we have shown that precipitating thiamine deficiency in the mother can lead to intrauterine growth retarded (IUGR) babies in the rat. These IUGR babies have been followed-up for their postnatal browth and for their learning ability. We found that the IUGR babies born of thiamine deficient mothers remained significantly lower in their body weight and size, and they were significantly deficient in their learning ability as judged from their exploratory behavior studies compared to their control counterparts. These data suggest that postnatal growth retardation and deficient learning ability, characteristic features of FAS, may be related to thiamine deficiency in utero. Since there are some strains of rats which are more susceptible to thiamine deficiency than others, these sensitive strains are now being studied to see if they have a transketolase with a high K_m for thiamine pyrophosphate (TPP). If so, these animals will be used as models to study predisposing genetic factors in FAS in more detail. To characterize these animals biochemically, we have developed a method for purifying the apotransketolase from various tissues to determine the K_m for TPP. Since the effects of thiamine deficiency is most pronounced in the brain and since most of the studies on transketolase are done on skin fibroblasts or RBC's, it will be important to show whether or not the K_m for TPP of the fibroblast or RBC enzyme is similar to that of the brain enzyme. The new purification technique is now being used to delineate this.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00910-06 HGB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

PI:	Anil B. Mukherjee, M.D., Ph.D.	Head	HGB, NICHD
Others:	Sondra Levin, M.D.	Clinical Associate	HGB, NICHD
	Tadahiro Kikukawa, Ph.D.	Visiting Fellow	HGB, NICHD
	A. Ghazanfari, Ph.D.	Visiting Fellow	HGB, NICHD
	Uwe K. Schumacher, M.S.	Chemist	HGB, NICHD

COOPERATING UNITS *(if any)*

Bryan Cowan, M.D., Dept. Ob/Gyn, University of Tennessee
Elliott Schiffman, Ph.D., NCI
Paul D. Wightman, Ph.D., Merck, Sharp and Dohme Research Laboratories, Rahway, NJ

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

NICHD, Bldg. 10, Rm. 10N314, 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.)*

Studies on uteroglobin continues. During the past decade several proteins have been characterized as exerting an antiinflammatory effect via inhibition of phospholipase (PL) activity. These endogenous phospholipase inhibitors include (i) lipomodulin, a 40K protein isolated from glucocorticoid-treated rabbit neutrophils, (ii) macrocortin, a 15K protein isolated from glucocorticoid-treated rabbit macrophages, and (iii) renocortin isolated from glucocorticoid-treated rat renomedullary cells. It has been shown that these proteins prevent inflammation by inhibiting phospholipase A_2 (PLA2), thereby blocking prostaglandin formation at a step proximal to arachidonic acid release. Since uteroglobin has been shown to be a progesterone-induced antiinflammatory protein in the uterus and corticosteroid-induced protein in the tracheobronchial epithelium of the rabbit, we investigated whether or not uteroglobin is also a phospholipase inhibitor. We found that uteroglobin inhibits PLA2 derived from porcine pancreas as well as from cultured mouse macrophages. A dose dependent inhibition of PLA2 by uteroglobin was observed with a maximum inhibition at a concentration of 20 μM of this protein. We speculate that previously observed effects of uteroglobin, including its immunomodulatory, platelet aggregation inhibitory and uterine antimotility effects, could be explained by one central effect of uteroglobin, i.e., PLA-2 inhibition.

In a related study we have recently discovered that in the human, a protein similar to uteroglobin could be identified by radioimmunoassay, Western blot and HPLC. The human protein has so far been localized in human tracheobroncheal, uterine and prostatic epithelium by immunofluorescence. Further characterization of this protein, including its function, is now being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00912-06 HGB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Gene Regulation and Cellular Differentiation

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

PI:	Janice Y. Chou, 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
	Jay Joshi, Ph.D.	Staff Fellow	HGB, NICHD
	Grace M. Young, M.D.	Medical Staff Fellow	HGB, 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:
4.0	3.5	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our studies have concerned regulation of gene expression during normal and abnormal differentiation processes. Tunicamycin was found to suppress placental alkaline phosphatase (PAP) biosynthesis in addition to inhibiting protein glycosylation. The degradation rate of the unglycosylated PAP monomer was unchanged. Tunicamycin suppressed PAP mRNA activity leading to the observed decrease in biosynthesis. Genes encoding PAP were cloned. Efficient isolation of these genes was accomplished by probing a phage λgt 11 recombinant DNA expression library with polyvalent antibodies directed against highly purified PAP.

α-Fetoprotein (AFP) synthesized by the temperature-sensitive (ts) fetal liver cells exhibiting a transformed phenotype was a glycoprotein of 65,000 daltons. The unglycosylated form of the 65,000-dalton AFP was a polypeptide of 48,000 daltons. Whereas the unglycosylated form of mature AFP from normal fetal liver was a polypeptide of 66,000 daltons. These two AFP variants were arised by translation of two mRNAs of 16S and 20S which were produced from a single AFP gene. The 16S AFP RNA differed from the 20S RNA at the 5' end.

The SV40 tsA mutant-transformed adult hepatocytes are ts for growth and differentiation. They synthesized greatly increased levels of albumin and transferrin and had greatly increased levels of albumin and transferrin mRNAs at 40°C (normal phenotype). These adult hepatocytes expressed 2 additional liver-specific functions, tyrosine aminotransferase (TAT) and fibrinogen. Such expression was temperature-sensitive and glucocorticoid dependent. Regulation of the expression of these liver-specific functions appears related; the studies should aid in the understanding of the differentiation processes.

HepG2 hepatoma cells produced two species of transferrin with apparent molecular weights of 74,500 daltons, predominantly extracellar, and of 72,000 daltons, predominantly intracellar. Human transferrin has been shown to be a single polypeptide of 73,200-79,500 daltons. Production of a second transferrin variant by HepG2 cells may indicate an altered mechanism in transferrin gene regulation.

Z01 HD 00054-11 Structural and Behavioral Analysis of Vocal Communication in Squirrel Monkeys
M. Biben, Ph.D.

Z01 HD 00062-09 Brain Mechanisms of Vocal Production in Squirrel Monkeys
J. D. Newman, Ph.D.

Z01 HD 00702-05 Genetics of Primate Vocal Behavior
J. D. Newman, Ph.D.

Z01 HD 01102-04 Behavioral Correlates of Endocrine Disorders in Children
Robert P. Klein, Ph.D.

Z01 HD 01104-03 An Observational Study of Parent-Infant Interaction in a Family Context
Frank A. Pederson, Ph.D.

Z01 HD 01105-02 A Follow-up Study of Mastery Motivation at 6-1/2 Years
Frank A. Pedersen, Ph.D.

Z01 HD 01106-02 Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity
Stephen J. Suomi, Ph.D.

Z01 HD 01107-02 Adaptation of Laboratory Reared Monkeys to Field Environments
Stephen J. Suomi, Ph.D.

Z01 HD 01108-01 Comparative Studies of Play Behavior
Maxeen Biben, Ph.D.

Z01 HD 01109-01 Infants' Visual Processing of Actions
James D. Demetre, Ph.D.

Laboratory of Comparative Ethology

The Laboratory of Comparative Ethology (LCE) carries out research that is focused on the development of behavior in humans, nonhuman 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 nonhuman primates are correlated with the results of longitudinal studies in human infants and families as well as results obtained by various neuroscience techniques. The LCE consists of 3 sections. The Comparative Behavioral Genetics Section (CBGS) investigates various processes underlying biological and behavioral development in rhesus monkey subjects by focusing on interactions between genetic and environmental factors that affect the course of an individual's ontogeny over a range of levels of analysis. The Brain, Behavior, and Communication Section (BBCS) studies the use of vocal signals by New World primates, both in terms of the physical characteristics of the signals and their information content, and in terms of brain mechanisms associated with vocal communication. The Child and Family Research Section (CFRS) examines cognitive, emotional, and psychosocial development in human infants and children, with special emphasis on determining the effects of early family experience on psychological development. Most of the nonhuman primate research projects are carried out at the NIH Animal Center near Poolesville, Maryland, while the human developmental studies are conducted on the Bethesda campus. Summaries of individual research projects from each LCE section follow.

Comparative Behavioral Genetics Section

The basic research approach in this section, headed by Dr. Suomi, involves long-term longitudinal developmental study of individual monkeys of known parentage who are reared in physical and social settings that are experimentally determined and periodically manipulated in a systematic fashion. Both behavioral and physiological data encompassing several levels of analysis are routinely gathered from each subject under controlled environmental conditions at predetermined age points from birth to maturity. Several long-term studies initiated or continued this past year have focused on individual differences in subjects' reactions to mild environmental challenges at various points during development. These differences in "stress reactivity" appear to have a genetic component, they can be detected very early in life, and they are relatively stable over major periods of development, although both the behavioral and physiological differences between subjects are largely masked when environmental challenges are absent.

The interaction between genetic and environmental factors affecting reactivity is reflected in one major study in which rhesus monkey neonates selected as potential "high reactors," on the basis of their genetic pedigrees, were cross-fostered to multiparous adult females of predetermined reactivity who also differed in their maternal style, as assessed by their care of previous offspring. These high-reactive infants were compared to control infants who were likewise foster-reared. Results of neonatal reflex tests and temperament ratings confirmed

the reactivity status of the infants, with high reactive individuals having more extreme scores on measures of orienting, muscle tone, behavior inhibition, and predominant state prior to one month of age. Nevertheless, the high reactive infants were effectively buffered from environmental challenges by their foster mothers, even those who were high reactors themselves. However, when these infants were briefly separated from their foster mothers at 6 months of age, they reverted to previous response styles, i.e., high reactive infants displayed more extreme behavioral, adrenocortical, and catecholamine responses to separation than did control infants. When reunited with their foster mothers, the high reactive infants once again displayed normal activity and social interaction patterns. Several months later the infants were placed in a small social group containing agemates and an elderly (>25 years) male-female pair ("foster grandparents"). The high reactive infants as a group were slow to adapt to peer group introduction, but the high reactive infant who had been raised by the foster mother judged most nurturant was the first individual to establish a close social relationship with the older monkeys, and as a result it became the highest ranking member of its peer group. Follow-up study of these cross-fostered monkeys will be continued at least through adolescence, and a replication is currently in progress.

Other long-term longitudinal studies examined juvenile and adolescent rhesus monkeys who were either mother-reared for their first 6 months of life or hand-reared in a neonatal nursery and placed in peer groups at one month of age, but whose social rearing environments had been otherwise identical after 6 months of life. When these monkeys were 8, 20, and 32 months of age they were each briefly separated from their social group. Analyses performed to date indicate that (a) there were no major early rearing condition differences in behavioral profiles during group housing, but (b) there were both major age and major early rearing condition differences in behavioral response to separation, with qualitatively different patterns displayed at the different ages and quantitatively more extreme reactions shown by early peer reared juveniles. (c) In contrast, there were no major rearing condition differences in physiological response to separation: both mother and early peer reared monkeys displayed marked increases over preseparation levels of plasma cortisol, heartrate, and CSF MHPG (a norepinepherine metabolite), moderate decreases in CSF norepinepherine (NE), small increases in CSF 5-HIAA (a seratonin metabolite) and no systematic change in the dopamine CSF metabolites HVA and DOPAC. Moreover, age differences in physiological reactions to separation were modest at best, with older subjects generally exhibiting more moderate changes from preseparation values. Thus, although brief social separation produced major changes in both behavior and physiological activity in juveniles of different ages and rearing backgrounds, the behavioral measures seemed more sensitive to differences in these variables, while the physiological measures were more robust across age and rearing background differences.

Detailed analyses of behavioral and physiological effects of the antidepressant drug imipramine administered to adolescent monkeys both during brief separation and during periods of group housing were also completed this year. Substantial individual differences in behavioral reactions to imipramine treatment during separation were found: most high-reactive subjects showed decreases in self-directed behavior and became more passive, whereas low-reactive adolescents (who had displayed little self-directed behavior prior to imipramine treatment) showed activity increases. Imipramine administered during periods of group living was

not associated with pronounced behavioral change in either high or low reactive monkeys. The physiological data presented a more complicated picture: while imipramine clearly reduced separation-elevated levels of plasma ACTH, it had no apparent effect on plasma cortisol levels. Imipramine given during periods of group housing significantly elevated levels of NE and lowered levels of the NE metabolite MHPG but had no consistent effects on either 5-HIAA or dopamine metabolite levels. Separation significantly elevated MHPG and 5-HIAA, and lowered NE, during placebo periods, while imipramine treatment during separation elevated NE above preseparation baseline levels (and reduced MHPG below those levels) without affecting separation-raised levels of 5-HIAA. No consistent drug or separation effects were found for HVA and DOPAC. Thus, the locus of physiological change produced by imipramine treatment during separation was apparently limited to NE among the monoamines and ACTH within the HPA axis.

Another project initiated this past year involved direct comparison of members of the LCE rhesus monkey colony with rhesus monkeys originating in other laboratories or feral colonies. The immediate purpose of such comparisons is to determine the generality of the various reactivity differences that we have already observed within the LCE colony. As a first step, a group of 6 juveniles was obtained from Dr. Seymour Levine's colony at Stanford. These subjects had been reared in social groups and had experienced short-term separations while still at Stanford; they were shipped to the LCE this past year. Of particular interest is the fact that all of the rhesus monkeys in the Stanford colony seem to be similar to the LCE's low reactive monkeys, at least in terms of their behavioral reactions to short-term separation. We are interested not only in comparisons of physiological reactions to challenge in these monkeys but also in any long-term advantages or disadvantages they might exhibit when integrated with LCE monkeys in mixed social groups. Such studies are currently in the planning phase.

Finally, CBGS personnel continued the longitudinal study of a group of 12-year-old rhesus monkeys and two generations of their progeny, all of whom now live year-round in a 5-acre enclosure on the grounds of the NIHAC. The 12-year-old adults were all laboratory born, hand-reared in a nursery, and subsequently put together as a mixed-sex peer group. Despite the fact that none of these now middle-aged monkeys (nor any of their progeny) have had any physical exposure to any other monkeys, since they were first moved outdoors as juveniles they have consistently exhibited the full compliment of species-normative social behavior and group organization reported to date for rhesus monkeys born and living in feral environments. Not only did all group members successfully adapt to the climatic extremes of the Maryland winter and summer, but also they demonstrated seasonally based changes in foraging patterns, diet, and social behavioral activities, including the emergence of a distinct breeding season that once again was consistent with existing data from rhesus monkey groups living in feral environments. Futhermore, the observed similarity of the present group's patterns of social organization and demographic dynamics to those of wild groups was extended this past year to include adolescent male peripheralization, with the group expulsion of the first male offspring to approach adulthood (this young adult male subsequently was successfully introduced to the previously mentioned group of monkeys from Stanford). These results serve to reinforce our previous impressions that major portions of rhesus monkey behavioral repertoires have deep-rooted genetic foundations.

Brain, Behavior, and Communication Section

This section, headed by Dr. Symmes, utilizes behavioral observations and detailed sound spectographic analyses to characterize the conditions under which specific patterns of vocalization are emitted by squirrel monkeys and to identify the information content of each distinct vocal signal. In addition, neuroanatomic and pharmacological techniques are utilized to experimentally modify the production of particular types of vocalization, while subtle species and subspecies differences in sound spectographic patterns of generically similar vocalizations are investigated in genetic studies that involve hybrid and back-crossing selective breeding techniques. The construction of outdoor group housing cages on the NIHAC grounds for the LCE squirrel monkey colony this past year has greatly facilitated the study of vocal exhanges in naturalisitic settings.

One study completed this past year focused on a type of vocalization, termed the "chuck," used in affliative contexts by adult female Gothic arch squirrel monkeys. The relatively high incidence of chuck exchanges between affiliated females was confirmed, and the temporal parameters of such exchanges clarified by higher resolution analysis. Socially popular monkeys uttered chucks which resulted in prompt chuck "answers" from others; less popular monkeys were answered more slowly, and they often repeated themselves. The latency-of-response distributions correlated significantly with ranked affiliative popularity. A significant new finding was that chucks used as answers were higher in peak frequency of several acoustic features than chucks preceded by above average intervals of silence (we have called them "question" chucks). Answers which terminated an exchange (i.e., which were the last in a sequence of 2 or more) were higher in frequency than those which continued an exchange, providing evidence that the original speaker detected and responded to the acoustic difference. Both findings are based on large samples of data and rigorous analyses of variance.

Dr. Masataka successfully carried out another study on the influence of separation distance on the characteristics of a different type of squirrel monkey vocalization, the isolation call or peep (IP). This work has been done utilizing the new outdoor habitats constructed near our laboratory at NIHAC. The results showed that duration of the IP (alone among standardized descriptors of the structural details of this call) varied positively with separation distance. Both juveniles serving as separated subjects (placed singly in a small restraining cage at varying distances from the habitat) and adults serving as home troop members used longer IPs at longer distances. The differences were significant for all monkeys involved. These two sets of findings are of considerable interest in that they provide the first evidence that important information can be conveyed via systematic variation within the same call type emitted by a particular individual, rather than by variation in the sequence of different call types. Such vocal flexibility has not previously been reported in the New World primate species.

Other BBCS studies conducted by Dr. Newman in collaboration with NIMH researchers focused on the production of vocalizations emitted by squirrel monkeys under more tightly controlled laboratory conditions. Two experiments employed behavioral pharmacological techniques to examine the role of specific neurochemical substrates in mediating vocal behavior. The first study utilized drugs that have affinity for binding alpha-adrenergic receptors. One such drug, clonidine, in a dose of 0.1 mg/kg i.m. consistently decreased or eliminated IPs in subjects

separated from conspecifics. Yohimbine in a dose of 0.2 mg/kg reversed the clonidine-related suppression of vocalization in this context, and, in addition, enhanced production of another vocalization, the twitter. In a second pharmacological study, benactyzine, an anticholinergic, was found to potentiate the induction of alarm calls given in response to a model predator. This same drug failed to significantly alter production of IPs. These findings indicate that benactyzine can increase alarm call rate in squirrel monkeys under defined laboratory conditions and may serve as a useful pharmacological probe to study chemical mechanisms mediating production of alarm calls. In a third study, performed in collaboration with Dr. Paul MacLean of NIMH, the role in vocal production of the medial frontal cortex was studied following anterior limbic and neocortical ablations. Postoperative testing completed on four animals provide evidence for a concerted involvement of pregenual and preseptal limbic cortex together with an as yet undefined area of adjacent neocortex in the normal expression of the IP when the subject is visually and acoustically separated from conspecifics.

Previous research in the BBCS had demonstrated consistent differences in the sonographic structure of IPs between Roman arch and Gothic arch (sub)species of squirrel monkeys, and a hybrid breeding program was initiated. This past year Dr. Newman analyzed IPs from Roman and Gothic hybrid infants through their first year of life. One cross-mating involved parents from sympatric populations (Loreto Province, Peru), while the other cross involved individual from geographically separate populations (Roman-arch male from Bolivia, Gothic-arch female from Guyana). As reported last year in a larger group of infant hybrids, the "sympatric" infant produced IPs classified as Roman (like the father, in this case), while the "allopatric" infant's IPs were more Gothic in character. The first viable F_2 hybrid from the project's breeding program was born during FY 85. It had the Roman-arch type of facial phenotype. Tape recordings of its IPs were made at 1 day, 1 week and 4 weeks of age. This infant consistently produced IPs typical of F_1 hybrids from sympatric matings, i.e., intermediate in acoustic character to IPs from the parents. This trait was particularly apparent in this individual, since the father (F_1 offspring of Bolivian x Guyanan cross) consistently produces IPs with a Gothic character while the mother (F_1 offspring of a sympatric Roman x Gothic cross) consistently produces IPs of the Roman phenotype. Addtional F_2 hybrid births are expected this year. The importance of genetic mechanisms for the development of normal communication and their involvement in communicative disorders is widely recognized but poorly understood. The availability of primate models for studying the genetic transmission of species-specific communication patterns provides a unique opportunity for analyzing the genetic mechanisms underlying the expression of communicative behavior in primates.

Finally, this past year Dr. Biben carried out a detailed analysis of play behavior in squirrel monkey juveniles. In this study of social play in 10 yearling squirrel monkeys of the Roman-arch variety, two types of play wrestling were identified which differed in the degree of roughness and were used with different frequencies by males and females. Partner choice and type of play were shown to be strategies used by animals to maximize their chances of winning in play interactions. By using some behaviors selectively to initiate play, animals were able to manipulate the type of wrestling that occurred. Both sexes initiated play more often with same-sex partners, and males responded more often than did females to initiation attempts by either sex. While dominance relationships

outside of play remained stable, role reversal characterized both male-male and female-female play, but not male-female play. Males dominated females both during and outside of play. These results suggest that social play, and particularly the strategies of role reversal and partner choice, may serve important functions in the socialization of young animals. Animals living in complex social groups should benefit from knowing when and how to assume different social roles later in life. This hypothesis will be tested directly in studies currently in preparation.

Child and Family Research Section

This section, headed by Dr. Pedersen, investigates the influences of early experience in the family on the cognitive, emotional, and psychosocial development of human infants and children, as well as how some neuroendocrine functions affect behavioral development. CFRS researchers utilize detailed behavioral observations of children interacting with family members in their natural home environment, structured play and test sessions in the LCE laboratory playroom, detailed interviews, and various standardized questionnaires to generate a longitudinal data base. This past year additional measures of saliva cortisol and telemetry-based heartrate and vagal tone indices were developed for inclusion in future longitudinal studies.

Several studies completed this past year under Dr. Pedersen's direction examined phenomena associated with maternal separations typically experienced by infants in our current society. One series of analyses highlighted distinguishing features of different types of separation experiences. Maternal separations associated with employment occurred more frequently, were of longer duration, began earlier in the baby's first year, and were more likely to occur in a patterned or predictable way than recurrent separations associated with nonreimbursed activities such as health and sports groups, classes, and volunteer work. In addition, caretakers for infants during employment-related maternal separation were less likely to be the infants' fathers, and care was more likely to take place in another home and with other children present than in nonemployment-related maternal separations. Of special interest was the finding that infants whose substitute care arrangements involved experience with peers were more likely to have secure attachment relationships with their mothers, employed or not, when they began their second year of life. This finding is noteworthy in view of other evidence linking early peer experience with greater autonomy and more advanced levels of play later during childhood and nonhuman primate data indicating that early peer experience can compensate in part for loss of mother via separation (in laboratory settings) or death (in feral environments).

The effects of recurrent maternal separation on fathers' relationships with their infants was examined in another study. Here, the interactions of fathers and their 1-year-old infants were compared between men who had relatively extensive experience with their babies in the first year on a one-to-one basis (in situations when the mother was out of the house) and men whose experiences with the baby rarely were independent of the mother's availability. On virtually every measure, men who had more extensive experience with babies on a one-to-one basis in the first year showed numerically higher rates of interaction when observed in a family setting. Significant differences were found in the men's vocalization rates (playful sounds) and in a rating of reciprocal positive

affect between father and infant. The infants also showed significantly higher rates of offering toys to their fathers, further supporting the reciprocal nature of these effects. Further analyses are being pursued to test whether the one-to-one experience had a truly causal impact on fathers or whether the results can be best explained as dispositional differences in fathers, a preference to interact with babies that manifests itself in both electing more one-to-one experiences and in higher rates of interaction as observed in the family setting.

Dr. Demetre this past year completed a major study investigating the cognitive processes utilized by infants in their processing of adults' manual actions and the nature of developmental changes that might occur in the manner of information processing utilized by the infants as they grow older. Three kinds of procedures were employed. First, an experimental procedure in which the experimenter performed various actions "live" for the infant under different conditions of prior exposure to the object in the static state was used to assess comparative processing. Second, a series of procedures involving presentation of visual stimuli via a video recorder was used to assess infants' sensitivity to perceptual and productive components of action. Third, joint play sessions of mother and infant with toys were recorded on videotape for subsequent assessment of infants' productive capacities and the specific details of maternal stimulation with objects. The measure of interest in the first two procedures was duration of visual fixation. Categorical and frequency data of infant and maternal manipulative behavior were coded for the third procedure. These included the frequency with which mothers and infants performed object-specific (demonstrative) acts, the variety of actions produced by the mother with any given object, and the extent to which the infant's manipulations of objects matched those of the mother in terms of content. Results analyzed to date from 9-month-old and 16-month-old subjects indicate that an adult's object-specific actions are attended to longer by 9-month-olds, but not 16-month-olds, following prior exposure to the object in a static state. This indicates that 9-month-olds process actions in terms of stimulus features of the object encoded in memory (comparative processing). Further experimental manipulation of the actions presented to infants indicates that the mere juxtaposition of a hand and an object previously experienced in isolation, and a hand moving an object previous experienced as static, are not sufficient in themselves to elicit comparative processing in infants of this age. These findings suggest that changes accompanying specific actions with objects alone are responsible for the eliciting characteristics of manipulative actions. Such actions served to highlight differentially or transform certain features of the object which are not readily decoded by infants when presented with static objects.

Dr. Klein's major research 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, it was found 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 during the past year have focused on 3 issues: (1) Closer examination of the previously reported relation between age and behavior problems revealed that children who did not receive treatment until 8 years old were at very high risk (85%) for clinical levels of behavior problems.

Dr. Klein is currently determining what characteristics of this group could be responsible for this finding. (2) There was no effect of treatment for reported behavior problems even though the treatment was effective in reducing sex steroid and gonadotropin levels. This suggests that the adjustment difficulties seen in this sample are not the direct result of hormanal action. (3) Comparison between the idiopathic cases and cases matched on Tanner stage data from an ongoing NIMH study of normal puberty showed several differences in behavioral adjustment. In several areas the probands showed evidence of greater adjustment difficulties than did the control cases, particularly with regard to hyperactivity. (4) In contrast to previous research, Dr. Klein found no evidence of verbal IQ superiority in a sample of girls aged 5 to 8 with idiopathic precocious puberty.

Finally, data collection for a long-term Child and Family Research Branch project was completed by University of Maryland contract employees. This project investigated the development of mastery motivation. Three of the studies employed the same sample of 75 children to study the interrelationships between cognition, motivation, and the environment at 6, 12, and 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. A separate study was conducted to investigate the expression of mastery motivation in Down syndrome infants. The current focus of analysis of data collected in the 6, 12, and 30 month studies is directed toward explicating the relationship between mastery motivation, competence, and social abilities. The results indicate that in 6- and 12-month-old infants, manipulative activities directed toward success with objects represent an important dimension of their behavioral repertoire. Moreover, these early manifestations of the motivation to be competent are an important aspect of later cognitive abilities. While these findings extend through to the early schoolage years, it appears that the quality of mother-infant attachment serves to mediate these relationships. With regard to the development of mastery motivation and social competence in Down syndrome (DS) infants, it appears that in comparison to a cognitively normative sample, DS infants are not as adept at integrating the object-related and socially-directed domains into ongoing streams of behavior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00054-11 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural and behavioral analysis of vocal communication in squirrel monkeys

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

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

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Comparative Ecology

SECTION
Section on Brain, Behavior and Communication

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.5	.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.)*

Continuing studies of vocal usage during affiliative behavior have yielded new and signficant findings. Considerably more is now known about the way adult female squirrel monkeys use chuck vocalizations in such affiliative contexts than at the time of publication of our first papers on this subject. We have described in detail the temporal parameters of sequences of chucks. Information theory analysis has demonstrated that at least two prior calls influence the probability of individual utterances. Study of the intervals involved in chuck exchanges and the structural details of the calls themselves have broadened our understanding of this communicative process. It is clear that socially popular monkeys (those preferred by several other group members as huddle partners) participate in more exchanges, and receive "answers" to their chuck calls with the shortest latencies. Less popular monkeys receive fewer answers and more often the calls of others follow at long latencies, i.e., they tend to repeat themselves more often. For all animals, several measures of chuck structure are altered depending on the position of the call in a sequence. Use of semi-natural outdoor habitats has permitted us to obtain new results demonstrating that the duration of the Isolation Peep depends both in adults and juveniles on the perceived distance separating them. Taken together these findings provide new and unique insights into the ways in which New World primates may vary the parameters of their communication system to convey information.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00062-09 LCE

PERIOD COVERED

October 1, 1984 to September 30, 1985

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)*

PI: J. D. Newman Research Physiologist LCE, NICHD

COOPERATING UNITS *(if any)*

LCS, NIMH (P. D. MacLean); CNB, NIMH (J. R. Glowa)
Johns Hopkins School of Medicine (J. C. Harris)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Brain, Behavior and Communication

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

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

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

This project investigates the effects of neurological and pharmacological interventions on the incidence and structure of squirrel monkey vocalizations emitted during vocalization-inducing contexts in a laboratory setting. This year, three separate studies were conducted. In one, the role in vocal production of the medial frontal cortex was studied following anterior limbic and neocortical ablations. Postoperative testing completed on four animals provide evidence for a concerted involvement of pregenual and preseptal limbic cortex together with an as yet undefined area of adjacent neocortex in the normal expression of the isolation call when the subject is visually and acoustically separated from conspecifics. The other two studies employed behavioral pharmacological techniques to examine the role of specific neurochemical substrates in mediating squirrel monkey vocal behavior. One of these studies investigated the role of drugs showing specific binding for alpha-adrenergic receptors. Clonidine in a dose of 0.1 mg/kg i.m. consistently decreased or eliminated isolation calls in subjects separated from conspecifics. Yohimbine in a dose of 0.2 mg/kg reversed the clonidine-related suppression of vocalization in this context, and, in addition, enhanced production of another vocalization, the twitter. In a second pharmacological study, benactyzine, an anticholinergic, was found to potentiate the induction of alarm calls given in response to a model predator. This same drug failed to significantly alter production of isolation calls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00702-05 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI: J. D. Newman Research Physiologist LCE, NICHD

COOPERATING UNITS *(if any)*

Duke University Primate Center

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Brain, Behavior and Communication

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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

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.)*

This project investigates the role of genetic factors in primate vocal development. Research strategies employed in these studies include analysis of vocal traits in hybrid offspring having vocally distinctive parents, comparing the vocal traits of full and half sibs with unrelated age-matched infants, and analysis of the effects of differential rearing on normal vocal development. This year, additional evidence was found in 2 hybrid squirrel monkeys studied through their first year of the different isolation call inheritance patterns between crosses involving vocally distinctive sympatric (Peruvian Gothic x Peruvian Roman) and allopatric (Guyanan Gothic x Bolivian Roman) populations. A pilot study of the effects of restricted rearing on vocal and other social behavior in squirrel monkeys suggests that exposure to conspecifics during the first year of life is not essential for normal expression of isolation and display calls, nor for participation in play and quiet affiliative interactions following introduction into a social group. An ongoing comparative study of prosimian isolation call characteristics obtained the first recordings from a 6-month old Lemur mongoz, and added data from a second infant Lemur fulvus sanfordi. Infant isolation calls from representative Lorisidae (lorises and galagos) were found to consist of single or multiple clicks, in striking contrast to the tonal isolation calls occurring in all other primates so far examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01102-04 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:	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:
3.6	1.1	2.5

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 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 during the past fiscal year have focused on 3 issues: (1) Closer examination of the previously reported relation between age and behavior problems revealed that children who did not receive treatment until 8 years old were at very high risk (85%) for clinical levels of behavior problems. We are currently determining what characteristics of this group could be responsible for this finding. (2) There was no effect of treatment for reported behavior problems even though the treatment was effective in reducing sex steroid and gonadotropin levels. This suggests that the adjustment difficulties we have seen in this sample are not the direct result of hormonal action. (3) Comparison between our idiopathic cases and cases matched on Tanner stage data from an ongoing NIMH study of normal puberty showed several differences in behavioral adjustment. In several areas the probands showed evidence of greater adjustment difficulties than did the control cases, particularly with regard to hyperactivity. (4) In contrast to previous research, we found no evidence of verbal IQ superiority in a sample of girls aged 5 to 8 with idiopathic precocious puberty.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01104-03 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:	J. D. Demetre	Visiting Fellow	LCE, NICHD
	R. L. Cain	Research Psychologist	LCE, NICHD
	J. D. Suwalsky	Research Psychologist	LCE, NICHD
	P. W. Berman	Guest Researcher	LCE, NICHD

COOPERATING UNITS *(if any)*

Parent and Child, Childbirth Education Associates,
University of Maryland (B. A. Rabinovich)

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:
3.00	2.80	.20

CHECK APPROPRIATE BOX(ES)

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

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

This project currently encompasses four areas of investigation based on several different samples with a total of approximately 200 families as participants. All studies were conducted with middle class families and first-born infants. The focal period of the research is from early infancy through the first two and a half years of life. Procedures vary with each sample, but include observations of mother-infant and father-infant interaction in the natural home environment, structured interactions in the laboratory, interviews, and questionnaires. The first area of inquiry concerns the effects of maternal workforce participation on the child's early experiences. Three studies were performed with employed and homemaker mothers, one involving time-sampling observations of mothers and fathers with their infant in the home, a second involving continuous behavioral records of mother-infant interaction both prior to and after mothers resumed employment, and the third involved structured laboratory observation of mother-toddler play. A second area of inquiry focused on normally occurring mother-infant separation experiences. A number of psychological dimensions were found to distinguish different types of separations, indicating that there exists considerable heterogeneity subsumed under the concept of "separation." Different psychological consequences were found with regard to security of attachment, distress in separation situations, and reactions to unfamiliar adults in separation contexts depending upon variation in past separation experiences. A third area of inquiry is concerned with the father's role in the family, both in "traditional" (single-earner) families and in families where both parents are employed. Distinctive aspects of father-infant interaction were identified for fathers who had more extensive experience on a one-to-one basis with their babies. The fourth area of inquiry concerns 3-person interactions, the mutual regulation of visual, vocal, proximity, and contact behavior of mothers, fathers, and infants depending upon the parents' psychological accessibility to the child and their degree of verbal engagement with one another.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01105-02 LCE

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
Other:	J. D. Demetre	Visting Fellow	LCE, NICHD
	F. T. Hunter	Research Psychologist	LCE, NICHD
	L. Martini	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

MRRC, NICHD (P. M. Vietze); Institute for Child Study, University of Maryland (R. H. MacTurk, M. E. McCarthy)

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:
1.0	.667	.333

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.)*

Several interrelated studies have investigated 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, and 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. A separate study was conducted to investigate the expression of mastery motivation in Down syndrome infants. The current focus of the 6, 12, and 30 month studies is directed toward explicating the relationship between mastery motivation, competence, and social abilities. The results indicate that in 6- and 12-month-old infants, manipulative activities directed toward success with objects represent an important dimension of their behavioral repertoire. Moreover, these early manifestations of the motivation to be competent are an important aspect of later cognitive abilities. While these findings extend through to the early school-age years, it appears that the quality of mother-infant attachment serves to mediate these relationships. With regard to the development of mastery motivation and social competence in Down syndrome (DS) infants, it appears that in comparison to a cognitively normative sample, DS infants are not as adept at integrating the object-related and socially-directed domains into ongoing streams of behavior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01106-02 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

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: C. E. Eisele Research Psychologist LCE, NICHD

COOPERATING UNITS *(if any)*
LCS, NIAAA (M. Linoilla); CNB, NIMH (T. R. Insel, R. D. Delizio); LN, NIMH (E. Murray); Department of Psychiatry, University of Wisconsin-Madison (G. W. Kraemer); Primate Laboratory, University of Wisconsin-Madison

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Comparative Behavioral Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.55	.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.)*

This project involves longitudinal study of rhesus monkey biobehavioral ontogeny, emphasizing investigation of individual differences in reactions to mild environmental challenges and determination of long-term developmental consequences under standardized rearing conditions. This past year 2 new studies were initiated, data collection was extended in 3 ongoing long-term studies, and major data analyses were completed in 2 other experiments, all of which is summarized as follows: (1) A neonatal assessment system, characterizing rhesus monkey newborn reflex development and temperamental stability, was validated on 4 independent infant samples and subsequently used to identify newborns at high risk for displaying extreme reactions to future environmental challenges. (2) Neonates identified as potential future high reactors (and low reactive control neonates) were cross-fostered with adult females who themselves differed in temperamental qualities and maternal rearing styles. Reactivity differences among infants were largely masked in the presence of their foster-mothers but reappeared when they were challenged via physical separation from these adult females. (3) Longitudinal study of older infants reared either by mothers or in all-peer groups revealed major rearing condition differences in behavioral reaction to separation challenge but similar patterns of physiological response, as assessed by levels of plasma cortisol and CSF monoamine metabolites. (4) Differences in social dominance among peer-reared juveniles were related to HPA reactivity differences, both of which were found to be associated with paternal pedigree. (5) High and low reactive juvenile and adolescent monkeys were compared in their biobehavioral response to the antidepressant imipramine, while (6) a new study examining their performance on cognitive tests of short-term memory under both baseline and challenge conditions was initiated. Finally, (7) direct comparisons with rhesus monkeys from different laboratories (and presumably different genetic pools) were begun.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01107-02 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adaptation of Laboratory Reared Monkeys to Field Environment

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

PI:	S. J. Suomi	Head	LCE, NICHD
Other:	P. O'Neill	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*
CNB, NIMH (G. DiGregorio)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Comparative Behavioral Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	.50

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

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

This project involves the longitudinal study of a group of 12-year-old rhesus monkeys and two generations of their progeny, all of whom live year-round in a 5-acre enclosure on the grounds of the NIHAC. The 12-year-old adults were all laboratory born, hand-reared in a nursery, and subsequently put together as a mixed-sex peer group. Despite the fact that none of these now middle-aged monkeys (nor any of their progeny) have had any physical exposure to any other monkeys, since they were first moved outdoors as juveniles they have consistently exhibited the full compliment of species-normative social behavior and group organization reported to date for rhesus monkeys born and living in feral environments. The past 12-month period was the first complete annual cycle that the present group had spent entirely in the same outdoor environment since its inception. Not only did all group members successfully adapt to the climatic extremes of the Maryland winter and summer, but also they demonstrated seasonally based changes in foraging patterns, diet, and social behavioral activities, including the emergence of a distinct breeding season (and subsequent birth season), that once again were consistent with existing data from rhesus monkey groups living in feral environments. Furthermore, the observed similarity of the present group's patterns of social organization and demographic dynamics to those of wild groups was extended this past year to include adolescent male peripheralization, with the group expulsion of the first male offspring to approach adulthood. These results serve to reinforce our previous impressions that major portions of rhesus monkey behavioral repertoires have deep-rooted genetic foundations. Additions to and modifications of the subjects' physical environment within the 5-acre enclosure were also completed this past year, and formal designs for a second enclosure and plans for its constituent monkey group were initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01108-01 LCE

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI: M. Biben Senior Staff Fellow LCE, NICHD

Other: D. Symmes Head LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Brain, Behavior, and Communication

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

This project uses the comparative method as a technique to better understand the functions of play in the behavioral repertoire of developing squirrel monkeys. Statistical analysis and manuscript preparation relating to data collected in the prior year was completed. In this study of social play in 10 yearling squirrel monkeys of the Roman-arch variety two types of play wrestling were identified which differed in the degree of roughness, and were used with different frequencies by males and females. Partner choice and type of play were shown to be strategies used by animals to maximize their chances of winning in play interactions. By using some behaviors selectively to initiate play, animals were able to manipulate the type of wrestling that occurred. Both sexes initiated play more often with same-sex partners, and males responded more often than did females to initiation attempts by either sex. While dominance relationships outside of play remained stable, role reversal characterized both male-male and female-female play, but not male-female play. Males dominated females both during and outside of play. Role reversal represents a strategy encouraging play between youngsters of diverse abilities and provides needed experience in a variety of social roles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01109-01 LCE

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Infants' Visual Processing of Actions

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

PI: J. D. Demetre Fogarty Visiting Fellow LCE, NICHD

COOPERATING UNITS *(if any)*

CRMC, NICHD (P. M. Vietze)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	0	.15

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 aim of the project is to investigate some of the cognitive processes utilized by infants in their processing of adults' manual actions. Individual studies in the project address four specific objectives: (1) to provide more detailed experimental confirmation of previous research indicating that infants at 9 months of age processed mothers' manual actions with objects as transformations of a static object; (2) to investigate 9- and 16-month-olds' sensitivity to the productive and perceptual complexity of actions observed; (3) to ascertain whether infants' sensitivity to the productive complexity of actions is related to their capacity for spontaneously generating complex actions; finally, (4) to establish whether infants' characteristic modes of processing information generated by adults' actions are related to the frequency and variety of mothers' manual actions during object-centered interactions. To date, the first specific research objective has been met, and the findings provide strong support for the hypothesis that the younger infants process adults' manual actions as transformations of a static object. Thus, the processing of an adult action is prolonged in 9-month-olds, but not in 16-month-olds, following prior exposure to the object in a static state. Most of the data addressing the remaining three issues are in hand, and they are currently being coded and analyzed.

Z01 HD 00073-14 Regulation of Immune Systems at the Cellular Level
Edgar E. Hanna, Ph.D.

Z01 HD 00918-04 Expression of Histocompatibility Antigens During Early Mammalian Development
Keiko Ozato, Ph.D.

Z01 HD 00920-04 Molecular Structure of Mouse Histocompatibility (H-2) Genes:
Keiko Ozato, Ph.D.

Z01 HD 01300-03 Enhancement of Immunogenicity of Capsular Polysaccharides Pathogenic Bacteria
Rachel Schneerson, M.D.

Z01 HD 01301-03 Human Immune Response to Polysaccharide-Protein Conjugate Vaccines
Rachel Schneerson, M.D.

Z01 HD 01302-03 Toxins of Pertussis: Isolation, Characterization and Mechanisms of Action
Ronald D. Sekura, Ph.D.

Z01 HD 01304-03 Protective Effect of Vi Polysaccharide Antibodies Against Typhoid Fever
John B. Robbins, M.D.

Z01 HD 01306-02 Pertussis Heat Labile Toxin (HLT): Isolation and Characterization
R. D. Sekura, Ph.D.

Z01 HD 01307-02 Pertussis Toxin: An Approach to a New Pertussis Vaccine
R. D. Sekura, Ph.D.

Z01 HD 01308-02 Conjugation of Pneumococcal Vi Polysaccharides with Carrier Proteins
S. C. Szu, Ph.D.

Z01 HD 01309-02 Bacterial Polysaccharides Cross-Reactive with Meningococcal Group A Polysaccharide
R. Schneerson, M.D.

NICHD ANNUAL REPORT
October 1, 1984 to September 30, 1985

Laboratory of Developmental and Molecular Immunity

The Laboratory conducts research into the developmental and molecular biology aspects of "natural" and immunization-induced immunity to antigens important in the pathogenesis of diseases of the newborn, infant, and young child. Three areas of interest can been identified: 1) Emphasis upon the immuno-pathogenic mechanisms of these infant and childhood bacterial diseases, development of vaccines for their prevention and characterization of the age-related immuno-regulatory mechanisms of the young host. Methods are devised in order to provide more rapid and accurate diagnosis of infectious diseases of infants, 2) Class I transplantation antigen genes, an essential recognition system for the recognition of cell associated antigens, are modified by site-specific mutagenesis in order to determine the structural basis for the immunologic polymorphism and function of these antigens at the DNA level. The activation and expression of paternal Class I transplantation antigen genes is being studied in developing embryos, 3) Lastly, the immuno-regulatory function of lymphoid cells and the effect upon these activities exerted by bacterial toxins of medical interest is being studied in vitro using hybridomas constructed of T-cells at various stages of their differentiation and development.

Robbins and Schneerson continue their work on the pathogenesis and prevention of invasive diseases of infants and children due to encapsulated bacteria. Their emphasis has been primarily on Haemophilus influenzae type b and pneumococci. These two bacteria remain the serious cause of morbidity and mortality in infants and young children, despite the availability of effective antibiotics and supportive therapy.

Recently, the H. influenzae type b polysaccharide vaccine, initially developed in the NICHD laboratories in the early 1970's, was licensed and received the recommendation of the American Academy of Pediatrics and the U.S. Public Health Service for "universal immunization of all children at 2 years of age". During this interval, clinical studies indicated the age-related and T-independent immunogenicity of this antigen which limits its use in young infants. Methods were then developed to covalently bind the H. influenzae type b polysaccharide to proteins in order to increase its immunogenicity and confer the properties of T-dependence. The H. influenzae type b polysaccharide-tetanus toxoid conjugate was the first of a series of prototype conjugate vaccines to be synthesized and evaluated in the laboratory. Following an extensive study of these conjugates in laboratory rodents, and in juvenile and then infant primates, it was concluded that these conjugates had both increased immunogenicity and T-cell dependent properties as compared to induce protective levels of antibodies in infant primates after using an immunization course, and dosage consistent with that used for routine infant immunization in the United States. This was of particular importance because of lesser reactivity of this non-human species toward this class of antigens as compared to humans. Two clinical studies were conducted with these conjugates. The first was in young adult volunteers at Davidson College in North Carolina. Two injections of the conjugates were given 3 weeks apart and the antibodies measured by radio-

immunoassay and a class specific ELISA in order to determine both the isotype and subclasses of the polysaccharide antibodies induced by the vaccines. All volunteers responded with a 4-fold or greater increase in type b antibodies which rose to about 180-fold above their pre-immunization level. These levels declined to about one-half of these values 6 months after immunization. This represents about a 20-fold increase in immunogenicity over that of polysaccharide alone. The conjugate induced antibodies had all the biologic properties associated with immunity toward H. influenzae type b and pneumococcus infection. The H. influenzae type b conjugate was used in young adults in Goteborg, Sweden, with defined immunodeficiency disorders involving one or two subclasses of IgG. Seventeen of the eighteen immuno-deficient patients responded with levels of antibodies indistinguishable from those achieved in the adult volunteers. Only one, an individual with a combined subclass and IgA deficiency, failed to respond with a 4-fold or greater increase in antibodies. Clinical studies in infants, ages 18 months to 24 months, are planned. The animal data and the volunteer studies strongly suggest that the connjugates will induce protective levels of antibodies in infants. Accordingly, epidemiologic information has been gained and preliminary contact with the appropriate authorities in Goteborg, Sweden, have been established in order to plan a double-blinded study in which the nearly synthesized H. influenzae type b polysaccharide conjugate vaccine and the pertussis toxin (toxoid) vaccine will be studied for both their immunogenicity and effectiveness in this country. Pneumococcai infections cause otitis media, a common and often chronic, infection of infants which is a major cause of acquired hearing loss in the United States. Clinical trials with pneumococcal vaccine have shown that immunization-induced capsular polysaccharide antibodies confer protection against otitis media. Yet, the current pneumococcal vaccine is not satisfactory for prevention of otitis media in infants and children, that age group with the highest attack rate, because of its age-related immunogenicity and its failure to induce a booster response (T-independent). Two approaches have been used to solve this problem of inducing pneumococcal immunity. The first, is to study the possibility of devising a species-specific, rather than a type-specific pneumococcal antigen. A covalent conjugate between a species-specific surface antigen, C-polysaccharide, and several carrier proteins was achieved using a novel method for binding these two different macromolecules. The C-polysaccharide was chosen because of previous reports indicating that antibodies to phosphocholine (PC) could protect mice against pneumococcal infection. The C-polysaccharide protein conjugate was injected into rabbits and induced antibodies to the otherwise non-immunogenic C-polysaccharide. Antibodies to the C-polysaccharide, as well as other components of pneumococci were induced by unencapsulated pneumococcus with an unusually long C-polysaccharide chain. The C-polysaccharide antibodies, induced by the C-polysaccharide protein conjugate, had no reactivity with PC. PC was present in the conjugate as shown by its reactivity with monoclonal PC antibody. The C-polysaccharide antibodies induced by this vaccine did not protect mice. Mice were protected by passive immunization with antibodies by immunization with unencapsulated bacteria. Yet, absorption of this sera and bacteria with the purified C-polysaccharide failed to protect its protective activity. These experiments showed that the PC-containing structure that induced protective immunity was probably not the C-polysaccharide and the search for other PC-containing structures is under way. Another approach was to create a more immunogenic capsular polysaccharide, of an optimum molecular weight and homogenous polysac-

charide, for synthesis into protein conjugates. The smaller, more homogeneous, polysaccharide has been synthesized in protein conjugates and its immunogenicity and other serologic properties are being defined.

Typhoid fever also is an example of an invasive disease due to encapsulated bacteria, Salmonella typhi. The capsular polysaccharide of this bacteria is called Vi antigen. The immunopathogenic role, and its potential for a vaccine, of the Vi antigen in typhoid fever has been reviewed by Robbins and his group. On the basis of this review, two clinical lots of a Vi polysaccharide prepared under the denaturing conditions, were evaluated by volunteers. The degree of adverse reactivity was shown to be related to the amount of LPS. The serum antibodies elicited by these two Vi Polysaccharides were shown to be equal to those observed after convalescence from typhoid fever and equal to those elicited by the current whole cell vaccine. In addition, the availability of a pure Vi polysaccharide has prompted reinvestigation into the serologic response to disease. It has been found that determination of the Vi antibody level has been both a reliable and rapid method for identifying asymptomatic carriers of S. typhi. Asymptomatic carriers are difficult to identify by conventional methods of culture. This diagnostic method has now permitted the identification of carriers and, therefore, a more effective control of typhoid than heretofore possible. A new method for covalently binding the Vi polysaccharide to carrier proteins, including cholera toxin, has been devised. This Vi-cholera toxin conjugate has superior immunogenic properties to the Vi polysaccharide alone and its clinical use, is being evaluated. Two clinical effectiveness trials of a Vi polysaccharide with low LPS content, have been planned and an effectiveness trial should be initated in early 1986.

The age-related development of protective antibodies to the capsular polysaccharides of common encapsulated bacterial pathogens, such as meningococcus, pneumococcus, and H. influenzae type b, has been shown to be largely stimulated by a continual interaction with non-pathogenic flora of the respiratory and gastrointestinal tracts. The origin of Group A meningococcal capsular polysaccharide antibodies, however, has never been clearly identi-fied. In collaboration with Egyptian investigators, two Escherichia coli with capsular polysaccharides (K antigens) were shown to be capable of inducing bactericidal and precipitating antibodies to the Group A polysaccharide. The structure of these two E. coli were identified. It was of interest that the more cross-reactive one, the E. coli K93 capsular polysaccharide, for no obvious structural relation to the Group A polysaccharide. The methods to study the sterochemistry of these two related polysacharrides, E. coli K93 and Group A meningococcus, are under way.

Pertussis remains a major health problem despite its rarity in the United States. Control of pertussis has been achieved by mass immunization with vaccines composed of whole inactivated Bordetella pertussis. While these vaccines have been effective in virtually eliminating pertussis, their use has come under increasing scrutiny because of the adverse reactions associated with their use. Sekura has developed methods for the large scale production of pertussis toxin (PT), an extra cellular protein excreted by B. pertussis. Investigations have indicated that serum anti-PT has an enzymatic action, ADP ribosylation, similar to other of the binding B. pertussis to mammalian cells was studied using the glycoprotein, fetuin, as a model compound. PT binding to fetuin was shown to be due to its interaction with the asparagine-linked oligosaccharide. A novel method was devised in order to inactivate its

oligosaccharide. A novel method was devised in order to inactivate its enzymatic (toxin) activity while retaining at least one-third of its antigenicity. The detoxified PT was shown to induce protection against intracerebral challenge with B. pertussis and to protect the immunized animal against the lethal effects of PT. A series of assays to monitor both the retained antigenicity, immunogenicity, and toxicity, were devised that could be used for standardization of a PT vaccine for clinical use. In addition, serologic methods for both the absolute measurement and biologic assay of antibodies induced by the vaccine and those after convalescence from disease, were established. It is planned to study this novel toxoided PT in sequential clinical studied involving adults first; then, infants and children. Ultimately, the pertussis toxoid vacxcine will be used in a double-blinded trial along with the H. influenzae type b vaccine in Goteborg, Sweden, to characterize its effectiveness in preventing trhese two common serious diseases of infants and children.

B. pertussis is only a pathogen for humans; pertussis has never been established in an animal species. B. pertussis secretes a variety of biolgoically active extra cellular materials, including adenylate cyclase and an activity referred to as heat labile toxin or dermonecrotic toxin. This later activity has never been characterized. Sekura and his associates have isolated this HLT from sonicates of the B. pertussis cells. They have found that HLT is an unstable protein and has extraordinarily high toxicity and unexplained propensity to cause involution of the spleen of sucking mice. The structure functions relation, and its immunopathogenic role is under scrutiny by a variety of techniques, including studying the effect of passive immunization with polyclonal and monoclonal antibodies.

The Class I major histocompatibility antigens are polymorphic membrane glycoproteins required for antigen recognition by T-cells. They are complex molecules composed of at least three domains. The strucutre function relationships of these Class I antigens were studied by site directed mutagenesis, in which the DNA sequence of a Class I gene was changed at desired positions by recombinant DNA technology. A mutant lacking the disulfide bridge in the domain most proximal to the membrane (external domain), was incapable of expressing the Class I antigen in the plasma membrane. The antigen was synthesized, as evidenced by its accumulation within the cell, but could not be expressed in the plasma membrane. This observation, and other kinetic studies, with other mutant antigens, led Ozato to proposed that the disulfide bridge in the third domain was essential for transport of the Class I antigen from the endoplasmic reticulum to the plasma membrane. Another mutant, lacking all the endolinked glycosylation sites, was produced by three step mutagenesis. The resultant mutant antigen was found to be expressed on cell surfaces, although at a reduced level. The expressed antigen was, however, reactive with both cytotoxic T-cells and a panel of antibodies specific for the Class I wild type antigen. This data indicated that carbohydrate moieties are not essential for the immunogenicity and immune function of the Class I antigens. This experimental approach, using site-directed mutagenesis and DNA recombinant technology, will be used to completely identify the structure function relations of the Class I antigens and will be extended to other histocompatibility antigens.

The ontogeny of Class I histocompatibility antigens has been the subject of numerous investigations. The experimental data resulting from these studies, which was based upon evidence with polyclonal antisera, was contradictory and

not generally accepted. Ozato has shown trhat the Class I antigens are expressed at about 9 to 10 days of fetal development in the mouse. This conclusion was based upon studies using the well-defined panel of monoclonal Class I MHC antibodies and several immunochemical assays. Homozygous mice, differing only at the Class I MHC locus, were used. Their expression can be accelerated by interferon, (including) alpha/beta or gamma). The paternal expression of Class I antigens after exposure of the fetus to antibody was studied. Paternal MHC Class I antibodies were administered to the fetus via the placental circulation by passive immunization of the mother. Cellular distribution of these passively administered antibodies were studied. The monoclonal antibodies were detected in various fetal tissues and inhibited the expression of these Class I antigens. This finding, using highly specific antibodies, with care to examine the quantitation of their inter-action with cell antigens, provides a powerful tool for studying the ontoeny of these structures and their effect upon immune function.

Another aspect of ontogeny of Class I antigens, was studied by using early embryo and embryonal teratocarcinoma cells. The ontogeny of the Class I antigens has been studied by using the model of retrovirus infection. It has been known that production of retroviruses does not occur in early embryo or teratocarcinoma cells. These teratocarcinoma cells and embryonic cells, from as early as 8 days after fertilization, could be productively infected by some mouse leukemia viruses of various interferon groups. There was no relation between the expression of the Class I antigens and susceptibility to infectivity. However, the administration of interferon, as was observed in vivo, could result in the expression of the MHC CLass I antigens in thse in vitro cells. It is planned to study trhe condition for an experimental system in which Class I gene expression can be inhibited. Several approaches are under consideration, including the construction of anti-sense DNAs for Class I genes which produce transcripts complimentary to the Class I gene message. These anti-sense DNAs are planend for introduction into fertilized mouse embroys in order to obtain transgenic mice in which Class I gene expression can be inhibited throughout the animal's life. In addition, the effect of passive immunization, with paternal histocompatibility monoclonal antibodies upon the expression of the Class I genes will also be studied.

Hanna's groupd studied the regulation and control mechanisms in immune systems and how microbial products, such as the streptococcal exotoxin (SPE) and pertussis toxin (PT), influence or deregulate these immune systems. Investigations are under way to: 1) understand the mechanisms by which thymus-derived TR-cells regulate antibody formation and secretion by interactions with bone marrow derived precursors (B-cells) of antibody forming cells, 2) to understand the interrelationships between the various T-cell phenotypes, from the precursor stage through development of helper and suppressor functions. A number of monoclonal lines and hybridomas which express regulatory T-cell functions (both helper and/or supressor) have been constructed, from fractionated spleen cells of wild type mice and nude mice. Some of these hybridomas, from nude mouse splenocytes, express surface molecule (Thy 1, Tla, Lyt 1, Lyt a, and Lyt 3) and functional phenotypes which classify them as model prescursors of regulatory T-cells.

To undersand how, effector or cytotoxic T-lymphyocyte (CTL) responses are regulated and whether other T-cells which regulate Ig formation by B-cells may also regulate CTL, a number of CTL clones have been derived by repeated

stimulation with the antigen and in the continuous presence of T-cell growth factors (TCGF). Some of these CTL clones are restricted in their recognition of $H\text{-}2K^{k}\text{-}^{TNP}$ and others are restricted in their recognition for alloantigens.

Experiments are also directed towards understanding how microbial agents such as SPE and PT deviate or divert thymus-derived T-cells towards different phenotypes or different functions. Previous experiments suggested that SPE deregulates the down regulation of T-cell dependent antibody responses. Current experiments, using monoclonal T-cell lines, show that the effective target of SPE responsible for the observed activity are precursor T-cells, rather than mature suppressor T-cells. Pertussis toxin is also suppressive for the development of B-cell (PFC) responsses. Cytotoxic T-lymphocyte (CTL) responses to alloantigens are also suppressed by both SPE and pertussis toxins. Since CTL-cloned lines are not suppressed by eigher SPE and PT, it is suggested that either CTL precursors or regulators of the CTL responses, may be the site of action by these toxins. Although SPE and PT suppress CTL responses, experiments to-date, suggest that these two toxins may have different cellular sites of action in the generation of CTL suppression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00073-14 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Immune Systems at the Cellular Level

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

PI:	Edgar Hanna	Head	LDMI, NICHD
Others:	Prince Arora	Staff Fellow	LDMI, NICHD
	Michael Walker	Biologist (Tech)	LDMI, NICHD
	Keiko Ozato	Research Microbiologist	LDMI, NICHD
	Ronald Sekura	Research Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)*
P. Skolnick, LBC, NIADDK; C.Hansen, VRS, DRS

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:
3.2	2.2	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.)*

We wish to understand regulation and control mechanisms in immune systems and how microorganisms influence or deregulate these systems. In order to do this we have constructed monoclonal lines of immunocytes (hybridomas) which express the functions of regulatory T-cells. Cytotoxic T-cell clones were derived by selective growth in the presence of T-cell growth factors (TCGF) and IL2 with the continuous presence of stimulating antigen(s). We have constructed hybridomas from splenocytes of nude mice bearing T-cell markers. Some of these express surface markers and functional phenotypes which allow us to classify them as model precursors of regulatory T-cells. Modulatory effects of bacterial toxins such as the streptococcal exotoxin, SPE and pertussis toxin (PT) have been tested in parallel on these cell lines in vitro. Our previous experiments suggested that SPE deregulates the down regulation of T-cell dependent antibody responses. Current experiments using the monoclonal T-cell lines show that the effective target of SPE responsible for the observed activity is a precursor of T-cells rather than mature suppressor T-cells. Both SPE and PT are suppressive for CTL responses. Since CTL clones are not suppressed, it is suggested that precursors or regulators of CTL may be the target of SPE in the effector T-cell immune system as well. Pertussis toxin is suppressive for both development of B-cell responses (PFC) and CTL. The target cells of PT and SPE appear to be different since PT is inactive on subpopulations of splenocytes containing precursors and active suppressors. At the molecular level, the genomes of the precursor-type T-cells from nude mice, appear not to be rearranged from germ-line or they are rearranged differently as compared with T-cells cloned from wild type.
The variable expression of T-cell surface markers by T-cell hybridomas has been associated with the coordinate expression of both allelic markers of the fusion parent cells (NFR x BW5147/AKR).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00918-04 LDMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	Keiko Ozato	Head	LDMI, NICHD
Others:	Yu-Jui Yvonne Wan	Visiting Fellow	LDMI, NICHD
	Bonnie Orrison	Chemist	LDMI, NICHD
	Susan Adeniyi-Jones	Visiting Associate	LDMI, NICHD

COOPERATING UNITS *(if any)*

A. Rein, LBI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland

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:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project aims at elucidating the role of major histocompatibility Class I antigen during embryonic development and in the maturation of the immune system. To this end we have studied the timing of Class I antigen expression in mouse embryogenesis throughout gestation, starting the egg cylinder stage by testing appearance of surface Class I antigens and of specific mRNA. A low, but significant amount of mRNA was detected in embryos later that day 9 (early somite, heart beating stage): concordantly, the earliest surface Class I antigens were detected in day 10 embryos. Class I gene activity in embryos remained much lower than in the adult until the time of birth. These findings led us to conclude that Class I genes are developmentally regulated, and become active only after primordial organogenesis. Interferons (IFNs) were found to be capable of inducing Class I antigen in embryonic cells which otherwise do not have mRNA or surface antigens, suggesting a developmental role of interferon for MHC gene activation. Subsequently, a number of permanent cell lines has been established from mid somite stage embryos by transformation with a retrovirus (HaSV). These cells showed either a low or no Class I antigen expression, in agreement with the characteristic observed in in vivo embryos. Further, these cells expressed a high level of the antigen upon treatment with IFN. In addition, undifferentiated embryonal carcinoma F9 cells representing very early embryos were found to respond to IFN. F9 cells normally express neither Class I message nor surface antigen; within 3 hours after addition of IFN, Class I specific mRNA becomes detectable. The majority of the cells display surface Class I antigen reaching at maximum 3 days after the treatment. Unlike retinoic acid which induces morphological differentiation as well, Class I antigens induction by IFN treatment is not accompanied by cellular differentiation. These cells will be used as an in vitro model system to study the mechanism of Class I gene activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00920-04 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	Keiko Ozato	Head	LDMI, NICHD
Others:	Jun-ichi Miyazaki	Visiting Fellow	LDMI, NICHD
	Toby Sivlerman	NRSA Recipient	LDMI, NICHD
	Ronald Lieberman	Guest Researcher	LDMI, NICHD

COOPERATING UNITS *(if any)*
E. 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:
0.45	0.45	0

CHECK APPROPRIATE BOX(ES)

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

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

Major histocompatibility (MHC) Class I antigens are polymorphic membrane glycoproteins that are necessary for antigen recognition by T-cells. To elucidate structure-function relationships of the antigen, we have employed site directed mutagenesis, in which DNA sequence of a Class I gene can be changed at desired positions. Expression and function of the mutant class I genes were examined in L cells after DNA mediated gene transfer. By using the mouse H-2L^d gene, we addressed the question of the role of highly conserved amino acids of the Class I antigen: those involved in the formation of disulfide bridges and in glycosylation. A mutant lacking the disulfide bridge in the domain most proximal to the membrane (3rd external domain) was generated by a single nucleotide substitution. We found that the mutant antigen is incapable of expressing the antigen in the plasma membrane, and that a large quantity of the antigen accumulates within the cytoplasm. This was verified by immunocytochemistry and immunoprecipitation domain specific antibodies. These studies also indicated that the mutant antigen is glycosylated and associated with β-2 microglobulin. These observations led us to conclude that the disulfide bridge in this domain, conserved throughout Ig gene super family evolution, is essential for transport of the antigen from the endoplasmic reticulum to the plasma membrane. A mutant lacking all the N-linked glycosylation sites was produced by a 3 step mutagenesis. The resultant mutant antigen was found to be expressed on the cell surface, though at a reduced level, and is reactive both with cytotoxic T-cells and with a panel of antibodies specific for the wild type antigen. This data indicated that carbohydrate moieties are not essential for antigenicity and immune function of the antigens. Quantitative measurement of cell surface versus intracellular mutant protein led us to conclude that carbohydrates are important primarily for efficient intracellular transport of the protein to the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01300-03 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enhancement of Immunogenicity of Capsular Polysaccharides Pathogenic Bacteria

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

PI:	Rachel Schneerson	Senior Investigator	LDMI, NICHD
Others:	Dietmar Tietz	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*
A. Chrambach, ERRB, NICHD; John Owens, NICHD; Philip Serwer, University of Texas Health Sciences Center, San Antonio, Texas

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:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

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

The first part of this project was reported by A. Chrambach, Project Number: Z01 HD 00165-09 LDMI.

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 epiglottitis and from carriers, was enriched utilizing their adherence properties to human RBC, as verified by EM.
Two of these Hib strains were grown on solid media homoginized and pili isolated by several cycles of isoelectric precipitation followed by ammonium sulfate precipitation. Final purification utilized preparative gel electrophoresis. Rabbit antisera were prepared with these pili preparations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01301-03 LDMI

PERIOD COVERED: October 1, 1984 to September 30, 1985

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

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

PI:	Rachel Schneerson	Senior Investigator	LDMI, NICHD
Others.	John B. Robbins	Head	LDMI, NICHD
	Zhen Wang	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*: G. Schiffman, State University, New York; J.C. Parke, Jr., Charlotte Memorial Hospital, North Carolina; J. Schlesselman, USHUS, Bethesda, Maryland; C. Bell, University of Illinois at Chicago

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:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Haemophilus influenzae type b is the leading cause of bacterial meningitis in infants and children and a major cause of septicemia, septic arthritis pneumonia and epiglottitis. Pneumococcus type 6A is a major cause of otitis media and the most frequent pneumococcal type causing meningitis and pneumonia. Anticapsular antibodies are protective; their induction by purified capsular polysaccharide is hampered by both their poor immunogenicity in the young age group and lack of anamestic response. In contrast, conjugates composed of these polysaccharides covalently bound to tetanus toxoid were immunogenic in laboratory mice and infant rhesus; this response could be boosted by further injections. Simultaneous injections of both conjugates with tetanus toxoid 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 TT, 50 µg/dose; Group 3: Hib-TT, 50µg + Pn6A TT, 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 100 µg dose. 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 antibodies increased 180 fold, Pn6A: 7 fold, and TT 10-20 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 levels to the vaccine components or the rate of antibody rise to the side effects of the vaccines. The isotype and biological activities of the conjugate-induced Hib antibodies have been analyzed and compared to those induced by the polysaccharide alone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01302-03 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:	Ronald Sekura	Research Chemist	LDMI, NICHD
Others:	Marie-Jose Quentin-Millet	Guest Researcher	LDMI, NICHD
	Yan-ling Zhang	Visiting Fellow	LDMI, NICHD
	Nathaniel 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, NICHD; R. Downs, University of Virginia; E. Hanna and K. Kratt, 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:
0.8	0.3	0.5

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

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

Bordetella pertussis, microorganism which causes the disease commonly known as whooping cough, produces several toxins (i.e., pertussis toxin (PT) and heat labile or dermonecrotic 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. The structure of the carbohydrate responsible for interaction with PT has been characterized and binding constants have been determined. The toxic action of PT is mediated by toxin catalyzed transfer of the ADP-ribose moiety from NAD to the adenylate cyclase regulatory component, N_i. This action of PT has been used as a probe to explore the role of N_i in regulation of cell function, and the changes of the regulatory component in response to desensitization. These studies have shown that PT catalyzed modification of N_i reduces receptor affinity and abolishes the action of GTP in producing the high affinity binding state without blocking the capacity of nonhydrolizable GTP analogs to mediate cyclase activation. Use of PT as a probe to examine N_i following glucogen desensitization of MDCK cells showed increased availability of N_i for ADP-ribosylation. This leads to the suggestion that hormone induced desensitization results from increased N_i or mobilization of N_i to pools that affect receptor mediated regulation of adenylate cyclase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01304-03 LDMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Protective Effect of Vi Polysaccharide Antibodies Against Typhoid Fever

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

PI:	John B. Robbins	Head	LDMI, NICHD
Others:	Shousun C. Szu	Senior Staff Fellow	LDMI, NICHD
	Rachel Schneerson	Senior Investigator	LDMI, NICHD

COOPERATING UNITS *(if any)*

H. Koornohof, South African Institute of Medical Research; I.L. Acharya, Infectious Disease Hospital, Kathmandu; R. Kumar, All India Institute of Medical Sciences; C.U. Lowe, OD, NICHD; M. Cadoz, Institute Merieux, Lyon France

LAB/BRANCH

Laboratory of Developmental and Molecualr 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	0

CHECK APPROPRIATE BOX(ES)

☒ (a) 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 in under-developed nations. The immunopathogenic role of the capsular polysaccharide of Salmonella typhi (Vi), the causative agent of typhoid fever, remains controversial. There is much evidence the serum Vi antibodies could confer protection against typhoid fever. Typhoid fever is a disease of humans only; there is no satisfactory animal model. Clinical studies are required therefore, to evaluate the effectiveness of Vi vaccination to induce antibodies against typhoid fever. Collaborative studies with Dr. H. Koornhof, South African Institute for Medical Research, Dr. I. L. Acharya, Infectious Disease Hospital Kathmandu, Nepal, and Dr. Ramesh Kumar, All India Institute of Medical Sciences, New Delhi, India, have been established to study the prevalence of Vi antibodies in the general population, the age-specific attack and the effectiveness of vaccines designed to induce Vi antibodies. A Vi antibody assay has been established as an accurate, sensitive method to identify carriers. Four surveys of Vi antibodies in populations with different attack rates of typhoid have been completed. The first measured Vi antibodies in U.S. armed forces recruits injected with the typhoid bacterial vaccine. Pre-immune Vi antibodies were < 0.2 ug/ml in 49/50. Three surveys in individuals of various ages in Chile, Eastern Transvaal, and Kathmandu, showed that adults had considerably higher Vi antibodies than those in the U.S. Vi antibodies elicited by the whole cell vaccine and by injection of Vi were comparable. Post-immunization levels of Vi antibodies were, however, lower than those induced by typhoid fever and those elicited by other capsular polysaccharide vaccines. Vi polysaccharide with lower LPS content has been prepared by the Institut Merieux and will be studied in Nepal and in Eastern Transvaal. A Vi-Cholera toxin conjugate has been prepared and standardized; it is about 15 times more immunogenic in mice than the Vi alone. Clinical studies with this new vaccine are being planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01306-02 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:	Ronald Sekura	Research Chemist	LDMI, NICHD
Others:	Yan-ling Zhang	Visiting Fellow	LDMI, NICHD
	Robin Roberson	Chemist	LDMI, NICHD
	Li Xiuru	Guest Worker	LDMI, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

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

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

Bordetella pertussis produces several protein toxins including pertussis toxin (see project Z01 HD 1302) and dermonecrotic toxin (heat labile toxin or HLT). HLT, injected subcutaneously into suckling mice, induces 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, homogeneous preparations of HLT have been obtained. These studies show that the toxin is a single polypeptide chain with a molecular weight of about 150,000.

The purified toxin has been subjected to amino acid analysis and has been used to produce antibodies. These antibodies have been used to document the purity of current toxin preparation. In addition, HLT antibodies neutralize toxic activity and high titer sera (1/20,000) have been obtained from immunized rabbits. HLT, in addition to mediating a dermonecrotic lesion, was shown to be lethal, induce a short term elevation (3 days) of white blood cells, and elicit a marked atrophy and "bleaching" of the spleen. The significance of these changes to possible biochemical mechanisms of action and their relation to the pathogenesis of pertussis are being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01307-02 LDMI

PERIOD COVERED: October 1, 1984 to September 30, 1985

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:	Ronald Sekura	Research Chemist	LDMI, NICHD
Others:	Yan-ling Zhang	Visiting Fellow	LDMI, NICHD
	Nathaniel Tolson	Biologist	LDMI, NICHD
	Birger Trollfors	Visiting Fellow	LDMI, NICHD
	Brett Acton	Biologist	LDMI, NICHD
	Robin Roberson	Chemist	LDMI, NICHD

COOPERATING UNITS *(if any)*: J. Shiloach and B. Kaufman, NIADDK; C. Johnson, NIAID

LAB/BRANCH: Laboratory of Developmental and Molecular Immunity

SECTION: Section on Bacterial Disease Pathogenesis and Immunity

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.67	1.07	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

.The incidence of pertussis has been controlled by use of our current whole cell pertussis vaccines. Recent advances in identification of pertussis toxin (PT) as a major protective antigen against pertussis affords an opportunity to produce a new vaccine with improved safety and efficacy. Project Z01 HD 01302 is directed at characterizing the biochemical action of PT; the current project concentrates on development of methods for production of PT, as well as methods for neutralization of toxic action and assessing the immune response to toxins thus neutralized. Growth conditions and purification methods scale production of PT under conditions that are suitable for human usage. B. pertussis cultivated in a 100 l fermentator, yields more than 200 mg of purified PT. This level of PT production makes practical the preparations of experimental vaccines in sufficient quantities to permit clinical trail. Assay methods for monitoring the toxicity and neutralization of toxicity of experimental vaccine preparation have been established. In addition, methods have been established to monitor the retained antigenicity of toxoided PT preparations, and to monitor immune response to PT-toxoids in mice, monkeys, and humans, and also to measure vaccine efficacy in mice and tissue culture. Experimental pertussis vaccines have been developed which show promise for use in humans. Pertussis toxoids, prepared in our laboratory, show only minimal levels of residual toxic activities, while antigenic activity is only partially reduced. Experimental vaccines prepared by absorption of pertussis toxoid onto aluminum hydroxide are highly antigenic and elicit a PT specific immune response. Immunization with this pertussis toxoid protects mice against both bacterial and toxin challenge, elicits serum antibodies which neutralizes PT in the CHO cell assay. These data predict that this pertussis toxoid will be effective in the prevention of pertussis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01308-02 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	Shousun Chen Szu	Senior Staff Fellow	LDMI, NICHD
Others:	John B. Robbins	Head	LDMI, NICHD
	Zhen Wang	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*
John L. Inman, Laboratory of Immunology, NIAID

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:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

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

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

Several monoclonal antibodies with phosophocholine (PC) specificity confer species-specific protection against virulent encapsulated pneumococci of several types. The cell-wall polysaccharide (C-Ps) is most likely to be the pneumococcal structure reacting with these PC antibodies. The C-Ps is of low molecular weight (c.a. 10,000) composed of a hexasaccharide repeating unit containing a ribitol phosphate ester at its reducing end and is non-immunogenic. The Vi capsular polysaccharide (Vi-P) is a potential protective antigen of Salmonella typhi, the causative agent of typhoid fever. The Vi alone, despite its unusually high molecular weight, is a poor immunogen in adult volunteers. Heterofunctional reagents, N-succinimidyl 3-(2-pyridil-thio)-propionate (SPDP) and succinimidyl 3-(2-iodoacetamide) (SIP) have been studied to devise methods for synthesis of polysaccharide-protein conjugates that could be considered for human use. The N-hydroxysuccinimide ester is the more reactive of the two functional groups of SPDP; accordingly, carrier proteins were derivated to about 6 moles SPDP/mole protein. No conformational change in the derivatized protein could be detected by circular dichroism measurement. The C-Ps was derivatized via its amino group with SPDP also. The Vi poly was then reacted with cystamine in the presence of a water-soluble carbodiimide to create a disulphide bridge. The disulfide derivatives of both C-Ps and Vi-Ps were reduced and the newly formed alipathic thiol groups were then allowed to react with the protein-SPDP derivative under thiol-disulfide exchange to form the disulfide linked Ps-protein conjugate. The resultant C-Ps and Vi-Ps-protein conjugates were considerably more immunogenic than the polysaccharides alone and are under study for their protective activites and as candidates for clinical studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01309-02 LDMI

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	Rachel Schneerson	Senior Investigator	LDMI, NICHD
Others:	Nabil Guirguis	Visiting Fellow	LDMI, NICHD

COOPERATING UNITS *(if any)*
J.D. MacLowry, CC; W. Egan, OOB; A. Bax, LCP, NIADDK; Ida & Frits Orskov, Statens Serum Institute, Copenhagen; E.C. Gotschlich, The Rockefeller University

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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

CHECK APPROPRIATE BOX(ES)

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

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

Similar to other encapsulated bacterial pathogens, serum anti-capsular polysaccharide antibodies confer immunity to invasive meningococcal diseases. Group A meningococcal diseases have a different epidemiological pattern than the other two major pathogenic meningococcal Groups B and C. In central Africa, Group A meningitis is endemic with a high frequency; in other parts of the world, Group A meningococci causes epidemics. In both situations, asymptomatic carriage of Group A organisms is low. In the U.S., Group A meningococcal meningitis or carriage have been virtually unknown for the past 35 years. Yet, most children and adults have protective levels of Group A meningococcal antibodies. Investigations to elucidate the origin of this "natural" immunity to Group A meningococcus revealed 11 E. coli strains of 645 stool samples from Egypt cross-reactive with meningococcal Group A. The cross-reactive antigens were the K51 and K93 capsular polysaccharides of these E. coli. Subsequently, K93 and K51 strains, identified by the anti-serum agar technique, were found among isolates from patients at the Clinical Center, NIH, and in a study of stool isolates from children in Copenhagen. The K polysaccharides were isolated and their structures elucidated: K93: 3)-D-Gal-(1-4)-D-GlcUA-(1-, acetylated at O-5 and O-6 of the galatosyl and K51: 3)-D-GlcNac-1-PO_4, acetylated at O-6. The cross-reaction between the K93 and the Group A meningoccal polysaccharide was unexpected since they share no monosaccharide components. Immunization of rabbits with E. coli K93 or K51 strains induced bactericidal and precipitating antibodies to Group A meningococci. These K93 and K51 E. coli strains did not synthesize enterotoxins and were non-invasive in in vitro assays. Absorption studies, using pre and post immune sera from individuals injected with the Group A meningococal polysaccharide vaccine, are planned to study the origin and specificity of "natural" and immunization-induced Group A meningococcal antibodies in inhabitants of the U.S. and Africa.

Z01 HD 00047-16 Biochemical Studies of Neuronal and Other Cell Types
Douglas E. Brenneman, Ph.D.

Z01 HD 00048-11 Studies of Transcriptional Control of Neurobiologic and Development Phenomena
Bruce K. Schrier, M.D., Ph.D.

Z01 HD 00064-09 Neurobiologic Studies of Neurons and Glia in Cell Culture
Phillip G. Nelson, M.D., Ph.D.

Z01 HD 00094-15 Pineal Regulation: Environmental and Physiological Factors
David C. Klein, Ph.D.

Z01 HD 00095-15 Pineal Regulation: Transsynaptic and Intracellular Mechanisms
David C. Klein, Ph.D.

Z01 HD 00704-03 Tetanus Toxin Effects and Localization in Neurons
(Inactive)

Z01 HD 00706-01 Physiological Studies of Nervous System Development In Vitro
Gary L. Westbrook, M.D.

Z01 HD 00707-01 Pharmacological Studies of Synaptic Transmission In Vitro
Mark L. Mayer, Ph.D.

Z01 HD 00708-01 Morphologic Studies of Neuronal and Non-Neuronal Cells in CNS Cell Cultures
Elaine A. Neale, Ph.D.

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

October 1, 1984 to September 30, 1985

The Laboratory of Developmental Neurobiology is concerned with mechanisms of central nervous system (CNS) function and of regulation of CNS development at the cellular, membrane, and molecular levels, with emphasis on the use of in vitro systems. The Laboratory, divided into two Sections and a Unit, is composed of five closely interacting research groups.

Information transfer in the nervous system is effected by the mechanisms responsible for synaptic transmission and this has been a focus for LDN activities. Results important for long-term modulation of synaptic efficacy have been obtained this year, with regard both to presynaptic transmitter release mechanisms and to postsynaptic receptor properties. Significant progress has been made on understanding neuron-glial relationships related to neuronal survival and development. A number of cell-specific immunocytochemical probes have augmented substantially our ability to analyze differential development of neuronal sub-populations in our culture systems. Successful cloning of the choline acetyltransferase gene opens up the possibility of rigorous analysis of the regulation of the expression of the important gene. A new concept of pineal-brain relationships has been introduced by the striking finding that processes from pineal cells extend into the nervous system. Progress has been made in the immunologic and molecular genetic analysis of the regulation of pineal gland metabolism.

1. Section on Neurobiology

This Section includes three closely interacting groups involved with physiological (P.G. Nelson), biochemical (D. E. Brenneman), and morphological (E.A. Neale) studies of neuronal development and function in cell cultures of the mammalian CNS.

Dr. Brenneman has now made a compelling case for a peptide-mediated interaction between neurons and glia that has a major impact on neuronal survival and maturation during a critical period of nervous system development. Vasoactive intestinal peptide (VIP) is released as a consequence of electrical activity by the VIP-containing neurons in our spinal cord cell culture system. This VIP acts upon glial cells and causes them to release a macromolecular factor which is required by neurons for survival and development. The evidence for this novel and important schema is as follows. Previous studies showed that a population of spinal cord neurons grown in culture died during a critical developmental period and that electrical blockade further decreased neuronal survival and expression of the cholinergic marker, choline acetyltransferase (CAT). Addition of VIP (at 10-12 - 10-8 M concentration) prevented both the naturally occurring cell death and that which was associated with electrical blockade. We have now shown that addition of VIP antiserum or a carboxy fragment of VIP (VIP_{10-28}) produces decreases in neuronal survival and CAT activity that are indistinguishable from those produced by electrical

blockade with tetrodotoxin (TTX). VIP_{10-28} acts at concentrations of 10^{-12} - 10^{-8} M. We have shown that high affinity VIP binding sites are present on non-neuronal, glial cultures, and conditioned medium from glial cultures stimulated with VIP (10^{-10}M) prevented TTX-mediated neuronal death, whereas conditioned medium from similar cultures not treated with VIP had no such effect.

This work is particularly exciting for two reasons: 1) it represents a concrete example of neuron-glial interaction of major significance for neuronal survival and development that lends itself to analysis at the membrane and and molecular level. As pointed out by one of the deans of experimental neuropathology in a recent review article on developmental malformation and mental retardation, "Elucidation of the molecular mechanisms that govern these multiple neuron-glial interactions is among the foremost challenges to clinical and cell biological investigation." 2) The activity-dependent neuronal survival that is mediated by glial-derived trophic material involves cholinergic neurons. Cholinergic systems in the mammalian central nervous system are becoming increasingly identified as playing a critical role in the expression of plasticity in the nervous system. Learning and memory deficits are associated with pharmacological or neuropathological interruption of these cholinergic systems, and understanding the molecular factors involved in their maintenance is of primary scientific and social significance to neuroscience.

It is a high priority, for the effective analysis of the culture systems we are using, to be able to identify clearly the different cell types in the cultures. Dr. Neale has achieved substantial progress in this regard with a number of cell specific immunocytochemical probes. Neurons can be distinguished from non-neuronal cells; cholinergic, GABA-ergic and various peptidergic neurons can be identified, as well as oligodendrocytes, astrocytes, and fibroblasts. Receptors (e.g., opiate and muscarinic cholinergic binding sites) can be quantitated both biochemically and radioautographically. Cholinergic neurons consist of both large motoneuron-like cells and smaller cells of various shapes; the number of these cholinergic neurons in a culture can be affected markedly by culture conditions. The number of astrocytes is reduced substantially in serum-free medium. Further exploitation of these powerful techniques is anticipated.

The physiology group has continued studies aimed at understanding nervous system function in terms of discrete physiologically identifiable membrane molecules, in particular ion channels and receptors, as well as cell biologic mechanisms such as internal Ca^{++} sequestering systems.

We had previously shown that major portions of the synaptic bouton apparatus mediating synaptic transmission may be inactive under physiologic conditions and would represent a synaptic reserve available for activation under appropriate circumstances. We have now shown that inadequate intracellular regulation of calcium ion concentration and the blocking effect of high $[Ca^{++}]_i$ on the voltage-sensitive calcium conductance mechanism may account for some of this synaptic inactivation. We use depolarizing voltage clamping pulses to induce calcium currents (I_{Ca}) which carry calcium ions into the neuron. Repetitive activation of this sort produces an inactivation of I_{Ca} due, at least in part, to an increase in $[Ca^{++}]_i$. This inactivation is much more prominent

in dorsal root ganglion (DRG) than in spinal cord (SC) neurons. We can also show that repetitive activation of DRG neurons is accompanied by a much greater reduction in transmitter output than is the case for SC neurons. DRG synaptic boutons contain a lower concentration of mitochondria than do SC boutons; mitochondria are related either directly or indirectly to calcium sequestration. We suggest that the ability to maintain an appropriate $[Ca^{++}]_i$ varies between different cells and for individual boutons, and represents a mechanism for regulating the long term functional status of synaptic structures.

The excitatory synaptic response evoked by interaction of presynaptically released transmitter with its postsynaptic receptor has been elucidated. Previous work had shown that two classes of excitatory receptors could be identified, those activated selectively by aspartate or n-methyl-D aspartate (NMDA) and those activated by kainate or quisqualate. The use of antagonists selective for these different receptors indicates that an excitatory amino acid receptor, but not the NMDA type, mediates fast excitatory neurotransmission between spinal cord neurons. The time course, voltage dependence and reversal potential of the excitatory synaptic currents have been examined. The synaptic current decays as a single exponential with a time constant of 0.5 - 1 msec; this sets an upper limit on the channel lifetime of synaptically activated channels. No voltage sensitivity of the decay time constant or channel conductance was detected between -80 and 0 mV. Calculations based on recent estimates of the conductances of single kainate or quisqualate activated channels suggest that about 300-750 channels are activated per synaptic site.

The ubiquitous distribution of NMDA activated channels and their powerful effect on neuronal excitability indicate the importance of understanding the properties of these receptors and their relationship to kainate activated channels. Previous work had shown that Mg^{++} ions enter and block the NMDA channel but have no such effect on kainate channels. Experiments with other divalent cations suggested that Ca^{++} ions might penetrate the NMDA channel but not the kainate channel. Direct experiments indicate that this is the case. This selective Ca^{++} ion permeability has important implications for the physiological role of amino acids beyond that traditionally ascribed to fast excitatory transmitters because of the broad metabolic regulatory role of intracellular Ca^{++}. It is of interest that recent evidence suggests that intracellular calcium activity is elevated during long-term potentiation (LTP, an important form of neural plasticity) and that NMDA receptor antagonists block LTP.

We have begun the study of the receptors and channels related to excitability and synaptic functions during early developmental stages in culture. This promising area has been opened up by technical advances using various types of patch recording electrodes. We have shown that the density of voltage-sensitive Na^+ channels increases markedly during the first week in culture, but the kinetics of Na^+ activation are not altered. A differential developmental pattern for excitatory amino acid receptors has been demonstrated with kainate activated channels showing a much more dramatic increase during the period of active synapse formation than do NMDA or quisquilate activated channels.

Infection of sensory neurons in culture with herpes viruses has revealed highly specific effects of the viral infection on functional membrane consti-

tuents. Na^+ channels, but not Ca^{++} channels, and specific K^+ channels were blocked by one strain of viruses. Cell fusion and electrical coupling were induced by other strains. Acyclovir, which blocks replication but not absorption or penetration of the viruses, prevented the electrophysiological effects of the viruses.

A membrane conductance mechanism important for regulation of neuronal excitability has been demonstrated in a subpopulation of sensory ganglion neurons. In those cells, entry of calcium ions during the action potential produces a large increase in chloride permeability, resulting in a prolonged post-spike depolarization.

We have begun physiological experiments directed at understanding central cholinergic function. As mentioned above, we can now visualize presynaptic cholinergic structures with choline acetyltransferase immunocytochemistry and postsynaptic muscarinic receptors using radioautographic techniques. We have developed a system incorporating cholinoceptive (and potentially cholinergic) superior cervical ganglion neurons into the spinal cord cultures. We have demonstrated post- and probably pre-synaptic muscarinic effects on the components of this system, but substantial further work is required and justified.

2. Molecular Neurobiology Unit

The Molecular Neurobiology Unit, under Dr. B.K. Schrier, has produced cDNA libraries, presumed to be differentiation-specific, by cascade hybridizations, and subsequent cloning characterization of sequences specific for the differentiated and undifferentiated state of NS20Y neuroblastoma cells is currently in progress.

A cDNA clone corresponding to the mRNA for human choline acetyltransferase has been identified by screening a λgt11 library prepared from mRNA of human basal ganglia with a monospecific polyclonal antibody to human CAT. The identified clone (λChat7) contains a 1100 bp. insert. A 160 Kd protein synthesized by the λChat7 lysogen was recognized by the anti-CAT antibody and by an anti-β glactosidase antibody. This fusion protein substantially inhibited staining by the antibody of the neurons in spinal cord cell cultures. The insert hybridized to mRNA in Northern blots of an RNA from CAT-producing neuroblastoma clones but not from neuroblastoma clones that did not express CAT activity. Availability of this probe for the choline acetyltransferase gene is particularly exciting in that it will enable study of regulation of the enzyme at the primary transcriptional level. This is a highly regulated gene and the involvement of central cholinergic systems in a number of highly significant disease states makes understanding of its genetic organization especially important.

3. Section on Neuroendocrinology

A single finding of the Section on Neuroendocrinology, under the guidance of Dr. David Klein, is of such importance for pineal neurobiology that it may take precedence over the contributions and impressive progress this group has made in several other areas this past year. That single discovery evolved from interest in the molecular similarities between the pineal gland and the retina.

Dr. Klein previously had initiated collaborations with investigators in the National Eye Institute with the intention of accelerating research on the pineal gland. The basis of this interest in pineal-retinal relationships is that the pineal organ in cold blooded vertebrates is a photoreceptor - a third eye - with morphological and biochemical similarities to the retina. He started to work with Dr. Igal Gery, an immunologist interested in a highly antigenic protein in the retina, the S-antigen. This protein, which is involved in regulating the cyclic nucleotide response to photostimulation in the retina, is found in the pineal gland but in no other tissues. With antiserum against S-protein, Dr. Klein initiated a series of collaborations with comparative pineal morphologists, Dr. Theo van Veen of the University of Lund, and Dr. Horst Korf of Julius Liebig University, who have used it to study the pineal gland and retinae of vertebrates and the photosensitive organs of several invertebrate classes. The antiserum has proven to be the best available reagent to specifically identify pineal cells, primarily because it is not present in brain. In the course of examining the distribution of the S-antigen stain in the mammalian pineal gland, Drs. Korf and Klein discovered that pineal processes were also identifiable with this reagent. These enigmatic processes had been thought to be short and terminate within the pineal gland. However, with the S-antigen antiserum, it was possible to trace these processes out of the pineal gland itself. Careful investigation revealed that these processes travelled through the habenula and were traced as far as the posterior commissure. The processes appeared to be neuronal in character: they had varicosities and bifurcations. Many questions remain to be answered regarding these processes. However, it is clear from this discovery that a new area of pineal research has opened. The pinealocyte had been thought of and studied as a neuroendocrine cell, communicating via chemical messages in the blood. Now the pinealocyte must be considered also as a neuron, capable of delivering high concentrations of chemicals directly to discrete brain areas.

The Section has made the following signifcant advances in understanding neural regulation in the pineal gland. David Sugden, a Visiting Associate, and Jiri Vanecek, a Visiting Fellow, have teamed up with Dr. Klein to investigate the atypical neural mechanism which regulates cyclic AMP and cyclic GMP in the pineal gland. Several years ago, Dr. Klein published preliminary evidence that in this tissue norepinephrine acts through two receptors to control these cyclic nucleotides, alpha-1 and beta-1 adrenoceptors. Using dispersed pinealocytes, it was possible to dissect this mechanism and unequivocally establish that in the case of both cyclic nucleotides, beta-1 adrenergic activation is a requisite, and that alpha-1 adrenergic activation potentiates this event. Further studies done with Drs. Wayne Anderson and Thomas P. Thomas of the National Cancer Institute established that the alpha-1 effect on cyclic AMP was mediated by a phospholipid product, diacylglycerol, and that it acted by promoting the association of a calcium-, phospholipid-dependent protein kinase with the membrane, with the resulting phosphorylation of a membrane protein. The mechanism through which cyclic GMP is regulated has not been elucidated. This development has important implications in neurobiology, because it provides a model to explain how neuromodulators might act.

A goal of this Section is to obtain the molecular tools required to determine how specific genes in the pineal gland are regulated. The Section has come closer to this goal through several efforts. First, it has been possible for

Joan Weller to establish a bovine pineal cDNA library, through the cooperation of Dr. William Strauss, LDN. This library has been screened with anti S-antigen antiserum and several clones were identified. Independently, Dr. Cheryl Craft, a Postdoctoral Fellow in the Section, has been working with Dr. Toshi Shinohara of the National Eye Institute to isolate S-antigen cDNA clones from retinal libraries. They have been successful, and it is expected that Dr. Craft will return to the Section with the skills required to pursue problems of pineal molecular biology independently. It is hoped that it will be possible to compare the pineal and retinal S-antigen cDNA to determine if they reflect the same or different genes. The goal of studying pineal gene products of special interest has been furthered through the efforts of Drs. David Sugden, Pierre Voisin and David Klein. Together, they have purified hydroxyindole-O-methyl-transferase, the enzyme which forms melatonin from N-acetylserotonin, and have prepared antisera. Weller is now using the antisera to screen the pineal cDNA library. Drs. Sugden, Klein and Voisin have also been able to obtain a partial sequence for the enzyme. Currently, Dr. Valentine Cena, a Guest Researcher in the Section, is continuing the isolation of this molecule using high performance liquid chromatography, and preparing sufficient amounts of fragments to allow the complete sequence of the enzyme to be determined. He is also examining the question of whether this enzyme is phosphorylated. Finally, the pineal enzyme of greatest interest to neurobiologists, N-acetyltransferase, is now being obtained routinely in highly enriched preparations. These preparations, from sheep and rat pineal glands, will be used to raise antiserum and to obtain the full sequence of the molecule. This enzyme is of special interest because its activity is increased 100-fold through an adrenergic-cyclic AMP mechanism involving both new gene expression and protein synthesis.

An intriguing study involving the pineal-retina question is being conducted by Dr. Tony Ho, a Visiting Fellow in the Section. This involves rhodopsin kinase in the pineal gland. Last year, Dr. Klein and Dr. Robert Somers of the National Eye Institute discovered that the pineal gland had about as much of this enzyme as the retina. However, the pineal has no detectable rhodopsin, and the question of the function of this enzyme was raised. To answer this, Drs. Ho, Somers and Klein are now in the process of determining whether there is a pineal substrate of purified retinal rhodopsin kinase, and the identity of this substrate. It is suspected that rhodopsin kinase in the pineal gland, as in the retina, phosphorylates a membrane-associated receptor. In the retina the receptor is rhodopsin; perhaps it is an adrenergic receptor in the pineal gland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00047-16 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical 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. Brenneman	Staff Fellow	LDN, NICHD
	G. Handelmann	PRAT Fellow	LDN, NICHD
Others:	G. Westbrook	Staff Fellow	LDN, NICHD
	M. Litzinger	Medical Officer	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD
	D. Warren	Bio. Lab Tech.	LDN, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

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.)*

Cell cultures prepared from fetal mammalian central nervous system were used to study the regulation of neuronal development. Mechanisms which relate to the role of electrical activity during development were investigated. Addition of 0.1 nM vasoactive intestinal peptide (VIP) to dissociated spinal cord cultures resulted in an increase in neuronal survival during electrical blockade. VIP antiserum and receptor blockade with VIP_{10-28} decreased neuronal survival to a similar extent as treatment with tetrodotoxin. Vasoactive intestinal peptide was shown to act indirectly through non-neuronal background cells to increase neuronal survival during electrical blockade. Conditioned medium obtained from VIP stimulated non-neuronal cultures produced an increase in neuronal survival as compared to that obtained from sister cultures not treated with VIP. Receptor binding studies indicated that VIP sites were present on non-neuronal cultures. Increases in cAMP levels were observed in background cultures treated with 10 nM VIP.

We have investigated the possibility that peptide neurotransmitters and neurohormones play a role in the development of their target organ. Early exposure to the neuropeptides was found to permanently affect the expression of neuropeptide receptors, and that this alteration in receptors was of physiological and behavioral importance to the mature animal. For opioid peptides, the developmental effects in rats parallel findings in children exposed in utero to opiate drugs, suggesting that a similar mechanism may operate in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00048-11 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

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

COOPERATING UNITS *(if any)*
M. Giovanni, NHLBI, B. Raj-Amaladoss, NHLBI; Y. Peng Loh, LNN, NICHD; D. Hilt, NHLBI, M. Nirenberg, NHLBI; B. Wong, LNN, NICHD; L. Hersh, Univ of Texas HSC at Dallas

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Molecular Neurobiology Unit

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.)*

(1) One of the cDNA clones otained from Xenopus anterior pituitary RNA by hybridization to mouse POMC cDNA was mapped with restriction nucleases and partially sequenced by chemical methods. One portion of the molecule has no homology to pro-opiomelanocortin mRNAs (POMC) of other animals, but contains a 134 bp region with 80% homology to a repetitive genomic sequence called REM1. There is also, within this same region, a stretch of 12 bp which is 100% homologous to a region in the 5' untranscribed region of the human POMC gene. The other half of the insert has been partially sequenced. An open reading frame contains a region of some amino acid homology to bovine POMC, but an expected area of high homology has proven difficult to sequence. (2) In the neuroblastoma project, libraries of cDNA presumed to be differentiation-specific obtained by cascade hybridizations, have been screened by hybridizations to cDNA probes made from undifferentiated and differentiated cell mRNA. Several colonies which appear to be differentiation-specific or enriched have been selected and are being characterized. (3) Sequences in one library each of cascade-purified cDNAs of undifferentiated and differentiated S20Y cells are being systematically examined as to abundance of the mRNA, specificity for the differentiation stage from which they came and for the presence or absence of restriction fragment polymorphisms in digests of DNA from cells in the two differentiation states as well as from mouse brain and liver. (4) Screening of the λgt11 cDNA library prepared last year from differentiated NG108-15 cell mRNAs with monoclonal antibodies directed against rat retina revealed a clone, λGDT7, with a 300 bp insert which corresponds to an antigen with a restricted distribution in the rat nervous system. This clone has been subcloned into M13 mp8/9 for sequencing. (5) A cDNA clone, λChAT7 was isolated by use of a polyclonal antibody to human choline acetyltransferase (ChAT) from a λgt 11 cDNA library (a gift from Dr. R. Lazzarini) made from mRNAs from human basal ganglia. Several lines of evidence indicate that this 1100 bp insert is complementary to ChAT mRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00064-09 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	P.G. Nelson	Head	LDN, NICHD
Others:	P. Guthrie	Staff Fellow	LDN, NICHD
	G. Westbrook	Staff Fellow	LDN, NICHD
	E.A. Neale	Physiologist	LDN, NICHD
	M. Litzinger	Med. Staff Fellow	LDN, NICHD
	M. Mayer	Visiting Associate	LDN, NICHD
	M. Jia, F. Wang	Visiting Fellows	LDN, NICHD

COOPERATING UNITS *(if any)*
P.F. Lemkin, NCI; P. Sonderegger, Univ. of Zurich; J. Moskal, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.91	2.91	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.)*

Profound inactivation of voltage-dependent calcium channels can be demonstrated with 2 Hz stimulation using voltage clamp techniques under conditions where Na and K channels are blocked in cultured spinal cord (SC) and dorsal root ganglion (DRG) neurons. Such inactivation is much more pronounced in DRG as compared to SC neurons. The inactivation is dependent both on steady membrane voltage and on the amount of calcium which enters the neuron. DRG, thus, appears to regulate intracellular calcium less well than SC neurons in response to activity related calcium ingress. DRG neurons also maintain transmitter output very poorly relato SC neurons. This deficit in function is accentuated in high external calcium. Synaptic boutons of DRG neurons have a relative paucity of mitochondria correlating with this deficit in transmitter output with repetitive activation. We propose that the inactive boutons described in earlier reports may be related to ineffective calcium sequestering mechanisms. These mechanisms may represent one source of long term modulation of synaptic efficacy.

Nitrendipine, a dihydropyridine binds with high and low affinity to cultured neurons, but in contrast to its blocking action for Ca^{++} channels in muscle, nitrendipine, even at micromolar concentrations, has little or no blocking action on neuronal Ca^{++} channels.

Infection of DRG neurons with various strains of herpes virus has different highly specific effects on neuronal excitable mechanisms. Fusion, with electrical coupling, may be induced and blockade of sodium but not calcium channels and of inward but not outward rectification result from viral infection. Acyclovir, which prevents viral replication, blocked the effects of the virus on membrane properties.

Chick and rat sensory neurons have voltage-sensitive calcium channels, and in a subpopulation of these cells, a large prolonged post-spike depolarization occurs. Voltage clamp experiments indicate that this after potential is due to a calcium-sensitive chloride conductance whose properties are described.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00094-15 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

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	Head	LDN, NICHD
Other:	D. Sugden	Visiting Associate	LDN, NICHD
	P. Voisin	Visiting Fellow	LDN, NICHD
	V. Cena	Guest Researcher	LDN, NICHD
	T. Ho	Visiting Fellow	LDN, NICHD

COOPERATING UNITS *(if any)*
P. Skolnick, NIAMMD; D. Jacobowitz, S. Markey, NIMH; J. Pierce, NIHLB; M.A.A. Namboodiri, Georgetown Univ.; R. Janowsky, U. of Penn.; M. Brightman, NINCDS.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neuroendocrinology

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 investigates the environmental and physiological regulation of the pineal gland. The major new findings made within the last year are: (1) The suprachiasmatic nucleus, which appears to contain the circadian clock which drives the pineal rhythms, was found not to drive the rhythm in oxytocin in cerebral spinal fluid of a subhuman primate. This is an indication that there are multiple circadian oscillators in primates. (2) Administration of hydroxytryptophan to sheep, which elevates melatonin in the blood, also elevates melatonin and N-acetylserotonin in the sheep pineal gland. This is an indication that hydroxy-tryptophan is probably acting to elevate melatonin by a mass-action effect on the pineal gland. (3) The pineal α_1-adrenergic receptors were found to be present before nerves invaded the gland, indicating that their development was independent of innervation, and either was part of a pineal developmental schedule, or trig-gered by circulating messenger chemicals. (4) Environmental and neural regulation of pineal α_1-adrenergic receptors was demonstrated. Both constant darkness and denervation caused an increase in receptor numbers. (5) It was discovered that 2-fluoronorepinephrine and 6-fluoronorepinephrine act as α- and β-adrenergic ago-nists, respectively, in controlling the pineal gland. This makes it possible to prepare mixtures of fluoroderivatives to norepinephrine which have graded α- or β- strengths.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00095-15 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pineal Regulation: Transsynaptic and Intracellular Mechanisms

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

PI:	D.C. Klein	Head	LDN, NICHD
Others:	P. Voisin	Visiting Fellow	LDN, NICHD
	D. Sugden	Visiting Associate	LDN, NICHD
	J. Vanecek	Guest Researcher	LDN, NICHD
	T. Ho	Visiting Fellow	LDN, NICHD
	L. Sugden	Visiting Fellow	LDN, NICHD
	C. Craft	NRSA Fellow	LDN, NICHD
	T. van Veen	Guest Researcher	LDN, NICHD

COOPERATING UNITS *(if any)*
A. Spiegel, K. Kirk, S. Beckner, NIAAMD; W. Anderson, T.P. Thomas, NCI; J. Pierce, NHLBI; M.A.A. Namboodiri, Georgetown Univ.; D. Goldman, C. Merrill, D. Jacobowitz, NIMH; H. Korf, Justis Liepsig Univ.;' R. Somers, I. Gery, T. Shinohara, NEI.

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.45	2.75	0.70

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

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

The goal of this project is to discover the molecular basis of neural regulatory mechanism in the pineal gland and to describe how the pineal gland functions. The approach is primarily biochemical and has yielded new information about pineal alpha-adrenergic receptors, how alpha-adrenergic receptors interact with beta-adrenergic receptors to control cyclic AMP and cyclic GMP, how phospholipid metabolism is involved in the control of cyclic AMP via a calcium, phospholipid-dependent protein kinase, has revealed that there are two amine N-acetyltransferases in the pineal gland, has developed new methods for the purification of pineal serotonin N-acetyltransferase and hydroxyindole-O-methyltransferase (HIOMT), has prepared antiserum against HIOMT, has prepared a bovine pineal cDNA library, and has started to screen for HIOMT clones. This project has described the development and photoneural regulation of α-adrenergic receptors, has described these receptors in sheep, has demonstrated that these receptors are of central importance in regulating melatonin production. A new infusion of concepts and tools into pineal research came from an active pursuit of collaborations with retinal scientists, which has led to the discovery of rhodopsin kinase in the pineal gland, the isolation of bovine S-antigen cDNA, the partial sequence of this protein, and the identifiction of the S-antigen in all pineal organs of all vertebrates. Perhaps the most important discovery of this program was that pinealocytes sent projections into the brain, which provided the first evidence that pineal cells of mammals may function as neurons, as do pineal cells of low vertebrates. Another activity of this project was to organize an international symposium on pineal-retinal relationships, in cooperation with the National Eye Institute.

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, 1984 to September 30, 1985

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)*

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 00706-01 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological Studies of Nervous System Development In Vitro

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

PI:	Gary L. Westbrook	Staff Fellow	LDN, NICHD
	P.G. Nelson	Head	LDN, NICHD
Others:	C. L. Mitchell	Guest Scientist	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*
A. B. MacDermott, LNP, NINCDS

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

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

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

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

New electrophysiological methods are being utilized to study the development of excitability and synaptogenesis of mammalian CNS neurons in vitro. Developing neurons, previously inaccessible to physiological investigation, have been examined using a variety of patch electrode techniques. Developmental changes in the action potential mechanism during the development of embryonic mouse spinal cord neurons in vitro have been found to be related to changes in the number and/or distribution of sodium channels rather than to changes in the kinetics of the sodium current. The appearance of excitatory amino acid receptors precedes the onset of excitatory synaptic activity, but the sensitivity to excitatory amino acids increases rapidly during synaptogenesis in culture. Different types of excitatory amino acid receptors exhibit differential developmental regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZOI HD 00707-01 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacological Studies of Synaptic Transmission In Vitro

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

PI:	M. L. Mayer	Visiting Associate	LDN, NICHD
	G. Westbrook	Staff Fellow	LDN, NICHD
Others:	P.G. Nelson	Head	LDN, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

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 physiology and pharmacology of excitatory amino acid receptors and their role in synaptic transmission in dissociated cultures of mouse spinal cord. Three receptor types exist: kainate, quisqualate and N-methyl-D-aspartate (NMDA). Magnesium ions cause a voltage-dependent block of the ion channels linked to NMDA receptors. Major new discoveries in the project were selectivity of the divalent cation binding site linked to the NMDA receptor ion channel. Two potent calcium channel blockers, nickel (Ni^{++}) and cadmium (Ca^{++}) ions, have strikingly different actions on responses to NMDA, Ni^{++} being a strong antagonist and Cd^{++} nearly ineffective. Reversal potential measurements suggest that calcium ions permeate through NMDA ion channels. Extreme membrane potential hyperpolarization may also force magnesium ions past the blocking site. Excitatory synaptic transmission in spinal cord cultures appears to result from activation of non-NMDA receptor types. The non-selective antagonist cis-2,3-piperidine dicarboxylic acid (PDA) reversibly blocked epsps while the selective NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (APV) was ineffective. The excitatory synaptic current decayed as a single exponential of time constant 0.5 - 1.0 msec. The decay time constant was voltage insensitive between -80 and 0 mV, and the peak current voltage relation was linear.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00708-01 LDN

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Morphologic studies of neuronal and non-neuronal cells in CNS cell cultures

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

PI:	Elaine A. Neale	Physiologist	LDN, NICHD
Others:	George A. Foster	Visiting Scientist	LDN, NICHD

COOPERATING UNITS *(if any)*

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

Immunohistochemistry has been used to identify various cell types in dissociated cell cultures of fetal mouse spinal cord. Neurons thought to be cholinergic; i.e., immunoreactive for choline acetyltransferase, develop this immunoreactivity after several weeks in culture and constitute a small percentage of the total neurons in these cultures. The morphologic features of the CAT-positive neurons are distinct from both GABA- and VIP-immunoreactive neurons.

LABORATORY OF DEVELOPMENTAL PHARMACOLOGY

Z01 HD 00136-17 Pharmacogenetics
Daniel W. Nebert, M.D.

Z01 HD 00137-11 Genetic Regulation of Drug-Conjugating Enzymes
Ida S. Owens, Ph.D.

Z01 HD 00500-07 Receptor Structure and Function
Howard J. Eisen, M.D.

Z01 HD 00503-01 Regulation and Expression of the UDP Glucuronosyltransferase Gene Family
Peter I. Mackenzie, Ph.D.

Annual Report of the Laboratory of Developmental Pharmacology
National Institute of Child Health and Human Development
October 1, 1984 through September 30, 1985

SUMMARY

The LABORATORY OF DEVELOPMENTAL PHARMACOLOGY studies the molecular mechanisms of gene expression involving 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 metabolize the innumerable foreign compounds that enter our body. Hundreds of drugs and other chemicals 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, limnology, and drug addiction, tolerance and toxicity. This Laboratory presently comprises three Sections and one Unit.

A. The Section on Pharmacogenetics and Molecular Teratology, under the direction of Daniel W. Nebert, M.D., is interested in the regulation and expression of genes encoding "Phase I drug-metabolizing enzymes," most of which represent the P450 proteins. The P450 gene superfamily is presently known to be composed of at least five gene families, and a protein from any of these five families has diverged more than 60% from a protein in any of the other four families: (1) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; in the lay press called "dioxin")-inducible; (2) phenobarbital-inducible; (3) steroid-inducible; (4) gene(s) responsible for cholesterol side-chain cleavage; and (5) genes responsible for the steroid C21-hydroxylase polymorphism. This Section has studied the TCDD-inducible P450 gene family in greatest detail, partly because of its relationship to polycyclic hydrocarbon-induced carcinogenesis, and partly because the inducer-receptor complex controlling the two genes in this family has been well characterized. In mouse the P_1450 and P_3450 genes have been isolated, sequenced and mapped. The two genes appear to reside adjacent to one another in the middle portion of mouse chromosome 9 and to be controlled by the same cytosolic receptor, yet exhibit striking differences in developmental and tissue specificity, differential sensitivity to inducer concentrations, and transcriptional versus posttranscriptional regulation. Cotransfection of pSV2-neo plus pSV0-cat-derived constructs containing upstream P_1450 sequences has led to the isolation of the promoter region, a negative control element (which interacts with a negatively autoregulated loop), a positive control element (presumably the inducer-receptor-associating region), and an upstream activation element (most likely an endogenous enhancer). The P450Coh protein, responsible for an inbred mouse polymorphism involving coumarin metabolism, was purified and a specific antibody was developed. This antibody will be used to study the mouse phenobarbital-inducible P450 gene family. The P450PCN protein, inducible by steroids such as pregnenolone-16α-carbonitrile, has been purified and a specific antibody was developed. A full-length cDNA clone has been isolated and sequenced. The protein sequences, deduced from the nucleotide sequences, allow us to conclude that the TCDD-inducible, phenobarbital-inducible, and steroid-inducible P450 gene families have each diverged from a common ancestral gene more than 200 million years ago and that the homologous

P_1450 and P_3450 genes separated from each other at least 65 million years ago. The human P_1450 gene and flanking regions has also been sequenced and mapped. 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_1450. 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. During the past year significant progress has been made in purification of the Ah receptor with the use of high performance liquid chromatography and affinity chromatography (polycyclic aromatic dyes linked covalently to agarose). We have continued to study mutant clones of mouse hepatoma Hepa-1 which do not express the Ah receptor ("r^-" phenotype); these clones and revertant clones are being used for "rescue" of the Ah receptor gene by DNA-transfection. We have identified a human cell line (hepatoblastoma $HepG_2$) that contains an Ah receptor with high affinity (K_d ~ 1 nM) for TCDD; aryl hydrocarbon hydroxylase (AHH) activity and human P_1450 mRNA are highly induced in this cell line by TCDD (EC_{50} ~ 1 nM). This cell line should provide a good model for studying the relationship between the Ah receptor and the induction of human P_1450 mRNA. Antibodies produced in this laboratory to the glucocorticoid receptor have been used to study the subcellular localization of glucocorticoid receptors in intact cells and to study glucocorticoid action in the mammalian brain.

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. Many highly fat-soluble substrates, converted to oxygenated products by the phase I cytochrome P450-dependent monooxygenases, are then detoxified by transferase through conjugation and, thus, transformation to highly water-soluble and excretable metabolites. We are particularly interested in understanding the genetic linkage in the regulation of certain monooxygenases and certain transferases by the Ah receptor in mice. We are also interested in induction by phenobarbital-like compounds. The regulation of the transferase enzymes is being studied by means of DNA, RNA and protein chemistry. Five mouse transferase clones have been isolated and shown to be 85% similar by nucleotide sequence analysis and cross-hybridization studies. These clones represent members of a subfamily with their unique sequences contained in the 3' region of the cDNAs. $pUDPGT_m$-1 (1800-bp insert) and $pUDPGT_m$-2 (1600-bp insert) differ by one nucleotide (converting an isoleucine to methionine). A third clone, $pUDPGT_m$-3, contains an unusually long insert with 4300 bp compared to other transferase cDNAs which encode mRNAs ranging from 1900 to 3000 nucleotides. The detection of 35 genomic clones (inserted into λEMBL-3) with sequence homology to $pUDPGT_m$-1 suggests that multiple and closely related genes exist and that the gene for $pUDPGT_m$-1 is most likely duplicated. At least two of these clones encode proteins with 50-kDa molecular weights, have one or two potential glycosylation sites, and contain hydrophobic transmembrane amino-acid sequences near the carboxy terminus. In order to study aromatic hydrocarbon-inducible transferase forms, a cDNA library

was recently constructed in λgt11 using 3-methylcholanthrene-induced mRNA. This library is being screened with previously developed mouse transferase antibody. Furthermore, human mRNA has been isolated and translated in vitro to produce a 48-kDa protein immunoprecipitable by mouse transferase antibody. A human cDNA library has been constructed and successfully probed with the insert from $pUDPGT_m$-1 to isolate and partially purify two human transferase cDNA clones. A human transferase form that is being purified appears to have similar properties to a mouse 3-methylcholanthrene-inducible form. Transferase studies at the molecular level will enable us to determine sites of regulation, the heterogeneity of the system, and substrate specificity of the different transferase forms.

D. The Unit on Recombinant DNA and the Conjugating Enzymes, under the direction of Peter I. Mackenzie, Ph.D., studies the regulation and expression of several subfamilies of the rat UDP glucuronosyltransferase gene family. Three cDNA clones encoding different forms have been isolated and sequenced. The mRNAs complementary to two of these clones ($pUDPGT_r$-1 and $pUDPGT_r$-3) are 85% similar by sequence analysis and are not elevated by 3-methylcholanthrene or phenobarbital treatment. They are transcribed from genes belonging to the same subfamily (according to Dayhoff's definition). The mRNA complementary to the third clone, $pUDPGT_r$-2, is only 65% similar in sequence to the other cDNAs. It is elevated 5-fold by phenobarbital and is transcribed from a gene belonging to a second subfamily. Sequence studies have also shown that the transferase forms of both subfamilies encoded by the three cDNAs contain signal peptide and membrane anchoring regions, and potential asparagine-linked glycosylation sites. In vitro translation studies indicate that the signal sequence is most likely cleaved during insertion into the endoplasmic reticulum. Experiments are in progress to express those transferase cDNAs which contain complete coding regions in transferase-deficient cells, in order to characterize each form by its catalytic activity and substrate specificity. The regulation of each member of these two subfamilies as a function of age, tissue distribution and administration of prototypic inducers will be investigated with the use of both cDNA and genomic clones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00136-17 LDP

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:
5.64	2.00	3.64

CHECK APPROPRIATE BOX(ES)

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

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

The "cytochrome P450" gene superfamily comprises a minimum of five gene families: (i) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; in the lay press called "dioxin")-inducible; (ii) phenobarbital-inducible; (iii) steroid-inducible; (iv) gene(s) involved in cholesterol side-chain cleavage; and (v) genes involved in the steroid C21-hydroxylase polymorphism. This laboratory has studied most intensively the TCDD-inducible P450 gene family, which has two members ($P_1$450 and $P_3$450 in the C57BL/6N mouse; P450c and P450d in rat; form 6 and form 4 in rabbit, respectively). In mouse $P_1$450 and $P_3$450 are but two genes in the [Ah] complex, a "battery" of at least a dozen genes activated by polycyclic aromatic inducers such as TCDD and presumably regulated by the Ah (TCDD) receptor. Many of these proteins are being purified, antibodies developed, and cDNA and genomic clones isolated and sequenced in order to understand regulatory expression of this gene battery believed to play an important role early in development. The $P_1$450 and $P_3$450 genes reside adjacent to one another near the Mpi-1 locus on mouse chromosome 9; their orthologs reside on human chromosome 15 and hamster chromosome 4. There are interesting differential transcriptional regulatory mechanisms for activation of the $P_1$450 and $P_3$450 genes, as well as striking developmental and tissue-specific differences in gene expression. Upstream $P_1$450 regulatory sequences include a promoter region, a negative control element (involved in a negative autoregulatory loop), a positive control region (associating with the inducer-receptor complex), and an upstream activating element (presumably an endogenous enhancer). One long-range goal of this laboratory is to develop assays, based on recombinant DNA technology, to assess the human Ah phenotype and other pharmacogenetic disorders. Such assays may predict who is at increased risk for certain types of environmentally-caused birth defects, cancers, and toxicity.

ATTACHMENT I - Others:

Warren A. Banks	Biologist (Tech.)	LDP	NICHD
Sanford W. Bigelow	Chemist	LDP	NICHD
Cynthia A. Edwards	Guest Researcher	LDP	NICHD
Hana S. Haleem	Guest Researcher	LDP	NICHD
Larry L. Heilmann	Jr. Staff Fellow	LDP	NICHD
Wayne E. Jackson	Stay-In-Schooler	LDP	NICHD
Shioko Kimura	Visiting Fellow	LDP	NICHD
Kristi L. Kotz	Federal Junior Fellow	LDP	NICHD
Lisa A. Neuhold	Biologist (Tech.)	LDP	NICHD
John A. Robertson	Visiting Fellow	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

F. J. Gonzalez, Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland 20205

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

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H. Westphal, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, Maryland 20205

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 00137-11 LDP

PERIOD COVERED

October 1, 1984 to September 30, 1985

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:	T. Kimura	Visiting Fellow	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

P.I. Mackenzie, Unit on Recombinant DNA & the Conjugating Enzymes, 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:
2.0	2.0	None

CHECK APPROPRIATE BOX(ES)

☐ (a) 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. Different members of the transferase system are known to be induced by a number of different types of effector compounds; two such compounds used in these studies are phenobarbital and 3-methylcholanthrene (3MC). Five mouse transferase cDNA clones have been isolated and shown to be 85% homologous by nucleotide sequence analysis and cross-hybridization studies. These clones represent members of a subfamily and possess unique sequences in their 3' regions. The pUDPGT$_m$-1 (1800-bp insert) and pUDPGT$_m$-2 (1600-bp insert) differ by one nucleotide (converting an isoleucine to methionine). A third clone, pUDPGT$_m$-3, contains an unusually long insert with 4300 bp compared to other transferase cDNAs which encode mRNAs ranging from 1900 to 3000 nucleotides. The detection of 35 genomic clones (inserted into λEMBL-3) with sequence homology to pUDPGT$_m$-1 suggests that multiple and closely related genes exist and that the gene for pUDPGT$_m$-1 is most likely duplicated. At least two of these clones encode proteins with 50-kDa molecular weights, have one or two potential glycosylation sites, and contain hydrophobic transmembrane amino acid sequences near the carboxy terminus. In order to study 3MC-inducible transferase forms, a cDNA library was recently constructed in λgt11 using 3MC-induced mRNA. This library is being screened with previously developed mouse transferase antibody. Also, human mRNA has been isolated and translated in vitro to produce a 48-kDa protein immunoprecipitable by mouse transferase antibody. A human cDNA library has been constructed and successfully probed with the insert from pUDPGT$_m$-1 to isolate and partially purify two human transferase cDNA clones. A human transferase form is being purified which appears to have similar properties to a mouse 3MC-inducible form.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00500-07 LDP

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	H. J. Eisen	Head	LDP, NICHD
Others:	A. K. Jaiswal	Visiting Fellow	LDP, NICHD
	D. W. Towne	Chemist	LDP, NICHD
	T. Cresteil	Guest Researcher	LDP, NICHD
	E. Zimmerman	Guest Researcher	LDP, NICHD
	M. E. Reichman	Expert	LDP, NICHD

COOPERATING UNITS *(if any)* D.W. Nebert & coworkers, Section on Pharmacogenetics and Molecular Teratology, LDP:NICHD:NIH

S.S. Simons, Jr., Laboratory of Chemistry, NIADDKD:NIH

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:
2.06	1.06	1.00

CHECK APPROPRIATE BOX(ES)

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

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

This project is focused on identification of the mechanism by which steroid hormones, drugs, and polycyclic aromatic compounds induce cytochrome P450 in mammalian tissues and cultured cell lines. Intracellular protein receptors have been identified for glucocorticoids (the glucocorticoid receptor) and planar polycyclic aromatic compounds (the Ah receptor). These compounds bind to their respective receptors; the ligand-receptor complexes interact with DNA in the cell nucleus and apparently activate directly the transcription of specific genes. Monoclonal antibodies and cDNA clones for the glucocorticoid receptor have been produced. A major goal of this project is to produce similar molecular probes for the Ah receptor. During the past year we have concentrated on the purification of the Ah receptor, and we have studied further the structural and functional homologies between the glucocorticoid and Ah receptors. We have found that certain covalently immobilized ligands (such as polycyclic aromatic dyes) can be used effectively for purification of both the glucocorticoid and Ah receptors. We have identified a human form of the Ah receptor in the hepatoblastoma $HepG_2$ cell line, and we are carrying out experiments to "rescue" the human Ah receptor gene by transfection of human genomic DNA into Ah receptor-deficient mutant mouse cells. Monoclonal antibodies and reverse phase HPLC have been used to isolate proteolytic fragments of the glucocorticoid receptor that represent the DNA and steroid-binding domains of this receptor. The HPLC methods developed initially for analysis of the glucocorticoid receptor have been useful particularly for purification and characterization of the Ah receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00503-01 LDP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
REGULATION AND EXPRESSION OF THE UDP GLUCURONOSYLTRANSFERASE GENE FAMILY

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

PI:	P. I. Mackenzie	Visiting Associate	LDP, NICHD
Others:	H. A. Privette	Bio.Lab.Tech.	LDP, NICHD

COOPERATING UNITS *(if any)* D.W. Nebert & coworkers, Section on Pharmacogenetics and Molecular Teratology, LDP:NICHD:NIH

I.S. Owens & coworkers, Section on Drug Biotransformation, LDP:NICHD:NIH

LAB/BRANCH
Laboratory of Developmental Pharmacology

SECTION
Unit on Recombinant DNA and the Conjugating Enzymes

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.27	1.0	0.27

CHECK APPROPRIATE BOX(ES)

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

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

The molecular mechanisms governing the regulation of the drug-detoxifying enzyme, UDP glucuronosyltransferase (transferase), and the structural differences between members of this family are being investigated in the rat. This animal, as exemplified by the Gunn rat, provides the only known animal model for investigating the defect in the glucuronidation of bilirubin and certain xenobiotics, characteristic of the Crigler-Najjar syndrome in humans. Three cDNA clones encoding different forms have been isolated and sequenced. The mRNAs complementary to two of these clones (pUDPGT$_r$-1 and pUDPGT$_r$-3) are 85% similar by sequence analysis and are not elevated by 3-methylcholanthrene or phenobarbital treatment. They appear to be transcribed from genes belonging to the same subfamily. The mRNA complementary to the third clone, pUDPGT$_r$-2, is only 65% similar in sequence to the other cDNAs. It is elevated 5-fold by phenobarbital and appears to be transcribed from a gene belonging to a different subfamily. Sequence studies have also shown that the transferase forms encoded by the three cDNAs contain signal peptide and membrane anchoring regions, and potential asparagine-linked glycosylation sites. In vitro translation studies indicate that the signal sequence is most likely cleaved during insertion into the endoplasmic reticulum. Experiments are in progress to express those transferase cDNAs which contain complete coding regions in transferase-deficient cells, in order to characterize each form by its catalytic activity and substrate specificity. The regulation of each form--as a function of age, tissue distribution and administration of prototypic inducers--will also be investigated.

Z01 HD 00056-10 Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones
Yoke Peng Loh, Ph.D.

Z01 HD 00058-10 Peptides in the Adult and Developing Vertebrate Nervous System
Harold Gainer, Ph.D.

Z01-HD 00705-04 Functional Organization of the Nerve Terminal
Harold Gainer, Ph.D.

NICHD ANNUAL REPORT

Laboratory of Neurochemistry and Neuroimmunology

October 1, 1984 to September 30, 1985

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. The approach of the laboratory is cell biological in nature, and hence utilizes a wide variety of techniques and concepts from a number of disciplines, e.g., physiology, biochemistry, morphology, immunology, and molecular biology. In particular, we study various secretory peptides, intracellular membrane systems, and cytoskeletal proteins which are found in these organ systems and which are essential to their functions (i.e., peptide biosynthesis and regulation, neuronal morphology and function, etc). A special emphasis is placed on the study of the cellular development of these organ systems.

The activities of the laboratory are divided into two sections and one unit.

I. Section on Functional Neurochemistry

A large number of neuropeptides have been identified 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 Section's goal is to study the cell biology of peptidergic neurons in the context of their regulatory functions in the nervous system. In particular, we specifically study the expression of neuropeptides during CNS development and their impact on the development of organismic functions.

Our studies are focused on two specific peptidergic systems in the hypothalamus, the oxytocinergic and vasopressinergic neuronal systems, and the LHRH neuronal system. We chose to concentrate on these systems because the neurons that constitute them represent excellent models of peptidergic neurons in the nervous system. The hypothalamo-neurohypophysial system is studied because the neurons that constitute it (i.e., the oxytocin and vasopressin magnocellular neurons) are prototypic 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. In addition, the magnocellular vasopressin neurons synthesize another neuropeptide, dynorphin, whereas the oxytocin neurons also synthesize CCK, and enkephalin. Some vasopressin neurons in the paraventricular nucleus also synthesize CRF. The phenomen of neuropeptide co-existence in these neurons is now well documented, and our lab is involved in elucidating the biological significance of, and mechanisms involved in generating this state of coexistence. The LHRH neurons found in the hypothalamus project principally to the median eminence. The secretion of LHRH into the portal blood system regulates the development and function of reproductive system. In addition, to being the objects of cell biological studies, the embryonic development of these two neuronal systems is currently also under intensive study. Our Laboratory has also recently introduced the developing Xenopus laevis embryo as a model system of neuronal development. Two projects are currently underway in this area: 1) investigation of the cell lineage and fates of distinct peptidergic neurons in the Xenopus hypothalamus (e.g., vasotocin, mesotocin, and LHRH neurons), and 2) the analysis of certain cytoskeletal proteins (e.g., neurofilaments, and microtubule-associated proteins) and their role in generating neuronal morphology and function in the developing brain.

Much of our past year's activity has been devoted to laying the ground work for these developmental studies. This includes the development of relevant monoclonal and polyclonal antibody reagents, establishing a sophisticated immunochemical and immunocytochemical (at the electron microscope level) technology, and generating a data-base for the biological model system. We have, with the efforts of Ms. S. House, succeeded thus far in generating 7 monoclonal antibodies against the neurophysins in the AVP and OT cells, 18 polyclonal antibodies against the AVP, OT, and LHRH peptides and their intermediate and prohormone forms, 5 monoclonal antibodies against neurofilaments in the squid giant axon, and over 50 monoclonal antibodies against Xenopus brain cytoskeletal proteins. These antibodies are currently being utilized in many studies ranging from the molecular to the morphological level, and are being evaluated for their phylogenetic cross-reactivities. Immunocytochemical studies by Dr. M.H. Whitnall using the neurophysin antibodies have shown that oxytocin and vasopressin cells in the fetal hypothalamus express their prohormones simultaneously and migrate to their brain nuclei at similar rates. However, they express the post-translation modification mechanisms which generate the peptide products, and their neurite outgrowth at dramatically different rates, the vasopressin neurons being considerably more precocious. Preliminary RIA studies by Dr. M. Altstein have confirmed these observations. Current studies are directed at understanding the mechanisms and biological significance of this differential behavior. Dr. S. Wray has examined LHRH neuron development in the rat from the neonate (2 days pn) to adulthood. Her observations are that smooth LHRH cells are transformed to irregular LHRH cells during puberty, and that this occurs independently of gonadal steroid feedback. Current efforts are being directed at determining whether specific synaptic inputs to the LHRH cells are modified during this dramatic morphological change. This involves the use of double-label E.M. immunocytochemistry.

We continue to develop our 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 various enzymes and peptides (precursors), to study the routing of these antigens through the membrane systems of the cell, and to identify pre- and post-synaptic cells. This has involved new technological developments by Dr. M. Whitnall, Dr. S. Wray, and Ms. S. 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 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, 4) show that the oxytocin precursor is lcoated in secretory vesicles during fetal development, and 5) show that CRF and AVP are colocalized in the same nerve endings and vesicles in the median eminence, and that this coexistence is enhanced greatly by adrenalectomy.

The Xenopus development program (Dr. B. Szaro and Dr. K. Conway) has succeeded in establishing breeding conditions in our laboratory whch allow for the study of wild-type and various mutant strains. Computer programs have been established for 3-dimensional analysis of neurons in brain, and immunological identification by Dr. Conway has revealed over 4000 AVT and MT neurons in the adult Xenopus brain. A 3-D map of their distribution is in progress. Dr. B. Szaro is analyzing the 2-D gel maps of Xenopus brain cytoskeletal proteins, and has succeeded in making potent antibodies against some of these proteins. These antibodies will be used in analysis of protein expression in development, and will be the basis of a molecular cloning strategy in the near future. Dr. M. Lang has studied the AVT receptor's development in the Xenopus kidney A6 cell line. He has demonstrated that the development of functional AVT (V-2) 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 AVT receptor become coupled to the adenylcyclase. This correlation is intriguing and suggests a common mechanism regulating both phenomena. Studies of the ligand selectivity of the A6 line AVT receptor showed that it is a novel V-2 receptor.

Recent studies on the squid axon model have continued to focus on 1) the Ca^{2+} activated protease's pattern of cleavage of its endogenous substrate, the neurofilament protein. We have found that the three molecular components; the neurofilament proteins, the protease, and the protein kinase are found in a complex in axoplasm. All are synthesized in the cell body, but the kinase acts only when the complex enters the axon, and the protease activates only at the nerve terminal. Thus, the genesis of neurofilament structure is determined topographically in the neuron, i.e., biosynthesis and assembly in the cell body, phosphorylation in the axon, and proteolysis in the terminal. This topographic organization of regulation of post-translational modification is currently under study with regard to mechanism.

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 emphasis has been on the ACTH/endorphin/αMSH family of peptides. Endorphin is an opiate peptide that is found in brain, pituitary and placenta. α-MSH is present in brain and pituitary, but in humans it is present in the pituitary only during pregnancy and in the fetus. This peptide has been implicated to have an effect on fetal growth and development. ACTH is a pituitary peptide which is traditionally known to stimulate steriodagenesis. However, it is also found in brain. All these peptides have been shown to have central nervous system effects and are thought to act as neurotransmitters and neuromodulators. The major focus has been to continue to study the enzymology and regulation of biosynthesis, packaging and secretion of this family of peptides. Within the past year two 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) has now been purified to homogeneity and characterized as a 70,000 molecular weight glycoprotein. It has a pH optimum of between 4.0-5.0 and is functional at the acid intravesicular pH. Purified PCE is inhibited by pepstatin A, but not by PMSF, DFP, (serine protease inhibitors), TLCK or EDTA. The thiol protease inhibitor dithiodipyridine, had a partial inhibitory effect. PCE specifically cleaved mouse pro-opiomelanocortin (POMC) between the basic residues and on the carboxyside of the ARG at Lys-Arg pairs to yield ACTH, β-endorphin and a 16K N-terminal glycopeptide. It also cleaved human β-lipotropin to yield β-MSH and β-endorphin and proinsulin to yield the A and B chains of insulin, suggesting that PCE can also cleave Lys-Lys and Arg-Arg pairs of basic residues in additon to Lys-Arg. PCE does not cleave single basic residues. Using human β-lipotropin as a substrate, PCE was found to have a Km = 0.5{M and V_{max} = 0.004{moles/{g protein/h. An enzyme with characteristics indistinguishable from the intermediate lobe PCE has been purified from bovine neural lobe secretory vesicles that cleaved the endogenous prohormones pro-vasopressin to yield their respective hormones. Generation of antibodies to PCE is now in progress. An Okayama-Berg bovine intermediate lobe c-DNA library has been prepared which will be ultimately used for screening for PCE clones, using an antibody to the enzyme as a probe. Other processing enzymes, e.g., a carboxypeptidase B-like enzyme that removes the C-terminal basic residues from the cleaved peptides has been detected in rat neural lobe, anterior lobe and intermediate lobe secretory vesicles. In addition, an aminopeptidase B-like enzyme which removes N-terminal basic residues has been partially purified from bovine 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. The dopamine receptor in the toad intermediate lobe has been pharmacologically characterized as being in the D2 category and is negatively coupled to adenylate cyclase. Presence of dopamine in the culture medium resulted in a decrease in intracellular cAMP and POMC synthesis in the intermediate lobe. This decrease in POMC synthesis was prevented by the addi-

tion of 8-Bromo-cAMP to the medium. Work is now in progress to determine the mechanism by which the cAMP activates and/or maintains POMC mRNA transcription and translation.

III. Unit on Neuronal Secretory Systems

The nerve terminal, is a highly specialized region of a neuron, separated from the neuronal soma by an axon, whose function is to release neurotransmitter substances when stimulated by an electrical signal carried by the axon. Thus the nerve terminal plays the central role in the nervous system function, that operates by signal transmission between cells, by means of secretion of neurotransmitters. Modulation of the quantity of the transmitter released at the terminal may form the basis for all central nervous system functions, including integration of information, long term information storage, and retrieval. Because of this complexity (cellular heterogeneity, and their complex organization), a basic understanding of the molecular mechanisms of nerve terminal function in the central nervous system is still lacking.

The program of the Unit on Neuronal Secretory Systems, established this year, is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. The hypothalamo-neurohypophysial system represents model central nervous system neurons because of their homogeneity and discrete localization, which are eminently accessible for experimentation. The nerve terminals of these neurons are discretely localized in the neurohypophysis, where they are accessible to experimental manipulations both in vivo and in vitro. These nerve terminals can be isolated from the neurohypophyses without contamination by the postsynaptic membrane, unlike nerve terminals from other regions in the central nervous sytem.

Over the past several years our Laboratory has been involved in the elucidation of the biochemical processes underlying the biosynthesis, and the post-translational modifications of the prohormones for vasopressin and oxytocin. This work led to the description of the secretory vesicle hypothesis, which states that the primary site of post-translational modifications of secretory proteins is the secretory vesicle, and that the vesicle provides the highly organized microenvironment for these biochemical reactions. Neurosecretory vesicles from posterior pituitaries were prepared in a highly purified, and stable form. These vesicles were shown to maintain an intravesicular pH of 5.5, by means of an electrogenic proton translocating Mg^{++}-ATPase on the membrane. The vesicle membranes were also shown to possess a b type cytochrome (cytochrome b561), which functions as an electron transporter to intravesicular semidehydro ascorbic acid, which is generated by the activity of the peptide α-amidating enzyme. The intravesicular ascorbic acid content was measured to be in the order of 20 mM. Recently, a preparation of highly purified nerve endings (neurosecretosomes) has been obtained in order to study the kinetics of neuropeptide secretion from these terminals in vitro. Because of the homogeneity, this preparation for the first time allows the study of intra-terminal calcium ion homostasis and membrane calcium channels and their physiological regulation. Work is underway to isolate different membrane systems from these nerve terminals in order to study the biochemical mechanisms of calcium ion transport and its regulation by intracellular and extracellular signals.

The optical studies of nerve terminal activity, begun in collaboration with Dr. B. Salzberg, continue. We have extended the voltage-sensitive dye work to mouse and rat pituitaries. These new studies revealed a light scattering signal correlated with the secretory process at the nerve terminals. In addition, we have developed two different secretion models: 1) an intact mouse posterior pituitary system stimulated electrically in vitro allowing radioimmunoassay of secreted neuropeptides following varied stimulation frequencies and pharmacological paradigms. Using this system, we have found that the dependence of secretion on extracellular calcium ions exhibits a relationship of 1 Ca^{++} per secretory event. 2) a neurosecretosome model (equivalent to synaptosomes from brain) but from the neurohypophysis, which will be used for basic studies on peptide secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00056-10 LNN

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Biosynthesis, processing & secretion of neuropeptides & pituitary peptide hormones

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

PI:	Y. P. Loh	Head	LNN, NICHD
Others:	David Parish	Visiting Fellow	LNN, NICHD
	Renu Tuteja	Visiting Fellow	LNN, NICHD
	Baldwin Wong	Microbiologist	LNN, NICHD
	Brenda Myers	Junior Fellow	LNN, NICHD

COOPERATING UNITS *(if any)*: Mark Goldman, NIMH; Michael Brownstein and Vivian Hook, LCB, NIMH; Hiroto Okayama, LMG, NICHD; James T. Russell, Miriam Altstein and Harold Gainer, LNN, NICHD.

LAB/BRANCH: Laboratory of Neurochemistry and Neuroimmunology

SECTION: Section on Cellular Neurobiology

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.28	1.0	1.28

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 at paired basic residues of the endogenous prohormones (pro-opiomelanocortin, pro-oxytocin and pro-vasopressin) to the active hormones, has been purified from bovine pituitary intermediate and neural lobe secretory vesicles. Purified PCE from both lobes appears to be identical and has been characterized as a ~70,000 dalton glycoprotein. PCE exists in a soluble and membrane associated form and was found to cleave pro-insulin to insulin as well. A carboxypeptidase B-like enzyme and an aminopeptidase B-like enzyme which function to remove the basic residues from the C- and N-terminal, respectively, from the peptide hormone, following the action of PCE, have been found in intermediate lobe and neural lobe secretory vesicles. The aminopeptidase B-like enzyme has been partially purified and characterized as a >75,000 dalton metallopeptidase. The regulation of biosynthesis of pro-opiomelanocortin (POMC) in the toad pituitary intermediate lobe by dopamine and cAMP was also studied. Pharmacological analysis indicates that the toad intermediate lobe dopamine receptor is of the D_2 category and negatively coupled to adenylate cyclase. Thus the dopamine acts, subsequent to binding to the receptor, by lowering the intracellular cAMP level which then results in a decrease in POMC synthesis in the tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00058-10 LNN

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters 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)*

PI:	Harold Gainer	Head	LNN, NICHD
Others:	Mark Whitnall	Staff Fellow	LNN, NICHD
	Kevin Conway, Ben Szaro	Staff Fellows	LNN, NICHD
	Susan Wray	PMA Fellow	LNN, NICHD
	Miriam Altstein	Weizmann Inst.	LNN, NICHD
	Shirley House	Biologist	LNN, NICHD
	Sharon Key	Biologist	LNN, NICHD
	Michael Lang	Special Expert	LNN, NICHD

COOPERATING UNITS *(if any)*: Drs. H. Pant and P. Gallant, Alcohol and Drug Abuse, NIAADA; Dr. R. Pruss, LCB, NIMH, Dr. J. Handler, LKE, NIAA.

LAB/BRANCH: Laboratory of Neurochemistry and Neuroimmunology

SECTION: Section on Functional Neurochemistry

INSTITUTE AND LOCATION: NICHD, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.7	5.0	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.)*

Seven monoclonal antibodies (MABs) against rat neurophysin have been produced and fully characterized. These MABS have been used in developmental studies in studies on the fetal rat hypothalamus. Differential expression of the specific properties of vasopressin- and oxytocin-neurons during development has been found. In addition, these MABs have been used to characterize the arginine vasotocin and mesotocin neurons in the Xenopus hypothalamus, where greater than 4000 neurons, which were found to contain these peptides, have been detected. Polyclonal antibodies have been generated against the various processing intermediates of vasopressin and oxytocin, as well as LHRH, and these are currently being used in RIA and immunocytochemical studies of the adult developing hypothalamus. Immunocytochemical studies have shown that vasopressin is coexistent with dynorphin in secretory vesicles in the neurohypophysis, and with CRF in the median eminence. The irregular (spinous dendrites) LHRH cells which develop during puberty, undergo this transformation independently of gonadal steroids. Ligand potency analyses of AVT (V2) receptors in the Xenopus A6 cell line and the toad bladder have shown that these amphibian systems contain a novel V-2 receptor. A large repertoire of MABs have been produced against neuronal cytoskeletal proteins in the squid axon and the Xenopus nervous system. Analysis of the protein kinase-calcium activated protease-neurofilament complex in the squid giant axon has shown that the protein kinase is similar to type II casein kinase, but with a preference for histone substrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00705-04 LNN

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)*

PI: Dr. James Russell Head LNN, NICHD

COOPERATING UNITS *(if any)*
David Njus, Dept. Biology, Wayne State Univ.; Patrick Fleming, Dept. Biochem., Georgetown Univ., Avril Somlyo, Penn. Muscle Inst.; Mark L. Mayer, LDN, NICHD; Gary Westbrook, LDN, NICHD; Harold Gainer, LNN, NICHD; Bryan Salzberg, Univ. of PA.

LAB/BRANCH
Laboratory of Neurochemistry and Neuroimmunology

SECTION
Unit on Neuronal Secretory Systems

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

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

CHECK APPROPRIATE BOX(ES)

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

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

The program of the Unit on Neuronal Secretory Systems is focused on studying the biochemistry and physiology of the nerve terminal using the neurohypophysial neuroendocrine cells as the model system. These include studies on the properties of the neurosecretory vesicle membranes, which take part in the exocytotic secretory process at the terminal, and has led to the development and consolidation of the secretory vesicle hypothesis in the Laboratory of Neurochemistry and Neuroimmunology. The neurosecretory vesicles were shown to have an intravesicular pH of 5.5 brought about by an electrogenic H^+ translocating Mg^{++}-ATPase on the membrane. The cytochrome b_{561}, also present on the vesicle membrane together with the intravesicular ascorbic acid, functions to transport electrons across the membrane and thus support a peptide amidating enzyme within the neurosecretory vesicles. Recently, a preparation of highly purified nerve endings (neurosecretosomes) has been obtained in order to study the kinetics of neuropeptide secretion from these terminals in vitro. Fractionation of membranes prepared from these neurosecretosomes led to the identification of non-mitochondrial Ca^{++}-sequestering membrane system inside the nerve terminals. Optical methods have been used to record action potentials in intact neurohypophysial nerve terminals, and have also revealed light scattering signals correlated with secretion.

LABORATORY OF MOLECULAR GENETICS

Z01 HD 00066-15 Control Mechanisms in Temperate Bacteriophage λ
Robert A. Weisberg, Ph.D.

Z01 HD 00067-17 Integrative Control of Macromolecular Synthesis
Michael Cashel, M.D., Ph.D.

Z01 HD 00068-14 Factors Influencing Genetic Transcription-Initiation and Termination
Robert J. Crouch, Ph.D.

Z01 HD 00069-13 Molecular Aspects of the Replication of Enveloped Animal RNA Viruses
Judith G. Levin, Ph.D.

Z01 HD 00071-13 Study of Adenovirus Gene Functions
Heiner Westphal, M.D.

Z01-HD 01001-03 Gene Organization and Expression in Drosophila
Igor B. Dawid, Ph.D.

Z01 HD 01002-03 Gene Expression During Embryonic Development of Xenopus Laevis
Igor B. Dawid, Ph.D.

Z01 HD 01003-03 Genetic Control of Oncogenesis
Hiroto Okayama, M.D., Ph.D.

Z01 HD 01004-02 Regulation of Amino Acid Biosynthetic Genes in Saccharomyces Cerevisiae
Alan G. Hinnebusch, Ph.D.

Laboratory of Molecular Genetics

In the report period the Laboratory of Molecular Genetics continued its research activities in the area of gene and genome structure, regulation of gene expression, mechanisms of recombination, and molecular aspects of developmental processes. As in the past, these research programs used a variety of different systems but relied on a commonality of basic interest, conceptual approach and technology.

This year saw the loss to the Laboratory of Molecular Genetics of a highly valued member of its staff; Jacob Maizel left the Institute for expanded responsibilities in the National Cancer Institute. The Laboratory of Molecular Genetics has thus lost its research program in the development of computer-assisted methodology for nucleic acid and protein sequence comparison and structural analysis. However, the capabilities for the application of existing methods in this area to ongoing research programs has been and will be maintained as it is indispensable for the work of every program within the Laboratory. The year also saw the gain of a research staff member in that Thomas Sargent, associated for several years with the program in developmental biology of Xenopus, has been promoted to position of senior investigator. In the future, Igor Dawid and Tom Sargent will guide this program as co-investigators.

Description of the research program in the Laboratory of Molecular Genetics

Developmental Biology Section

This section encompasses four distinct research programs using different biological systems. The program on Gene Expression during Embryonic Development of Xenopus, headed by Igor Dawid and Tom Sargent, continued to study a group of genes that are activated for the first time during blastula to gastrula stages of Xenopus. Several of these genes, which are named DG genes (for Differentially expressed in Gastrula), have been analyzed by sequencing, localization studies in the embryo, and determination of their developmental profiles.

Determination of abundance of 30 DG RNAs during development has shown that all are restricted in their expression to embryonic or tadpole stages. Most DG RNAs persist in the embryo for just one to a few days and thus have a very restricted developmental profile; a few continue to be present throughout tadpole life but largely disappear during metamorphosis. Thus, no DG gene studied so far is expressed in the adult, showing that the DG set of genes is quite distinct in its developmental properties. In contrast, genes that are activated at the neurula stage, several hours later than DG genes, frequently continue to be active in the adult.

Three of the most abundant DG RNAs have been identified as mRNAs for epidermal cytokeratins. Cytokeratins are protein subunits of intermediate filaments that are abundant structural components of epidermal and epithelial cells. The three cytokeratin genes encode distinct but related proteins that have all the structural features also found in cytokeratins of mammals and birds that are being studied in other laboratories. The cytokeratin RNAs have been localized in the epidermis by in situ hybridization and by analysis of RNA from dissected embryos. Genomic DNA corresponding to one of the epidermal keratin genes has been isolated and sequenced, revealing an exon/intron arrangement closely similar to that of a recently analyzed human cytokeratin gene.

DG42 is a gene that is expressed for less than one day during blastula to early neurula stages, during which time the corresponding RNA becomes quite abundant. This RNA has been localized to the endoderm, providing a very early endodermal marker which has not been previously available. DG42 has been sequenced and its properties are being investigated further by attempting to generate antibodies against its predicted protein product.

Differentiation in the Xenopus embryo, as in other embryos, depends on factors and structural properties inherited in the egg and on information generated by the interaction between embryonic cells. The relative importance of these two types of determinants in the differentiation of the three germ layers has been investigated by using regionally specific genes as molecular markers. Keratin gene expression has been used to signify ectodermal differentiation, DG42 as the endodermal marker, and muscle-specific actin genes as mesodermal marker. The use of molecular markers is preferable to cytological observation since they allow earlier and more objective evaluation of differentiation potential of embryonic cells. Cell-cell interaction during cleavage and blastula stages was prevented by dispersion of the cells after abolition of adhesion by culture in calcium/magnesium free medium. The results of these experiments indicate that the initial differentiation of the ectoderm and endoderm is independent of cell interactions and thus embryonic induction, but the establishment of the mesoderm requires such interactions. The use of these newly established molecular markers will allow a more detailed analysis of developmental mechanisms than has been possible heretofore.

The program on Gene Organization and Expression in Drosophila, headed by Igor Dawid, has focused on the molecular-genetic analysis of the locus fs(1)h, a maternal effect homeotic gene of considerable developmental interest. The function of this gene is required in the mother to allow normal segment determination in the progeny. In addition, the locus has functions during larval and pupal stages. In establishing segment identity, the fs(1)h locus interacts with other homeotic loci in the fly, notably the trithotax (trx) locus and some members of the bithorax complex. The fs(1)h locus has been cloned by chromosomal walking and the limits of the locus have been established by genetic and transcriptional analysis. In the ovary, RNA molecules of 7.6 and 5.9 kb are transcribed from the fs(1)h locus; these RNAs persist for the first few hours of postfertilization development but rapidly decay thereafter. It is likely that these RNAs mediate the maternal effect of the locus. Other RNA molecules of 8 to 9 kb are found in larval stages at very low abundances, and yet another RNA (2.4 kb) occurs in pupae. Several cDNA clones corresponding to parts of the fs(1)h locus have been isolated, but due to the large size of the RNAs no full-length clones have been obtained so far.

In the course of analysis of the fs(1)h locus a new family of repeated sequences in Drosophila has been found. These sequencers, named pen, are short, interspersed in the genome, and transcribed into RNA in a developmentally regulated way. These properties are unusual in Drosophila and, therefore, the pen family is being studied in some detail. Copies of the repeat originating from different genomic regions have been isolated and are being sequenced. Chromosome in situ hybridization is being used to determine whether the location of pen repeats correlates with any known gene loci.

The research program on Genetic Control of Oncogenesis headed by Hiroto Okayama is engaged in a study of genes that are involved in the steps that lead to cellular transformation. The approach is based on the earlier work of this research group in which a method was developed that allows the isolation of cDNA based on their functional expression after introduction into cultured cells. The following experiment was carried out: RNA from SV40-transformed human cells was transcribed into cDNA and cloned in a vector that supplies the necessary controls to lead to the expression of the information content of the cDNAs when they enter a mammalian cell. The vector also contained a separate selectable marker (the neo gene) which allows preliminary selection of transfected cells. After introduction of such a cDNA library into NIH 3T3 cells and selection with G418 the cells were cultured to observe their growth regulation. Several clones of cells were obtained that did not exhibit the contact inhibition of growth characteristic of the original 3T3 cells. DNA from these cells proved able to transmit this growth property to fresh cells. While a few of these transformed cells carried SV40 T antigen most did not; they may thus be carrying new oncogenes. One of the putative oncogenes has been isolated and others are being recovered at present. Their detailed analysis will be carried out subsequently.

The research program headed by Alan Hinnebusch is concerned with the regulatory pathway in yeast called general amino acid control. This system is responsible for the coordinate regulation of at least 30 unlinked genes involved in amino acid biosynthesis in response to starvation for single amino acids. A hierarachy of control genes has been established by genetic and molecular studies, suggesting that 3 genes, GCN1, GCN2, and GCN3, encode negative regulators for GCD1, which in turn is a negative regulator of GCN4. The GCN4 product is a positive regulator of the ultimately affected genes like the HIS4 gene. The regulation of expression of GCN4 has now been shown to occur at the translational level. This was accomplished primarily by generating fusion genes in which the 5' untranslated part of the coding region of the GCN4 gene has been fused to β-galactosidase to provide an easily assayed product. Culture under conditions of amino acid starvation and in various genetic backgrounds has shown clearly that the primary level of regulation of the fusion product is translational. This regulation is mediated by 4 short open reading frames in the 5' untranslated region of the GCN4 mRNA; it does not depend on the GCN4 promoter but does depend on wild type GCN2, GCN3, and GCD1 products. These conclusions are based on the fact that a GCN4 gene fusion from which the 4 short reading frames have been removed is constitutively derepressed, but a construct which contains the normal 5' untranslated region but a foreign promoter is regulated normally. The 4 short open reading frames are not equivalent, the largest effect being exerted by the first (5'-proximal) reading frame.

In a separate program in collaboration with Drs. Breviario and Dhar the transcriptional regulation of the yeast RAS genes has been studied. Yeast contains two similar RAS genes which are not fully equivalent. Recent results in this laboratory have shown that the two RAS genes are differently regulated by carbon source, possibly explaining the fact that a ras2- mutation shows a phenotype whereas a ras1- mutation does not.

Section on Molecular Regulation

The research group headed by Michael Cashel is concerned with the regulation of bacterial metabolism by nutritional factors and attempts to dissect molecular mechanisms that coordinately regulate the expression of many genes in response to such factors. The role of ppGpp in the regulation of ribosomal RNA synthesis has long been a focus of this laboratory's research; this work has been expanded to include studies on the metabolism of ppGpp and on ppGpp effects on histidine operon expression.

In the ribosomal RNA operon the focus of research has been on anti-termination, a feature of ribosomal transcripts to read through normally efficient terminators. This feature is shared with bacteriophage λ transcripts, and recent experiments have shown that the nusA, nusB, and nusE genes, known to be involved in λ anti-termination, are also involved in the case of ribosomal transcripts. Anti-terminated transcripts are terminated by super-terminators. A search for other genes required for supertermination was carried out and yielded mutations in three locations. One of these has been identified as the rpoB subunit gene of RNA polymerase. A series of overlapping deletions in the superterminators has been prepared which will be used in dissecting the functional determinants of this region.

A second series of experiments carried out by this research group was directed towards an analysis of the spoT gene in E. coli; this gene is responsible for the degradation of ppGpp. Different mutants in spoT have been obtained and combined with various relA mutants. In this way, different levels of intracellular ppGpp have been generated in cells cultured in the same medium. This experiment allowed a test of the effect of changing ppGpp levels on growth rate without changing nutritional conditions. An inverse correlation between ppGpp level and growth rate was found. The spoT gene has been cloned and sequenced, which will allow the generation of null mutations and a further study of its properties.

The regulation of histidine biosynthesis in Salmonella is affected by ppGpp. Two histidine regulatory mutations have been studied and shown to be alleles of gyrA and gyrB, the genes encoding the two subunits of DNA gyrase. This observation suggests that superhelix density affects transcription of the histidine operon.

The research group headed by Robert Crouch is concerned with RNA processing and enzymes involved in nucleic acid metabolism. RNaseH, an enzyme that degrades the RNA strand of RNA/DNA hybrids, has been the focus of research in this group. In E. coli rnh mutants which also carry a recB temperature sensitive mutation are temperature sensitive for growth. Using this complementation test

several mutant RNaseH genes from E. coli, the RNaseH gene from Salmonella typhimurium, and a complementing DNA sequence from yeast which probably is the yeast RNaseH gene, have been isolated.

In a separate program, the mechanism of chicken rRNA processing is being studied. Binding of the small nuclear RNA U3 to rRNA precursor has been shown by gel co-electrophoresis. The rRNA precursor appears to have several U3 binding sites, supporting the view that U3 RNA is involved in rRNA processing.

Section on Animal Viruses

This section is headed by Heiner Westphal. Two distinct research programs are carried out by this Section, one of these concerned with the regulation of adenovirus metabolism, the other with the regulation of genes introduced into the germline of mice.

In a collaboration with Martin Rosenberg and his colleagues, the functions of the adenovirus E1A gene product have been studied. The wild type and various modified E1A proteins have been produced in E. coli and were injected into cultured cells. The major properties assayed were complementation of growth of a mutant virus lacking the E1A gene, and translocation of the E1A product to the nucleus. Deletion analysis has demonstrated that the translocation function is encoded by a region close to the C terminus of the E1A protein, whereas complementation of mutant virus required a contiguous region in the interior of the protein.

The second program carried out by this section has shown the most gratifying progress during the past year. Transgenic mice have been generated with several different genes and expression of the introduced genes has been observed in a number of cases. This approach allows the study of the function of genes and regions of genes in the normal life cycle of the animal and thus promises important insights into mechanisms of developmental regulation of gene activity. In the recent past, transgenic mice have been obtained carrying gene constructs with protions of the lens αA-crystallin gene (in collaboration with J. Piatigorsky), the α2(I)-collagen gene (collaboration with B. de Crombrugghe), and the RSV-LTR. Constructs containing relatively short segments from the 5' region of the crystallin and collagen genes were expressed in a spatially and developmentally correct fashion: The crystallin construct was expressed only in the fiber and epithelial cells of the lens while the collagen construct was expressed most intensely in the tail. Constructs carrying the RSV-LTR were expressed in muscle and connective tissues, again in agreement with the known tissue preference of the virus. In addition, several mouse strains have been generated which carry mutations apparently induced by the integration of foreign DNA. The continued application of the transgenic methodology may be expected to yield further important insights.

Unit on Viral Gene Regulation

This research group is headed by Judith Levin and is concerned with the analysis of replication in enveloped RNA viruses, specifically MuLV. The structure and mechanism of action of reverse transcriptase has been the focus of recent work. To allow further study of this question, portions of the viral pol gene have been cloned in E. coli. By the use of suitable expression

vectors fusion proteins between parts of the pol gene and β-galactosidase have been produced, and were used to generate antibodies in rabbits. These sera react with reverse transcriptase and with the pol gene-encoded endonuclease derived from virus-infected cells.

In a related project, revertants of a reverse transcriptase mutant named clone 23 have been isolated. Several revertants have been obtained and will be studied by molecular methods.

Microbial Genetics Section

This section, headed by Robert Weisberg, is concerned with mechanisms of genetic recombination. The major system of study is bacteriophage λ and its interaction with the host. Recombination of the phage and the host chromosome is mediated by specific sites called attachment sites. The central regions of the attachment sites have sequence homology that mediates pairing required for recombination. Mutations that change this central region interfere with homologous pairing and consequently with recombination. Recent experiments have shown that in such mutants there is no accumulation of Holliday structures. Holliday structures are X-shaped, four stranded DNA molecules believed to be intermediates in genetic recombination. Therefore, the strand exchange leading to the formation of the Holliday structure is dependent on homologous pairing.

Endonuclease I of bacteriophage T7 cleaves Holliday structures and is required for genetic recombination. Studies on endonuclease I have shown that the symmetry of cleavage of the four-stranded DNA molecule depends on homologous pairing. Holliday structures containing wild type and mutant attachment sites yield only two rather than four products after cleavage with endonuclease I. It appears likely that the effect of the mutation is to limit the extent of branch migration in the Holliday structure.

Site specific recombination has also been studied in the λ-related bacteriophage HK022, a phage with distinct attachment site from λ. In spite of the difference between the two phages, HK022 produces a xis protein that is completely interchangeable with the λ product. Phage HK022 has also been studied with respect to termination of transcription. A phage-encoded termination protein, called Nun, has been detected and shown to prevent the transcription of early λ proteins. Interactions between Nun, host factors called nus, and λ sites called nut, suggest that termination and anti-termination have steps in common.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00066-15 LMG

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control Mechanisms in Temperate 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
	Bernard de Massy	Visiting Fellow	LMG, NICHD
	Jacques Oberto	Visiting Fellow	LMG, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Molecular, NCI (Dr. Max Gottesman).
Department of Biology; Brookhaven National Laboratory; Upton, N.Y.
(Dr. F.W. Studier)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Microbial Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2	0.4

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

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

Recombination between bacteriophage λ and its host Escherichia coli (site-specific recombination) is effected by a pair of reciprocal strand exchanges that occur within special sequences called attachment sites. We have previously shown that base substitution mutations in the central segment of an attachment site interfere with recombination by preventing homologous pairing between the mutated site and its wild type partner. Our recent results suggest that the same mutation also prevents conversion of a Holliday structure -- an X-shaped DNA molecule that is a postulated intermediate in recombination -- to recombinant products. It therefore appears possible that a direct interaction between homologous regions is also necessary for this step in the reaction.

We previously showed that endonuclease I of bacteriophage T7 cleaves Holliday structures. We have now extended our original observation, made with a short cruciform DNA substrate, to several other substrates, including Holliday structures formed from phage λ attachment sites. We have made the surprising observation that introduction of a base substitution mutation into the central region of the λ Holliday structure affects the symmetry of cleavage: instead of obtaining all four products in equal yield, only two products are obtained. It appears likely that the effect of the mutation is to limit the extent of branch migration of the Holliday structure.

We have discovered that HK022, a temperate coliphage related to λ produces a protein that causes RNA polymerase to terminate transcription when it encounters specific sequences in the early region of the phage λ chromosome. Termination occurs at or near sequences called nut sites and requires host encoded proteins called nus factors. Nut sites and nus factors are also required for a contrasting process: antitermination of transcription by the λ N protein. It is of considerable interest that termination and antitermination of transcription appear to have mechanistic steps in common.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00067-17 LMG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
	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:
2.2	2	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project continues to be concerned with how cells coordinately regulate the expression of their genome during growth. Focus continues on processes mediated by guanosine 3', 5' bis-pyrophosphate (ppGpp).

Ribosomal RNA transcription is exemplary of ppGpp inhibitory activity. We have found that ribosomal transcripts are modified (anti-termination) so as to ignore normal termination signals. This alteration depends on a sequence near the ribosomal promoter region that is genetically and physiologically homologous to an equivalent modification in phage lambda. Special terminator regions (called super-terminators) can stop an anti-terminating transcript. We have isolated such a region and constructed a large set of sequenced overlapping deletions that should enable functional dissection of the region. We have also isolated two mutants result in specific defects in super-termination, that terminate normally.

New mutants of the major enzyme responsible for ppGpp degradation, encoded by the spoT gene, have been isolated and exploited to yield ten-fold variations in ppGpp basal levels during balanced growth; previously ppGpp variations could be studied only during starvations. We find an inverse correlation between growth rate and ppGpp levels. The spoT gene has been sequenced and extragenic suppressors of its function isolated that are important in analysis of ppGpp synthesis and decay.

We have both genetic and physiological indications that histidine operon expression typifies a process positively regulated by ppGpp at the promoter level. Two histidine regulatory mutations (his W333 and hisU1820) have been mapped to the genes for gyrase subunits (gyrA and gyrB) suggesting super-helicity of DNA templates is important for his operon regulation as well as for ribosomal operon transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00068-14 LMG

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	B. Stevens-Klapholz	Visiting Associate	LMG, NICHD
	D. Drakeford	Biologist	LMG, NICHD
	M. Walters	Guest Worker	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:
3.1	1.7	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.)*

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. A mutant of rnh (Ribonuclease H) of E. coli is poorly viable in combination with recBC mutants. We have taken advantage of this observation to construct a double mutant (rnh, recBts) that is temperature sensitive for growth. Screening DNAs from a variety of sources has permitted us to clone (1) mutant E. coli rnh genes (2) a Salmonella ribonuclease H gene and (3) a DNA from Saccharomyces cervisiae. In a second area, we have demonstrated that U3RNA (a nuclear small RNA) can form relatively stable complexes with rRNA precursors. The latter observation supports the idea that U3RNA is involved in rRNA processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00069-13 LMG

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 Levin	Research Biochemist	LMG, NICHD
Others:	John P. Quinn	Visiting Fellow	LMG, NICHD
	Stella C. Hu	Chemist	LMG, NICHD
	Michael Seddon	Bio Aid	LMG, NICHD

COOPERATING UNITS *(if any)*
NCI - FCRF (Don Court); Basic Research Program, LBI, NCI-FCRF (Alan Rein); PRI-FCRF (Martin Zweig)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Unit on Viral Gene Regulation (Developmental Biology Section)

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1	1.4

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

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

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. Portions of the MuLV pol gene have been expressed in E. coli. The expression system consists of (i) a vector carrying the bacteriophage λ P_L promoter and sequences encoding the first 13 amino acids of the λ cII protein fused (out-of-frame) to the β-galactosidase coding region and (ii) a lac- host with a temperature-sensitive λ repressor. Recombinants containing in-frame inserts of the reverse transcriptase and endonuclease coding regions have been isolated. At 42°C these clones express large amounts of high molecular weight lac Z fusion proteins, which have been injected into rabbits to elicit antibody production. Analysis of wild-type and mutant virions by immunoprecipitation and Western blot techniques has demonstrated that the antisera react specifically with two proteins present only in wild-type MuLV: the 80K reverse transcriptase; and a 47K protein which we can now identify as the viral endonuclease. Proteins expressed by clones engineered to eliminate lac Z expression can be partially solubilized with high salt and detergents, and some reverse transcriptase activity has been detected in extracts of clones containing the reverse transcriptase coding sequence. In other studies, revertants of the frameshift pol mutant clone 23 have been isolated. Efforts are underway to make molecular clones of these revertants to determine the mechanism by which the mutation is repaired. Experiments involving in vitro mutagenesis of selected regions of the MuLV genome have also been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00071-13 LMG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Regulation of Gene Expression in the Adenovirus System and in Transgenic Mice

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 Associate	LMG, NICHD
	P. Overbeek	Staff Fellow	LMG, NICHD
	B. Krippl	Visiting Fellow	LMG, NICHD
	K. Mahon	Staff Fellow	LMG, NICHD
	A. Dey	Visiting Fellow	LMG, NICHD
	S. Lai	Chemist	LMG, NICHD

COOPERATING UNITS *(if any)* NCI, NIH (B. de Crombrugghe); FCRF (G. Vande Woude); NEI, NIH (J. Piatigorsky); SKF Laboratories, Philadelphia, Pa. (M. Rosenberg); NIAID, NIH (K. Bernstein); Brookhaven National Labs (J. Dunn); U. of Heidelberg, W. Germany (P. Gruss)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Animal Viruses

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4	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.)*

Our laboratory investigates mechanisms of gene control in mammalian cells in culture and in the whole animal. One of two major projects, dealing with the E1A regulatory function of adenovirus, has been completed. The E1A function acts as a transcriptional modulator 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 microinjected the proteins into mammalian cells and measured their ability to migrate to the cell nucleus and to activate an adenovirus E1A deletion mutant. Information for nuclear localization and for viral gene activation has been shown to be encoded by distinct domains of the adenovirus E1A gene. Our second project involves insertion of specific gene constructs into mouse embryos. Spatial and temporal control of the expression of the inserted genes is examined in the resulting transgenic animals. So far, we have analyzed mice carrying three different chimeric gene constructs. Each of these contains the gene for bacterial chloramphenicol acetyl transferase (CAT) under the control of a promoter/enhancer region derived from either the mouse αA crystallin or α2(I) collagen gene or from Rous sarcoma virus (RSV). The temporal and spatial control of CAT expression in mice carrying the αA crystallin-CAT or the α2(I) collagen CAT construct reflects that of the genuine mouse genes from which the 5' flanking sequences of the chimeric genes were derived. In mice carrying the RSV-CAT construct CAT expression is preferentially directed to muscle and connective tissue. This reflects the disease specificity of sarcoma viruses. Finally, we have begun to analyze one RSV-CAT transgenic strain which is characterized by a dominant trait of embryonic lethality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01001-03 LMG

PERIOD COVERED
October 1, 1984 to September 30, 1985

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. Rebbert	Chemist	LMG, NICHD
	B. Mozer	Biologist	LMG, NICHD
	F. Forquignon	Visiting Associate	LMG, NICHD
	S. Haynes	Guest Researcher	LMG, NICHD
	J. Owens	Computer Specialist	LMG, NICHD

COOPERATING UNITS *(if any)*
Centre Genetique Moleculaire, CNRS, Gif-sur-Yvette, France (M. Gans and F. Forquignon)

LAB/BRANCH
Laboratory of Molecular Genetics, NICHD

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	1.2	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 maternal effect developmental gene fs(1) has been studied by genetic and molecular techniques. Mutations in this locus result in homeotic transformations in the progeny of mutant females, and thus this locus represents a maternal gene that is involved in the specification of segment identity in Drosophila. The fs(1)h locus has been cloned by chromosomal walking and the region corresponding to the gene has been identified by the localization of 4 mutations within a stretch of 13 kb. Transcription mapping has shown that the fs(1)h locus is expressed in a doublet of RNAs of similar size of 7.6 kb and a 5.9 kb RNA during oocyte development; these transcripts correlate with the maternal function of the locus. A different transcript of 2.4 kb has been identified in pupae, correlating with additional functions known to be carried out by fs(1)h at this stage of development. Cloned cDNAs representing parts of the ovarian transcripts of fs(1)h have been isolated and are being sequenced.

The expression of the ribosomal insertion which interrupts more than half of the rDNA in Drosophila has been studied. The DNA intercalating drugs chloroquin and ethidium bromide stimulate the transcription of the insertion, suggesting that the transcriptional repression of insertion sequences are very sensitive to the degree of torsional stress in the chromatin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01002-03 LMG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
	E. Jonas	Visiting Fellow	LMG, NICHD
	A. Cheng	Biologist	LMG, NICHD
	G. Michaels	Staff Fellow	LMG, NICHD
	J. Owens	Computer Specialist	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

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.15	5.55	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Molecular events during embryogenesis in Xenopus laevis are being studied with the aid of gene sequence isolated from a subtracted cDNA library. This library contains copies of only those RNAs that are absent from the oocyte and are accumulated in the embryo between blastula and midgastrula stages; these are named DG RNAs. The analysis of a selected group of about 30 DG cDNA has resulted in the following findings.

Three cDNA clones representing some of the most abundant embryonic RNAs have been identified by sequence analysis and comparison as encoding epidermal cytokeratins. Two of the genes represent type I keratin genes, the third is a type II keratin gene. The two type I genes, named DG70 and DG81, are related but quite distinct sequences. They have different developmental profiles in that DG70 is restricted to an earlier stage than DG81. The genomic DNA encoding DG81 has been isolated and sequenced. Its intron/exon arrangement is closely similar to that of a recently analyzed human type I keratin gene.

Several DG RNAs have been localized in the embryo. The keratin genes DG70, DG76 and DG81 are localized in the ectoderm and later the epidermis. DG42, an RNA that is abundant for just a short period of gastrula and neurula development, and one other RNA have been localized to the endoderm. These cDNA clones, together with an α-actin clone, have been used to study the significance of cell interactions in early development for differentiation of different lineages. Evidence has been obtained for the requirement of cell interactions for differentiation of the mesoderm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01003-03 LMG

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

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

PI:	Hiroto Okayama	Visiting Scientist	LMG/NICHD
Others:	Masashi Kawaichi	Visiting Associate	LMG/NICHD
	Claudie Chen	Biologist	LMG/NICHD
	Suzanne Rousseau	Biologist (Tech)	LMG/NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

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 is strongly suggested that oncogenes transform cells through a common pathway, which perhaps involves most of the cellular growth regulatory systems including cell-cell recognition coupled to growth control, growth factor production and cell cycle controls. In attempting to elucidate this pathway, we have initiated studies to molecularly clone and characterize cellular genes whose activation leads to induction of full or partial transformed phenotypes in cells, by employing the cDNA expression system we recently developed (Okayama, H., and Berg, P., Mol. Cell. Biol. 5, 1136-1142). This approach comprises the following steps: 1) construction of a cDNA clone expression library with the mRNA from SV40-transformed human fibroblast; 2) transduction of the library into NIH3T3 cells; 3) screening of colonies that show lack of growth contact inhibition; 4) recovery of the integrated cDNA into E. coli for characterization. Transfection of 10^7 NIH3T3 cells with the library (1.5×10^6 independent clones) yielded 62 colonies that showed lack of contact inhibition: 36 are perhaps transformed by integrated cDNA, 24 by spontaneous transformation, and 2 by SV40 T-antigen. The integrated cDNAs from the 36 colonies are being recovered into E. coli for characterization. One cDNA clone recovered is able to induce the same transformed phenotype in NIH3T3 cells but has no detectable homology to the major known oncogenes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01004-02 LMG

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Regulation of Amino Acid Biosynthetic Gene 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:	Ernest Hannig	Staff Fellow	LMG, NICHD
	Satoshi Harashima	Visiting Associate	LMG, NICHD
	Alice Ma	Biologist	LMG, NICHD
	Peter Muller	Visiting Fellow	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:
3.8	2.6	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our work focuses primarily on a regulatory system in the yeast Saccharomyces, known as general amino acid control, which governs the transcription of a large number of amino acid biosynthetic genes in response to amino acid availability. Our recent results extend our previous finding that the proximal trans-acting positive effector in this system (GCN4) is itself regulated and this regulation occurs at the translational level. Analysis of a GCN4-lacZ gene fusion indicates that all other known general control trans-acting factors exert their effects indirectly as translational effectors of GCN4. This was shown directly by the fact that their effects on GCN4 expression are independent of the GCN4 promoter. GCN4 mRNA contains a cis-acting negative element which mediates the translational control of GCN4. This site encompasses 4 short open-reading-frames (ORFs). We have generated point mutations in the AUG codons of these ORFs and preliminary results suggest that these sequences are the primary regulatory elements in the GCN4 mRNA leader. Interestingly, the ORFs are not equivalent in their effects and the first ORF seems to be critical for establishing the regulatory effects of the trans-acting factors on GCN4 translation. We have also isolated four new complementation groups of mutations which inactivate or bypass the GCN4 short ORFs. One of the groups is closely linked to GCN4 and may represent cis-acting constitutive alleles of GCN4. Finally, we are attempting to produce antisera specific for the protein products of GCN4 and its positive regulator encoded by GCN3. These antisera will be used to determine the cellular localization and for purification of these proteins. We are also involved in collaborative work on the expression of the yeast homologues of the RAS cellular oncogenes and the RNase H gene of yeast.

LABORATORY OF THEORETICAL AND PHYSICAL BIOLOGY

Z01 HD 00040-10 Statistical and Mathematical Studies and Modeling of Drug Receptor Interaction
Peter J. Munson

Z01 HD 00165-10 Isolation and Characterization of Protein Hormones
Andreas Chrambach, Ph.D.

Z01 HD 00171-09 Electrophoretic Methodology
Andreas Chrambach, Ph.D.

Z01 HD 00189-04 Computer Programs for Analysis of Laboratory and Clinical Data
David Rodbard, M.D.

Z01 HD 01400-03 Clinical Applications of Stable Isotopes
Alfred L. Yergey, Ph.D.

Z01 HD 01401-03 Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry
Alfred L. Yergey, Ph.D.

Z01 HD 01402-02 Protein-Cytoplasmic Matrix Interaction; Ligand Binding Kinetics During Endocytosis
Nahum D. Gershon, Ph.D.

Z01 HD 01403-02 The Three Dimensional Organization of Cells and Anatomical Components
Nahum D. Gershon, Ph.D.

Z01 HD 01404-02 Characterization of Opioid Receptors in Brain and Peripheral Tissues
David Rodbard, M.D.

Z01 HD 01405-01 Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus
David Rodbard, M.D.

NICHD ANNUAL REPORT
October 1, 1984 through September 30, 1985

Laboratory of Theoretical and Physical Biology

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 chromatograpy/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.

Section on Theoretical Biology

The Laboratory of Theoretical and Physical Biology has made several important contributions during the past year. These include: The pharmacological characterization of multiple subtypes of the opioid receptors in adrenal medulla, designated K1, K2, K3. The existence of a very high affinity "mu-1" receptor subtype in brain has been established in our laboratory. These laboratory findings have been made possible by our development of new mathematical statistical and computational methods for studying dose response curves in the presence of multiple ligands (the "multi-ligand" design), the combination of modelling and biochemical approaches, computerized methods for optimization of experimental design, for analysis and validation of results. Related applications include the characterization of histamine receptors in lung and lymphocytes. Other programs, developed to analyze enzyme-substrate-inhibitor systems, have been used to characterize enzymes important for cancer chemotherapy and catecholamine transport in chromaffin cells.

A major activity and accomplishment has been the development of several innovative computer programs to assist physicians, paramedical personnel and patients with home monitoring of blood glucose and self-adjustment of insulin dosage. These programs for the IBM-PC and Apple II classes of microcomputers, provide data storage and retrieval, graphical and statistical analyses, and "expert" consultation regarding adjustment of insulin dosage, timing of glucose

measurements, and when the patient should call the physician. Human engineering factors have been optimized to facilitate acceptance, and the programs have multiple fail-safe features. We have developed a general flexible approach for development of customized treatment plans ("algorithms") for individual patients. The computer enables us to examine the discrete and continuous "glucose profile" to evaluate median circadian patterns, or changes by day of week with appropriate weighting and smoothing. The program provides a detailed analysis and interpretation of the glucose profile. The ancillary documentation for the programs has developed into a book, explicitly describing a versatile new approach to management of patients with type-I diabetes. These programs are now being evaluated in controlled clinical trials at medical centers throughout the country, with special reference to their use in children, adolescents, and during pregnancy.

A major new activity has been the development of several new approaches to analysis of sequential hormonal measurements, for detection of "pulses" of episodic secretion. These methods are objective, statistically valid, rational, simple, sensitive and reliable, and have been incorporated into interactive computer programs for the DEC-10 and IBM-PC. They permit the evaluation of the "half-life" of the hormone, and estimation of the instantaneous rate of hormone secretion. These programs are now being applied to multiple areas of clinical investigation with extensive collaborations at NIH and throughout the world.

Continued progress has been made in the 3-dimensional reconstruction of images from light- and transmission electron microscopy, with applications to the "whorl bodies" of endoplasmic reticulum and Golgi apparatus of hormonally sensitive hypothalamic nuclei, and to other applications in the cell biology of normal and neoplastic tissues.

Section on Macromolecular Analysis

The Section on Macromolecular Analysis has continued its studies on the methodology of polyacrylamide gel electrophoresis, isoelectric focusing and related techniques. Pilot studies are examining the feasibility of new approaches to "two dimensional macromolecular mapping". A major effort has been made to obtain stable pH gradients for isoelectric focusing, avoiding problems of low conductance. The combination of soluble and immobilized ampholyes appears to offer significant improvement over previous methodology. Analysis of available data for migration of viruses in very dilute agarose gels, has revealed "non linear Ferguson plots", inconsistent with most previous data from gel electrophoresis. This has led to theoretical studies, with modification and extension of the application of the "Ogston model". It appears that the effective gel-fiber radius and coating or density vary systematically as a function of gel concentration. New methods have been developed for electrophoretic fractionation of cell organelles and vesicles, and for multimillion molecular weight immunogens using agarose electrophoresis. An improved apparatus for rapid staining and destaining of gels has been developed. The theoretical analysis of isoelectric focusing using multiple mono- and bivalent buffer constituents has been pursued.

Unit on Metabolic and Mass Spectroscopy

The Unit on Metabolic Analysis has made several important advances in the application of liquid-chromatography-thermospray mass spectrometry (LC-MS) of compounds of biochemical and medical interest. New methods have been developed

for measurement of deuterated glucose and cortisol in human plasma, and are now being applied to clinical investigation of the role of cortisol metabolism in normal puberty, and biochemical analysis of the pathophysiology of various inborn errors of metabolism (glycogen storage diseases). Studies of metabolism of stable isotopes of calcium in neonates (to examine the role of vitamin D supplements), in pregnancy, lactation and disorders with ectopic calcification are continuing with multiple collaborators at NIH and throughout the country.

LC-MS has now been applied to a host of compounds, including the carnitine derivatives important in fatty acid and drug metabolism, studies of glucuronide derivatives of drugs, and a novel amino acid (hypusine).

Pilot studies have indicated the feasibility of new approaches to characterization of oligo- and small polypeptides, and these methods are now being applied to growth factors derived from neoplasms.

In summary: the LTPB has had an extremely productive year with its wide ranging multidisciplinary program for development of new strategies, biochemical methods, and computer programs, to permit improved analysis of receptor systems, purification and characterization of protein and polypeptide hormones, metabolism of metals and organic compounds in man and in vitro, and for utilization and interpretation of laboratory and clinical data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00040-10 LTPB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical and Mathematical Studies and Modeling of Drug Receptor Interaction

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

PI:	Peter J. Munson	Mathematical Statistician	LTPB, NICHD
Others:	D. Rodbard	Head	LTPB, NICHD
	R. Lutz	Visiting Scientist	LTPB, NICHD
	R. Cruciani	Visiting Fellow	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	R. Staton	Statistitican	LTPB, NICHD
	M. Beveridge	Guest Researcher	LTPB, NICHD
	G. Pesce	Guest Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.13	1.0	.13

CHECK APPROPRIATE BOX(ES)

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

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

We have continued to develop, refine and apply theoretical and statistical techniques which permit the characterization of mechanisms of hormone-receptor interaction. These techniques include optimized design to reduce the amount of experimental material required and maximize the utility of results obtained in each experiment. Analysis techniques utilizing non-linear least squares have been developed which allow simpler, more informative experiments to be performed. These techniques have been applied to the study of opiate receptor systems wherein a further characterization of subtypes of the mu receptor were obtained, and to characterization of subtypes of the kappa receptor in bovine adrenal medulla. A Monte-Carlo study of the validity of these statistical techniques was completed, showing them to be valid in the situation of mu-1, mu-2 opiate subtypes.

A parametric means of characterization of asymmetric dose-response curves was developed and tested extensively on practical problems. An alternative, "non-parametric", spline-based method of characterizing such curves, testing parallelism, and calculating potency is currently under development.

A new statistical method for detecting non-randomness was developed and tested. This method is an alternative to the commonly used mean-square-successive differences test.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-10 LTPB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
Others:	G. Kapadia	Guest Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)*

LDMI, NICHD (D. Tietz, R. Schneerson, and J. Robbins); NIADDK, NIH (M.H. Gottlieb); INSERM, unite 36, Paris (C. Auzan, P. Corvol, J. Menard); Genetech Inc., South San Francisco CA (A. Jones).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

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.)*

1) Covalently linked protein-polysaccharide conjugates exhibit in agarose gel electrophoresis a particle size of 20 nm relative to spherical virus standards. Differences in net charge and size between preparations were distinguished by 2-dimensional agarose gel electrophoresis. Correlation between these differences and immunogenic potency is being attempted. 2) Clathrin-coated brain and liver vesicles exhibit radii of 40 and 53 nm respectively in agarose gel electrophoresis, in agreement with values from electron microscopy. Free mobilities of 0.76 and 0.61 10-5 cm2/s/V were found and measure surface net charge. 3) Inactive renin from amniotic cultures is larger and more basic than active renin by the criteria of quantitative gel electrophoresis. These properties make it likely that inactive renin is prorenin. Under conditions of electrofocusing, each reveals 2 charge isomeric species. 4) Des-angiotensin I-angiotensinogen is optimally separated from angiotensinogen at the transient state of electrofocusing. 5) Angiotensins I, II and III separate as cationic charge isomers in PAGE and can be analyzed jointly at 30 %T in the Rf-range 0.3 - 0.8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00171-09 LTPB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	B. An der Lan	Guest Researcher	LTPB, NICHD
	P. Bocek	NAS Exchange Scholar	LTPB, NICHD

COOPERATING UNITS *(if any)*
LDMI, NICHD (D. Tietz); DRS, NIH (J. V. Sullivan); INSERM, unite' 36, Paris, France (C. Auzan, P. Corvol, J. Menard)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Macromolecular Analysis

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.)*

1) In pursuit of the goal of a 2-dimensionally steady-state macromolecular map, the field strength across a pH 4-10 gradient was made more even by the addition of carrier ampholytes to Immobiline gels. This eliminates a) lateral zone spreading; b) the pile-up of protein zones at the edge of low current regions around neutrality, c) the retention of proteins in the sample zone, and d) allows for pH and voltage measurements not or difficultly feasible without such addition. 2) To obviate the considerable gel length associated with flat pH gradients over the wide pH range (4-10) and the resulting need for very high total voltage across such gels, a diagonal mode of Immobiline electrofocusing was devised. 3) The effective radius of the agarose fiber was determined in the order of 1 nm, using high agarose concentrations suitable for the sieving of proteins. This shows that the reduction in fiber radius from about 18 nm, found with viruses, to the same value previously determined for hydroxyethyl-agarose is not due to hydroxyethylation. 4) The divergent effective fiber radii of agarose found with viruses and proteins were reconciled by the demonstration of non-linear Ferguson plots for both, and thus increasing KR, decreasing r, increasing 1' and decreasing fiber volume (ml/g) with increasing agarose gel concentration. 5) Ferguson plots were derived for electrophoresis in linear, uncrosslinked, liquid polyacrylamide. Sieving is qualitatively similar to that obtained with crosslinked gels. 6) The potential application of the capillary apparatus with optics for sieving of macromolecules with liquid polyacrylamide was initiated. 7) A device for rapid diffusion of gels was constructed by use of which the staining time of SDS-gels can be reduced to 1.5 h or less. A similar device for the silver staining of cylindrical gels is being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00189-04 LTPB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Computer Programs for Analysis of Laboratory and Clinical Data

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

PI:	D. Rodbard	Head	LTPB, NICHD
Others:	P. Munson	Mathematical Statistician	LTPB, NICHD
	R. Staton	Statistician	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	K. Oerter	Guest Researcher	LTPB, NICHD
	M. Beveridge	Guest Researcher	LTPB, NICHD
	M. Jaffe	Guest Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)* CC, NIH (J. Foy); NCI, NIH (B. Chabner, and C. Allegra); NIADDK (H. Pollard); Univ. of Virginia School of Medicine (Rogol, Veldhuis); Univ. of Florence (G. Forti, M. Serio, M. Pazzagli); Hershey School of Medicine (K. Oerter); Kantonspital, (R. Lutz).

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:
1.0	.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 space provided.)*

This laboratory continues its tradition of developing novel computer programs for analysis of clinical and laboratory data. We have developed multiple new approaches for analysis of episodic hormone secretion in man, experimental animals, and in in vitro perifused cell systems. These methods are statistically valid, objective, reliable, sensitive and yield new physiological information including the instantaneous rate of hormone secretion and the half-life or decay constant(s) for hormone metabolism and degradation. Other programs include improved methods for Lineweaver-Burk and Dixon-plot analysis of enzyme-substrate-inhibitor systems, radioimmunoassays, bioassays, radio receptor assays, X-ray inactivation and dissociation studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01400-03 LTPB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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. Estaban	Visiting Fellow	LTPB, NICHD

COOPERATING UNITS *(if any)* HGB, NICHD (J. Sidbury); Lab. of Mathematical Biology, NCI (D. Covell); Dept. of Ped., Washington Univ. Med. School, St. Louis, MO. (Laura Hillman); Dept. of Clinical Nutrition/Gastroenterology, University of Chicago Med. Sch. (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	.5	1.0

CHECK APPROPRIATE BOX(ES)

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

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

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. 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. The acquisition of a new instrument has permitted the measurement of isotope ratios with greatly expanded accuracy and precision. We are now able to detect a 2-2.5% change in the natural ratio of 42Ca/48Ca with a relative standard deviation of 0.5%. The protocol has been expanded to include pregnancy and lactation as areas for study, and the first participant in this part of the program has been studied. Population statistics for a group of normal children have been obtained for the mean residence time of Ca in plasma (11.1 + 2.5 hrs) and fraction of Ca absorbed from diet (0.45 + .2) using a non-compartmental model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01401-03 LTPB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Biological Applications of Thermospray Liquid Chromatography/Mass Spectrometry

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

PI:	A. Yergey	Head	LTPB, NICHD
Others:	D. Liberato	Staff Fellow	LTPB, NICHD
	N. Esteban	Visiting Fellow	LTPB, NICHD

COOPERATING UNITS *(if any)*

Div. of Ped. Met., Dept. of Ped., Duke Univ., Durham, NC (D. Millington and C. Roe); LMG, NICHD (J. Sidbury and J. Muenzer); DEB, NICHD (J. Booth, J. Winterer); LDN, NICHD (D. Brenneman); NRL, Chem. Div. (R. Colton, D. Kidwell).

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.)*

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 quantification of cortisol in human plasma. Development of stable isotopic tracer methods using d3-cortisol prior to use in studies of cortisol production rates in children. Tracer levels are readily measured at 1-4% of total plasma cortisol. 3) Separation and quantification of glucose in human plasma prior to use of uniformly labelled C13 labelled glucose (98 atom % excess) as tracer for production and turnover rate studies in type I glycogen storage disease. 4) Identification of a variety of glucuronide and glutathione conjugates that were intractable to mass spectrometric analysis by other methods (Protocol 85-CH-15 and 85-CH-33).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01402-02 LTPB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein-Cytoplasmic Matrix Interaction; Ligand Binding Kinetics During Endocytosis

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

PI:	N. D. Gershon	Visiting Scientist	LTPB, NICHD
Others:	K. R. Porter	Guest Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)*
DCRT, NIH (B. Trus); FIC, NIH and Department of Biology, University of Maryland, Baltimore County, Catonsville, MD; ELTA Electronics Industries, Ashdod, Israel (B. H. Aizenbud).

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:
.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 objectives of this work are 1) to determine the volume of the cytoplasm taken by the cytoplasmic matrix under different external osmotic environments, and how it affects the diffusional motion of proteins inside the cytoplasm and 2) determine the rate of intake of ligands during endocytosis.

The cytoplasmic matrix is composed of a number of filamentous systems (microfilaments, intermediate filaments, microtubles, and the microtrabecular lattice). The use of the image analysis method that we develped demonstrated that the fractional volume of the cytoplasmic matrix is rather low (16% -21%). These values and the pore dimensions indicate that the cytoplasmic matrix slows down the diffusion of proteins to a great extent by transient binding.

Refinement of the binding analysis showed that most of the matrix binding sites are free. The binding constants values point out that although the association-dissociation process can occur very quickly, most of the proteins are bound to the cytoplasmic matrix. We initiated the study of the effect of the external osmotic environment on the volume of the cytoplasmic matrix.

The binding rate of a diffusing ligand to membrane receptors was calculated as a function of the internalization (endocytosis) rate. It was shown that when the internalization rate is relatively slow, the effect of the lateral distribution of the receptors on the ligand intake is not large compared with the situation where the internalization occurs instantly.

The meaningfulness of this work lies in the fact that measuring the volume fraction of the cytoplasmic matrix can shed light on molecular transport through the cytoplasm. In addition, the amount of surface area associated with the cytoplasmic matrix which was estimated is important in understanding the role of hydrated water in the physiology of the cell. The result on the rate of intake of ligands by membrane receptors with different internalization conditions can shed light on different situations in endocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01403-02 LTPB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)*

P.I.:	N. Gershon	Visiting Scientist	LTPB, NICHD
Others:	N. Esteban	Visiting Fellow	LTPB, NICHD
	K. Porter	Guest Researcher	LTPB, NICHD

COOPERATING UNITS *(if any)* Univ. of Arkansas (D. Mattison); DCRT, NIH (B. Trus); FIC, NIH (K. Porter); Dept. of Biol., Univ. of MD, Baltimore County (K. Porter and N. McNiven); Dept. of OB/GYN, Yale Univ. Sch. of Med. (F. Naftolin and H. Sakamoto); LCS, NIMH (D. Jacobowitz and J. Hamill).

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:
.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.)*

1. More samples of the cell center (the microtubule organizing centers (MTOC) and the centrioles) were reconstructed from electron micrographs of serial sections of pigment cells of Holocentrus ascensionis. The method for three-dimensional reconstruction was further developed using the color capability of the computer graphics system to enhance parts of the cell center and a hidden-line algorithm was implemented. 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. The outer surface of part of the ER, or whorl body (WB), was reconstructed from twenty sections. It was found that adjacent layers in the WB are equally spaced. We have initiated a study involving image analysis techniques to determine whether an intervening layer of sugar-like material is responsible for this constant gap. 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. This reconstruction will be used to evaluate the effects of teratogens and to follow the migration of germ cells in embryos. Algorithms and programs were devised to digitize serial sections, to align them and reconstruct them into a single three dimensional image. These studies will further our understanding of how the cell acquires its shape, and organizes its organelles. 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 01404-02 LPTB

PERIOD COVERED

October 1, 1984 - September 30, 1985

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:	P. Munson	Mathematical Statistician	LTPB, NICHD
	R. Cruciani	Research Chemist	LTPB, NICHD
	G. Forti and G. Pesce	Guest Researchers	LTPB, NICHD
	M. Maggi	Visiting Fellow	LTPB, NICHD
	G. Forti	Guest Researcher	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD
	R. Staton	Statistician	LTPB, NICHD

COOPERATING UNITS *(if any)*

LNN, NICHD, (H. Gainer and M. Lang); University of Florence, Depts. of Endocrinology and Andrology; NHLBI (H. Keiser); NIADDK (H. Pollard); Imperial College, London UK (E. Barnard); and Univ. Sherbrooke, Canada (E. Escher).

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:
2.6	1.5	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.)*

We have used quantitative ligand binding studies to characterize the multiple types and subtypes of opioid receptors in rat brain, bovine adrenal medulla, cultured chromaffin cells, and other peripheral tissues. We have demonstrated the existence of the previously known mu, delta and kappa receptors, and in addition the existence of mu-1 and mu-2 subtypes, and of kappa-1, kappa-2, kappa-3 subtypes with distinctive pharmacological profiles. This has been made possible by use of computer simulation to optimize experimental design for experiments involving the simultaneous presence of multiple ligands, the combined use of pharmacological blockade and computer modelling of multiple data sets simultaneously with appropriate constraints.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01405-01 LTPB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus

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

PI:	D. Rodbard	Head	LTPB, NICHD
Other:	N. Esteban	Visiting Fellow	LTPB, NICHD
	N. Pernick	Guest Researcher	LTPB, NICHD
	M. Jaffe	Guest Researcher	LTPB, NICHD
	V. Guardabasso	Visiting Fellow	LTPB, NICHD

COOPERATING UNITS *(if any)*

University of Virginia (Dr. Steven Pohl); University of Pittsburgh School of Medicine (Dr. Alan Robinson).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have developed computer programs to assist physicians, diabetes educators, other paramedical personnel, and patients with intensive insulin therapy. The programs provide analysis of the ambulatory circadian glucose profile, using manual entry and/or verified data from recording or "memory" glucose reflectance meters. Graphical and statistical displays are accompanied by detailed written description, with advice and explanations for alterations of insulin dosage, timing, type, or dietary changes. Extensive human engineering and fail-safe features are provided. Nonparametric statistics and principles of exploratory data analysis are combined with techniques for transformation, weighting, smoothing and interpolating using advanced spline methods. The programs also provide analysis of patient compliance, and are educational for both patients and physicians.

OFFICE OF THE SCIENTIFIC DIRECTOR

Z01 HD 00093-11 Mechanism of Action of Nerve Growth Factor
Gordon Guroff, Ph.D.

Z01 HD 01500-03 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 00093-11 OSD

PERIOD COVERED
October 1, 1984 to September 30, 1985

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.	C. Greiner	Guest Researcher
	H. Kuzuya	Vis. Scientist	K. Fujita	Guest Researcher
	P. Lazarovici	Vis. Associate	Y. Matsuda	Guest Researcher
	N. Nakanishi	Vis. Fellow	E. Yavin	Guest Researcher
	T. Hama	Vis. Fellow		(Courtesy)
	J. Tanner	Fed. Jr. Fellow		

COOPERATING UNITS *(if any)*
Department of Neurology, University of Chicago, Chicago, IL
Department of Neurobiology, Weizmann Institute, Rehovot, Israel
Department of Biochemistry, Tohoku Dental University, Koriyama, Japan

LAB/BRANCH
Office of the Scientific Director

SECTION
Section on Growth Factors

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	5.75	2.25

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

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

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 survival and development of the sympathetic and sensory nervous systems. Nerve growth factor controls the expression of specific genes in target neurons and, in that way, directs their development. The molecular mechanism by which the factor controls gene expression is not known. Our studies are focused on the intracellular events which follow the binding of nerve growth factor to its receptor. We have used PC12, a cell which differentiates in response to the factor. We have developed three cell-free phosphorylation systems, one cytoplasmic, one nuclear, and one ribosomal, which reflect the action of nerve growth factor on the cells. We have separated the components of the soluble system, kinase and substrate, and purified each. We have found that a decrease in kinase activity, rather than a decrease in substrate, is responsible for the overall decreases in phosphorylation. We have seen that the components, kinase and substrate, are present in many tissues, not just those that are nerve growth factor sensitive. We have purified the substrate from brain and are developing antibodies against it. We have explored the role of other, known kinases and have evidence that an early step in the action of nerve growth factor on phosphorylation involves protein kinase c. We are also exploring the nerve growth factor-induced changes in DNA. Our effort is to correlate these changes with changes in the phosphorylation of nuclear proteins, or with alterations in transcriptional activity and nuclear morphology. Finally, we are looking at the transcription of a specific gene, the gene for the epidermal growth factor receptor. The binding of epidermal growth factor is decreased in cells treated with nerve growth factor. We would like to know if this decrease occurs at the transcriptional level. If so, we want to clone the gene and study its functional state in nerve growth factor-treated cells. The aim of these studies is to describe the actions of the factor at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 01500-03 OSD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adenovirus(Ad) and SV40:Molecular Mechanisms of Transformation and Tumorigenicity

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. Matthews	Staff Fellow	OSD, NICHD
	K. Akagi	Visiting Fellow	OSD, NICHD
	M. H. Haddada	Visiting Fellow	OSD, NICHD
	N. Tuteja	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 Biomathematics, UCLA School of Medicine, Los Angeles, CA (E. Landaw)

LAB/BRANCH
Office of the Scientific Director

SECTION
Section on Viruses and Cellular Biology

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.

ANNUAL REPORT

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Epidemiology and Biometry Research Program, NICHD

The Epidemiology and Biometry Research Program conducts research on a broad range of topics which include low birth weight, preterm delivery, intrauterine growth retardation, high risks pregnancies and specifically diabetes in pregnancy, specific aspects of obstetrical management such as the use of Cesarean section, infant mortality, Sudden Infant Death Syndrome, and also studies of infant feeding practices.

A substantial part of the research activity of the Program has increasingly focused on research about the epidemiology of low birth weight and its related problems of preterm delivery and intrauterine growth retardation. This is so because low birth weight babies account for 75% of all deaths during the neonatal period and about two-thirds of all deaths during the first year of life. More importantly time trends show only an about 10% reduction in the risk of low birth weight during the last decade with no reduction in the risk of very low birth weight in this country, that is babies who weigh less than 1500 grams.

The research is conducted in collaboration between the Epidemiology and the Biometry branches and the Office of the Director and representatives from universities, state health departments, city health departments as well as private groups such as the Greater Washington Research Center here in Washington, DC, and the Ford Foundation.

Office of the Director

With the Office of the Director, EBRP, taking the lead a multi-risk-factor intervention is currently in its pilot phase in Washington, DC, called Better Babies Project which involves collaboration between the Greater Washington Research Center, the City Health Department, and the EBRP. The project is targeted in a geographical area of Washington, DC, defined by census tracts and will provide a package of services which include outreach to identify all pregnant women in the target area early during their pregnancy and link them with medical and social services, and offer interventions for smokers, drinkers, and underweight women, and also training to recognize early signs of premature contractions in an effort to diagnose premature labor early and at a time when it can still be prevented. The impact of the interventions will be evaluated by measuring the rate of low birth weight for all women in the target area and comparing these to other areas of the city which are comparable in population composition and in risk of low birth weight. This design has been chosen to get around the problem of self-selection where evaluation of the impact of services is only measured upon the participants in the program. The project is unique in that the funding for the services and the interventions is provided exclusively from 15 different private foundations. The Office of the Director has taken responsibility for the design of the project and its evaluation.

The Office of the Director has provided the lead in the design of the Congressionally mandated study to determine whether any long term effects are associated

with exposure to the chloride deficient formula Neo Mull Soy in 1979. Extensive efforts are still underway to generate an unbiased sample of children exposed to the chloride deficient formula in 1979 by use of hospital discharge diagnoses obtained through the collaboration of CPHA and also surveys of pediatricians in two states, namely Oregon and Pennsylvania. The questionnaire instruments for the project have been developed and pretested and progress has been made in selecting appropriate tests for the evaluation of these children. Because of an observation of a clinician on the West Coast of a possible link between exposure to the chloride deficient formula and a specific behavioral aberration called over-focusing, a small number of children with exposure to the chloride deficient formula and a group of control children are to be blindly evaluated by a team consisting of a pediatric neurologist and a developmental psychologist. Thereafter we plan a pilot study of a sample of about 20 exposed children and 20 controls and possibly siblings of the exposed children to test the logistics and other aspects that we will be facing in the national study of several hundred children before implementing the national study. An interesting finding from the analysis of the data generated from an earlier evaluation of a group of children at NIH who were exposed to the chloride deficient formula and suffered hypochloremic metabolic alkalosis is the very strong relationship between duration of exclusive use of the formula and motor development, specifically fine motor development at about two years and also at around four years. It is expected that the national study will be in the field sometime during 1986.

A large scale case-control study of low birth weight has been completed in Washington, DC, entitled D.C. Perinatal Study. This project involved all low birth weight births in the six major hospitals of Washington, DC, and control births for the period of February 1, 1984 through January 31, 1985. The six major hospitals included cover the births of about 90% of DC residents. This is one of the few geographically based case-control studies of the problem of low birth weight ever conducted. Data are currently being transcribed and error checked and should be ready for analysis in a short time.

The Office of the Director has also provided leadership in projects involving exploration of intergenerational effects of low birth weight and has actively pursued the identification of other data sets both in this country and abroad that could be used for research in this area. In collaboration with the Columbia group data from the Dutch famine have been summarized and show that women who were exposed in utero to the severe famine which was prevalent in Holland from the period of October 1944 through April 1945, themselves were at greater risks of delivering low birth weight children. This is, to our knowledge, the first indication for the possible transmission of environmental effects intergenerationally and not just genetic effects. We are currently pursuing with Dr. Olav Meirik, an obstetrician at the University of Uppsala, the use of a data base in Sweden for intergenerational studies.

The Office of the Director also has been actively involved and provided the stimulus for research on ethnic differences in low birth weight by providing the data base and by encouraging staff to explore the study of ethnic differences. An important finding is the inability to account for the marked differences in low birth weight between whites and blacks by controlling for the major currently known risk factors of low birth weight such as maternal age, parity, education, prenatal care utilization, and also maternal weight and weight gain and adverse health habits such as smoking and drinking. The findings from this

study point out the way for further investigations to clarify the reasons for the marked differences in low birth weight between blacks and whites.

The study of infant feeding practices among Bedouin tribes living in the Negev, an area south of Beer Sheva, Israel, has been completed in collaboration with Ben Gurion University. Analyses are in progress to document the changes over time in infant feeding practices in relation to changes in lifestyles, that is moving from a nomadic to a sedentary lifestyle, a change which is taken place for some of the tribes and of the relationship between infant feeding practices and physical growth and development and morbidity during the first year of life and the identification of determinants of breast feeding.

Other Professional Activities:

Dr. Berendes has continued to serve on the Institute of Medicine Committee on the Prevention of Low Birth Weight. He is a member of the Assistant Secretary's for Health Interagency Task Force on the Prevention of Low Birth Weight and in charge of a subcommittee which is to evaluate and advise the Department with respect to the specific recommendations from the Institute of Medicine report. He also is leader of a task force on low birth weight to re-evaluate the health goals for 1990.

He was appointed to the Board of Directors of the Better Babies Project Inc. and recently to an advisory committee on the Study Group for Complications of Perinatal Care.

Dr. Berendes participated in a special meeting of the Ford Foundation which focused on the cost benefit evaluation of interventions to effect the rate of low birth weight. He was involved and chaired part of an international meeting in Evian, France, on the Prevention of Preterm Births - New Goals and New Practices in Prenatal Care which was held in May 1985. He was the author or co-author of several papers presented at the meetings of the Society for Epidemiological Research, the American Public Health Association, the Pediatric Society and the International Congress of Nutrition.

He lectured upon invitation at the D.C. Society for Home Economics on the "Prevention of Low Birth Weight," the March of Dimes in New York on the "Epidemiology of Low Birth Weight and Strategies for its Prevention," at Johns Hopkins University about "Epidemiology of Low Birth, What Do We Know?", to the Healthy Mother, Healthy Babies Coalition on "Health Habits. How Do They Effect the Risk of Low Birth Weight?" and at the University of North Carolina on "Risk Factors Associated With Low Birth Weight".

Dr. Berendes has continued his appointment at Howard University as Clinical Professor of Pediatrics and at Johns Hopkins University as Senior Associate in Epidemiology.

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Epidemiology and Biometry Research Program, NICHD

Office of the Director

Publications

Forman, Michele R., Meirik, Olav, Berendes, Heinz W.: Delayed Child Bearing In Sweden: J.A.M.A. 252: No. 22, 3135-3139, December 14, 1984.

Berendes, Heinz W.: Epidemiological Aspects of Sudden Infant Death Syndrome and Future Directions of Research. International Symposium Sudden Infant Death Syndrome, February 22-24, 1984, Santa Monica, California, in press.

Z01 HD 00318-05 A Prospective Study of the Frequency and Duration of Infant Feeding Practices
G. G. Rhoads

Z01 HD 00323-05 District of Columbia Perinatal Study
H. W. Berendes

Z01 HD 00325-04 Neural Tube Defects and Folate
J. L. Mills

Z01 HD 00326-04 Premature Thelarche in Puerto Rico
J. L. Mills

Z01 HD 00329-03 Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.
H. W. Berendes

Z01 HD 00331-02 Diabetes in Early Pregnancy Project (DIEP)
J. L. Mills

Z01 HD 00332-02 The Risk of Adverse Pregnancy Outcome Following Cervicitis During Pregnancy
G. G. Rhoads

Z01 HD 00333-02 Congenital Anomalies and In Vitro Fertilization (IVF)
J. L. Mills

Z01 HD 00334-02 Low Birth Weight Across Generations
M. A. Klebanoff

Z01 HD 00336-02 Coitus in Pregnancy: Is it Safe
M. A. Klebanoff

Z01 HD 00337-02 Vomiting during Pregnancy
M. A. Klebanoff

Z01 HD 00338-02 Childhood Nutritional Experience and Subsequent Reproductive Performance
M. A. Klebanoff

Z01 HD 00339-02 Race, Age, Socioeconomic Status and Low Birth Weight
M. A. Klebanoff

Z01 HD 00340-02 Ethnic Differences in Birth Weight and Length of Gestation
P. H. Shiono

Z01 HD 00341-02 Cesarean Childbirth Rates in the U.S.
P. H. Shiono

Z01 HD 00342-02 Dietary Intake of Pregnant Women
N. Kurinij

Z01 HD 00343-02 The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins
H. W. Berendes

Z01 HD 00344-02 Long Term Effects of Infant Formulas Deficient in Chloride
H. W. Berendes

Z01 HD 00345-01 Food Supplement and Dietary Intake in Women of Childbearing Age
N. Kurinij

Z01 HD 00346-01 Time Trends in the Incidence of Biliary Atresia
M. A. Klebanoff

Z01 HD 00347-01 Nonresponse and Misclassification Bias
P. H. Shiono

Z01 HD 00348-01 The Use of Oral and Other Contraceptive and Congenital Abnormalities
P. H. Shiono

Z01 HD 00349-01 Chromosomal Abnormalities and Contraceptive Use Around the Time of Conception
P. H. Shiono

Z01 HD 00350-01 A Prospective Study of Congenital Malformations and Maternal Smoking
P. H. Shiono

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Epidemiology Branch
EBRP/NICHD

The research program in the Epidemiology Branch is focused predominantly in three subject areas: 1) low birth weight, 2) teratologic and genetic problems, and 3) nutrition. In addition the Branch is involved in specific projects on premature thelarche, cesarean section, nausea and vomiting of pregnancy, early precursors of atherosclerosis, and methodologic problems of interest in reproductive epidemiology.

Low Birth Weight

Infant mortality among blacks in the inner cities, including Washington, DC, remains about twice as high as in the nation as a whole. There is some evidence that this problem may be worsening. For instance, infant mortality in the District of Columbia rose from 18.2 per 1000 births in 1983 to 21.2 in 1984. Much of the excess loss in the inner cities is due to an higher rate of low birth weight. It is well known that low birth weight is more common in black women than in white and is a particularly severe problem within the inner cities. A comparison of birth weights among different race/ethnic groups in the patient population subscribing to the Kaiser-Permanente Health Plan of Northern California showed low birth weight to be a much less frequent outcome in this middle-class, insured population than in the nation as a whole. The low birth weight rate for these insured blacks was 7.7% as compared to a national rate of 12.5% and for whites was 3.6% as compared to a national rate of 5.7%. The Kaiser data suggest that much more favorable rates of low birth weight are achieveable, but that even in that middle class population with equal access to medical care, an approximate 2:1 excess for blacks persists. Adjustment for a variety of risk factors which were measured in the Kaiser study did not change this result.

The Branch is involved in several projects aimed at exploring the reasons for the differences in low birth weight frequency in different socioeconomic and race groups. Within the District of Columbia field work was completed this year on a case-control study of low birth weight. Cases occurring in six District hospitals accounting for about 85% of such births to District residents were interviewed. Control women with normal birth weight babies were also studied. A variety of demographic, lifestyle and medical factors will be examined to identify particular characteristics in this group of predominantly black women which predispose them to low birth weight.

In another inner city initiative the Branch has collaborated with several private sector organizations in the Better Babies Project, a three year effort aimed at reducing the rate of low birth weight infants in a target area in the District of Columbia. Outreach workers will attempt to identify as many pregnant women in a specific target area of the District as early as possible in

their pregnancies and will encourage them to begin prenatal medical care, improve the frequency and total number of their prenatal visits, improve their adherence to health and medical advice and link them with specific interventions designed to reduce prematurity, smoking, and social stress. EBRP is providing advice on study design and types of intervention and will be responsible for evaluating the impact of the project.

The excess of preterm and low birth weight births in lower socioeconomic groups has suggested the possibility that infection might play a causal role. Pathologically defined chorioamnionitis is known to be much more common in preterm than in term births, but the literature relating carriage of particular vaginal or cervical organisms to the onset of labor has been confused. A major project to examine these issues, funded by CRMC and NIAID, is being largely coordinated by Branch staff. More than 2000 women have been enrolled in five medical centers across the country with eventual enrollment projected to be between 10,000 and 15,000. Vaginal and cervical cultures are being performed on participants during the second trimester of pregnancy. A variety of organisms are being sought. Outcomes are being monitored in terms of subsequent complications of pregnancy, intrapartum events, and perinatal outcome. Women carrying Group B streptococci, Chlamydia trachomatis, and a fraction of those carrying Ureaplasma urealyticum will be invited to participate in a randomized trial of long term erythromycin therapy (1 gram daily) in order to assess its prophylactic effect.

In a related but smaller project the Branch has been investigating the usefulness of cervicitis as a possible marker for preterm birth. Approximately 800 women attending the Johns Hopkins University prenatal clinic have been enrolled in a study including careful observation and photographs of the cervix in the second trimester of pregnancy. Cultures for multiple organisms were also taken. Follow-up of the women is just being completed and the data are expected to be ready for analysis next year. Results so far suggest that cervical inflammation is difficult to define in a reproducible way, which is likely to make it difficult to use the concept clinically. The association of various aspects of the cervical appearance with organism prevalence is being investigated.

If infection is an important precursor of preterm birth, then the ways in which cervical/vaginal seeding occurs would be of considerable importance. Sexual transmission is an obvious (and in some cases proven) possibility for some organisms, and the relation of the frequency of coitus to pregnancy outcome is therefore of considerable interest. In two separate analyses recently completed by the Branch no important association was found. One of these was based on information gathered at the time of delivery from approximately 10,000 women in Jerusalem, Israel, and the other was based on more than 30,000 women participating in the Perinatal Collaborative Project in this country. In the latter study the information about frequency of sexual intercourse was collected prior to the time of confinement. It is surprising that in these large studies no effect should be found and it suggests, perhaps, that the role of infection as a cause of prematurity may be a limited one.

A striking feature of low birth weight is its tendency to recur across generations. For instance, a recent analysis of Perinatal Collaborative Project data showed that women weighing 4-5.9 pounds at birth were at 3.5 times more likely to have a low birth weight infant themselves than were women weighing 8 pounds or more at birth. Conversely the women weighing 4-5.9 pounds were only 14% as

likely to have a macrosomic infant (greater than 4000 grams). It appears, therefore, that the effect of maternal birth weight on infant's birth weight operates through a shift of the entire distribution rather than specifically on low birth weight. What is not clear from these studies is the extent to which this cross-generational similarity in birth weight is mediated by the rate of intrauterine growth as opposed to the length of gestation. In order to determine which of these mechanisms is operating, it will be necessary to acquire data sources from the early 1960's in which both length of gestation and birth weight (and perhaps other confounding factors) were recorded for subjects who can be traced and whose own reproductive performance can be assessed at the present time. Data of this type are being assembled from a health district in Sweden which maintained a low birth weight registry in the 1950's. A "souces sought" was issued this year to determine the availability of other appropriate cohorts. A number of investigators from around the U.S. responded enthusiastically and it appears likely that a more formal project in this area would be feasible and worthwhile.

The effect of smoking on low birth weight is well known but the attributable risk related to this factor has, perhaps, not been fully appreciated. It appears to differ according to socioeconomic status and accounts for as much as 30% of low birth weight births in the lower socioeconomic strata. In a recent analysis of the Kaiser-Permanente data it was found that alcohol intake was also associated with lower birth weight primarily because of intrauterine growth retardation. While this has generally also been considered to be the mechanism through which cigarettes affect birth weight, there is some evidence that smoking is associated with more preterm deliveries as well. In the Kaiser data women who smoked one or more packs of cigarettes daily were 60% more likely to deliver before the 32nd week than were non-smokers. This association persisted after adjustment for a variety of confounding factors and suggests that the negative influence of smoking on reproduction operates through several mechanisms.

Teratology/Genetics

The Branch has continued its work on a number of projects relating to the etiology and prevention of congenital malformations. A variety of malformations are more common in births to diabetic women and it is clear that these are determined (based on their embryology) in the first 6 weeks after conception. The Diabetes in Early Pregnancy project has recruited women before or within 21 days of conception to identify early pregnancy in 422 diabetic pregnancies and 494 control women. Upon confirmation of pregnancy the status of the diabetic women was assessed and they were taught to monitor their blood glucose levels at home on a daily basis. Blood was collected on a weekly basis through the first 12 weeks of pregnancy so that metabolic control was closely monitored. Recruitment and follow-up of the patients were completed this fiscal year and the data are currently being edited and prepared for analysis. Data on the frequency of spontaneous abortions in the two groups have been examined and show an overall frequency of 14-15% in both. Thus, in these fairly well controlled diabetic women no excess of spontaneous abortions was noted. Comparisons of malformations between the diabetic (n=349) and control women (n=391) whose pregnancies went to term and the relation of these outcomes to metabolic control in the first few weeks of pregnancy await completion of data editing.

The Branch is assisting the CRMC in coordinating a large study to compare the new method of prenatal diagnosis, chorionic villus sampling (CVS), with amniocentesis. CVS is done between 8 and 12 weeks after the last menstrual period and provides results between one and two months earlier than does amniocentesis. The NICHD study involves seven medical centers and will attempt to recruit several thousand women seeking prenatal diagnosis. Early attempts to have a randomized study have been abandoned for lack of recruitment and the final details of a non-randomized comparison are being worked out. All patients having CVS at the participating centers will contribute data to assess the accuracy of the procedure, which will be compared to published data in the literature as well as to the accuracy experience with amniocentesis at the centers. Patients at average obstetric risk who live within one to two hours driving distance of the centers and who have a baseline ultrasound showing a viable pregnancy of 49-90 days gestational age will be asked to participate in the safety study. Many of these women will choose CVS, but a considerable effort is to be expended at each center to recruit such women who seek amniocentesis early enough to participate as controls. Life table analysis will permit examination of loss rates in early pregnancy following CVS and in women (up to 16 weeks) who have had no diagnostic procedure. Following 16 weeks, the comparison would be between CVS and amniocentesis. It is hoped that it will be possible for the centers to karyotype spontaneous abortions occurring prior to prenatal diagnosis (mainly in the amniocentesis group) so that the frequency of euploid losses can be measured in both groups. Follow-up of the course of pregnancy and examination of the newborn infants is also planned. Spontaneous abortion rates in the DIEP participants who had an ultrasound showing a normal, viable pregnancy at about 8 weeks was only about 3%. Since half of these losses could be expected to be aneuploid, a background rate of euploid losses in such women may be as low as 1.5%, explaining the highly favorable CVS statistics which have been reported in the literature to date.

It has long been known that the incidence of neural tube defects (NTD) is subject to some environmental influence which must account for the variation in frequency of this malformation over time and between populations. Recent reports from Great Britain have suggested that periconceptional vitamin supplementation may prevent NTD and have implicated folate more specifically as the active ingredient. The Branch has initiated a case-control study in Illinois and California in cooperation with Northwestern University and the California State Department of Health. It is hoped to recruit several hundred neural tube defect cases over the next two years as well as two groups of control women: those having an ostensibly normal pregnancy as well as a group having a fetus or child with a major medical problem. Cases and controls will be interviewed about three months after birth (or prenatal diagnosis) with special reference to their use of supplementary vitamins around the time of conception. Interviews will be done by telephone.

The relation of congenital malformations to contraceptive use was a major thrust of the Kaiser-Permanente Birth Defects study which was carried out in the late 1970's under contract. Some of the basic findings were never published by the contractor and the Branch has made progress this year in completing these analyses. Among 33,545 newborns whose mothers had been questioned during pregnancy about use of contraceptives around the time of conception there were 597 (17.8/1000) with major malformations. The rate in mothers who had used oral contraceptives prior to conception was 17/1000 as compared to 15 and 20/1000 in

the groups who had used other methods or no birth control, respectively, prior to conception. These differences were not statistically significant (n.s.). There were 850 babies exposed to oral contraceptives in utero and the ratio of observed to expected cases of major malformations was 1.24 (n.s.) if the mother was a non-smoker and 2.98 (p=.03) if the mother smoked one pack or more of cigarettes daily. There were no significant changes in malformation rates following failures of intrauterine devices, spermicides, or rhythm contraception. No increase in risk for chromosomal abnormalities was found among women who used oral contraceptives prior to becoming pregnant or among women who experienced oral contraceptive breakthrough pregnancies. Two cases of trisomy 18 were observed among 814 infants born after in utero exposure to oral contraceptives and two cases of trisomy 21 occurred in 338 births following failures of rhythm contraception. There were no cases of trisomy 21 or 18 among the 1,569 women using spermicides at the time of conception.

Two large data bases were used to explore the possibility that smoking might be associated with birth defects. The Kaiser-Permanente study was used to formulate hypotheses by relating each of a large number of birth defects to the frequency of smoking. Significant positive associations were observed for ventral hernias and for "other major gut abnormalities." Significant negative associations were found for six other malformations. To determine if these findings were an artifact of multiple comparisons seven of these eight malformations were tabulated by smoking status for women in the Collaborative Perinatal Project. Only one of these relationships could be confirmed which was between smoking and hemangiomas. The relative risk for hemangioma was 0.8 in the Collaborative Perinatal Project and 0.8 in the Kaiser study. However the prevalence of hemangiomas was diagnosed much more frequently in the Kaiser study (3.9%) than in the Collaborative Perinatal Project (0.5%). The negative association could not be explained by differences in ethnic group. It was concluded that smoking is unlikely to be responsible for an increase in congenital malformations.

Specific analyses of the possible effect of spermicide exposure around conception were also carried out. Multiple malformation rates in women using spermicides only before or after their last menstrual period were 3.8 and 4.8/1000 respectively. For control groups the corresponding rates were 5.4 and 6.4 (not significant). No pattern of malformations was found in spermicide-exposed infants. The risk of preterm delivery and of low birth weight delivery and of spontaneous abortion were no higher in women exposed to spermicides than in women using other methods of contraception.

Nutrition

The Branch has continued to be involved in several projects relating to nutrition during pregnancy and childhood. Because it is well known that relatively few American women breast feed their infants for the recommended four to five months, a study of black and white women has been carried out to investigate the underlying reasons for this. Approximately 1200 primiparae were interviewed during the first few days postpartum to ascertain their infant feeding plans and the factors which led them to choose breast feeding or bottle feeding. These women are being followed through the first year with a series of interviews to ascertain when they actually stop breast feeding and their reasons for stopping. Data from the hospital interviews and from interviews conducted at one and three

months post-partum are in the final editing stages and should be ready for analysis next year. Ethnic differences in the rate of breast feeding are evident with 85% of the white/Hispanic women breast feeding compared to only 49% of black women giving birth in the three hospitals selected for the study. Major reasons for choosing to breast feed are "better/healthier/more nutritious way to feed the baby" (62% of breast feeders); whereas major reasons for choosing to formula feed are "return to work" (22%), "not wanting to breast feed" (16%), or "convenience" (10%). In a pilot survey a number of women who stopped breast feeding early complained of "insufficient milk" and it is hoped that the full analysis of the present data will explicate this perception.

In another study of infant feeding Bedouin women in the Negev desert of Israel have been interviewed to ascertain their infant feeding practices. Data have been collected on about 5,000 infants of whom 2500 were identified at birth with a subsample followed for a period of 5-8 months. Another sample of children were identified at six months of age and infant feeding histories were obtained retrospectively with follow-up of a sample up to 18 months of age. Information collected include demographic data, medical and reproductive history, and cultural information about the particular Bedouin tribe. Health information about the children is collected from the mother and from medical records which are unusually complete for a population in the early stages of westernization. Data collection is now accomplished. Analyses are in progress and focus 1) on the relationship between women's work, the support and help available during the early months of her child's life, and their effect on the choice of infant feeding practice; 2) on the relationship between maternal health characteristics during pregnancy and around the time of birth as well as birth weight and child health characteristics and their effect on choice of infant feeding; and 3) a comparison of infant feeding data collected prospectively from birth and that collected retrospectively from six months. The latter is of some interest since almost all infant feeding studies that have been done thus far have obtained information retrospectively.

In an analyis of data from the National Health and Nutrition Examination Survey the relationship between dietary adequacy and food supplement use has been explored. Among 3,227 non-pregnant women aged 15-41, 25% used dietary supplements regularly and 67% of these consumed some form of multivitamin. Women using food supplements consumed significantly more dietary protein, phosphorus, iron, potassium, thiamin, and niacin than did non-users of supplements. Dietary differences remained after adjusting for socioeconomic, geographic, and anthropometric differences between the supplement usage groups. It appeared that supplement users were the individuals who least needed such supplements. Other preliminary analysis on the NHANES data include an assessment of the diets of pregnant women and an effort to study body mass index measures for obesity in children.

Because of an error in production in the infant formula industry in the United States in 1978 and 1979, some thousands of children were exposed to formula which was deficient in chloride. The Branch has been involved in a Congressionally mandated study to evaluate the long term effects of this exposure on the children both to assess what long term medical needs they may have, and to take advantage of this unique episode to examine the impact of acute electrolyte derangement on subsequent growth and development. Case finding has been in active progress this year based on a review of records from the Centers for

Disease Control and on systematic selection of potential cases supplied by the Commission on Professional and Hospital Activities. A survey of pediatric nephrologists and pediatricians from several states and a review of records from various lay groups who assembled informal registries are leading to the identification of additional cases. Progress is also being made on the selection of a test battery which will cover the appropriate areas of investigation. Preliminary analysis of children studied at the NIH Clinical Center at the ages of two and four years has suggested the possibility of some cognitive deficits. For a variety of methodological reasons these findings need confirmation. A more detailed exploration will be attempted in 1986 using the newly ascertained cases and a series of appropriate controls.

Childhood nutrition is generally recognized to have some influence on the onset of puberty. It is well known that large and especially obese children tend to go into puberty early while children who are sick or malnurished tend to have late onsets of puberty. There is some evidence that the average age at menarche has decreased since the 17th and 18th centuries in western countries, a change which is widely attributed to improved health and nutrition. An exploratory study has been carried out on a cohort of 80 boys in Berkeley, California, whose nutritional intake and anthropometric measurements have been carefully documented since infancy. These boys were assessed for pubertal status at age 14 and the findings were related to their early nutritional measures. No differences in nutrient intake could be documented between the boys who were at an advanced stage of puberty as compared to those who were just starting. On the other hand the more advanced boys were found to have been heavier as infants and throughout childhood. The extra weight appeared to be attributable to an increase in length and lean body mass as well as to a slight increase in skinfold. The data suggests that in boys growth in infancy bears some relation to the onset of puberty although it is not clear whether both are affected by some common genetic/hypothalamic/endocrine factor or whether the effect of body size on the onset of puberty is simply traceable to a much earlier age than has generally been recognized.

Other Projects

Branch staff has been involved in a number of other research projects which do not fit directly into one of the above general areas. In cooperation with the American College of Obstetricians and Gynecologists the Branch is undertaking an analysis of a survey of U.S. hospitals conducted by ACOG concerning updated information on Cesarean section. The rates of Cesarean section in the United States have continued to increase since the Consensus Conference held by NICHD five years ago. In the prospective study of infant feeding which was described above, 31% of primiparae delivering in three Washington Metropolitan area hospitals had Cesarean sections. The survey seeks to establish the distribution of Cesarean rates in approximately 400 sampled U.S. hospitals, to get updated information on vaginal birth after Cesarean, and to inquire about current indications for Cesarean and some practices surrounding the procedure. In addition rates for Cesarean section are being obtained from the Commission on Professional and Hospital Activities which should include information about indications and about vaginal birth after Cesarean.

A study of nausea and vomiting of pregnancy was carried out based on the Collaborative Perinatal Project. Information on vomiting was recorded at each

prenatal visit in that study. The frequency of vomiting was common, affecting 37% of women during weeks 1-4, reaching a peak of 44% during weeks 5-8 and decending to 29% by weeks 13-16. Vomiting was more common among primigravidas, younger women, women with less education and among those who did not smoke. Miscarriage and stillbirth was less common among women who vomited (relative risk = .70, p=.002) than among those who did not. Women who experienced vomiting carried their pregnancies an average of 1.5 days longer ($p<.001$) and were 17% less likely to deliver before 37 weeks (p=.004). Overall, there was evidence that vomiting was a normal occurrence in the first trimester and, if not excessive, compatible with outcomes which were slightly more favorable than among non-vomiters. These findings are of interest in relation to levels of particular hormones which are believed to be responsible for nausea and vomiting of early pregnancy.

Work carried on in the Branch has led to two methodological investigations during the year. Study design considerations for the problem of vitamins and neural tube defects revolved around the feasibility of doing a randomized trial versus a case-control study. While a randomized trial would have a number of methodologic advantages, a number of practical issues limited its attractiveness. The case-control approach permited much more statistical power and allowed an assessment of the effect of vitamins in women having their first child with a neural tube defect rather than only studying high risk women with a prior affected child as had been suggested for a randomized trial. A paper was drafted examining other situations in which case-control studies might be useful for the evaluation of preventive health modalities.

The implications of response bias for the assessment of the effect of smoking and alcohol use on spontaneous abortions was carefully investigated in a unique data set. Information on tobacco and alcohol use had been obtained independently at the time of abortion on a sample of women who subsequently were sent a mailed questionnaire addressing these same issues. The kinds of biases that differential response rates (to the mail questionnaire) can create were carefully documented and are being prepared for publication.

Other Professional Activities

Dr. Mills has continued to pursue his interest in pediatric endocrinology and has collaborated on an investigation of premature thelarche in Puerto Rico. While an epidemic has been suspected on the island, it has not been thoroughly documented and no source of estrogens in the foodstuff has been identified. He also participated in the 2nd International Workshop Conference on Gestational Diabetes and in the Work Group on Pregnancy and Fetal Malformation of the National Diabetes Advisory Board.

Dr. Mills served as Visiting Professor in the Department of Community Medicine, Memorial University of Newfoundland, St. Johns, Newfoundland, Canada. In addition to didactic sessions with students and faculty members he presented formal lectures on diabetes in pregnancy, alcohol effects on birth weight, and early fetal losses. He has continued to teach both at Johns Hopkins University where he was promoted to Associate and at the University of Pittsburgh Graduate School of Public Health. He also served as a consultant to the Environmental Epidemiology Center at the latter institution, advising them on enviornmental risks in pregnancy. Finally, Dr. Mills has kept active in the clinical area

working both in the NIH Clinical Center, Pediatric Endocrinology Clinic, and at the Pediatric Diabetes Clinic at the Naval Hospital.

Dr. Rhoads has continued his interest in atherosclerosis and has consulted with the National Heart Lung and Blood Institute on several of their projects. In addition he has been actively working on the atherogenic implications of Lp(a) lipoprotein, which is genetically controlled and shows considerable promise of being useful for identifying children who are at risk of atherosclerotic disease in later life.

Dr. Willoughby is assisting the Family Study Section, Division of Cancer Etiology, NCI, in currently designing a study of HTLV-III positive pregnant women in order to study the perinatal and postnatal transmission of the virus and outcome of children of such women. The study population is a group of present or past drug abusing pregnant women and Haitian women attending special clinics in Brooklyn, New York. The drug abusing group has a seropositivity rate of about 50% while the Haitians have one which is much lower. Seronegative women will act as controls. Dr. Willoughby is actively collaborating with the Family Study Section of the Division of Cancer Etiology in the design of the intake questionnaire and the planned follow-up of the children born to cases and controls.

Because of the increasing encroachment of private litigation on epidemiological research, Drs. Willoughby and Rhoads have undertaken a review of the experience of governmental and university research workers whose work has been interrupted by subpoena to appear in court and to produce their data for judicial review. They are collaborating with the Drug Epidemiology Unit of Boston University School of Medicine in a scholarly review of the relevant literature, developing a compendium of cases in which a judicial decision has been reached regarding the legal status of epidemiological research and are attempting to draft a proposed solution to the opposing demands of the need for risk research versus the rights of litigants.

Dr. Willoughby served as the NIH representative to the Technical Advisory Committee for the Indian Health Service Comparison Methodology project. The Technical Advisory Committee provided the overall direction for the comparison of availability, use, cost and efficacy of health services to American Indians with other U.S. population. She has also served as the NIH representative to the committee planning the Conference on Intergovernmental Options for Improving Preventive Health Care Services for Adolescents.

Dr. Shiono is serving as a representative on the Working Group on Human Reproductive Outcomes which is organized by Child Trends Inc. The Working Group is funded by the Environmental Protection Agency and the National Science Foundation. Its purpose is to assist the EPA in considering steps it might take to strengthen its capability for assisting reproductive effects of environmental contamination.

Ms. Kurinij has been collaborating with Dr. Marta Axelson of the University of Maryland, Department of Food and Nutrition, in examining food classification systems used by nutritionists. A study was conducted among 51 college students who were asked to rate 23 foods representative of the Four Food Groups. A multidimensional scaling analysis was used, and it was found that respondents

grouped foods by criteria other than nutrient composition. Results indicate that consumers' criteria for grouping foods should be considered when developing food guides for nutrition education. A manuscript has been prepared for publication.

Dr. Klebanoff served as a member of the steering committee for the Conference on Smoking and Reproductive Health, sponsored by Family Health International, to be held in San Francisco on October 15-17, 1985. He attended the Workshop on Environment and Adverse Reproductive Outcomes: Surveillance and Epidemiologic Methods in Atlanta, November 1984. In addition, he was a member of the Abstract Review Committee, Epidemiology Section, American Public Health Association.

Presentations:

Mills, J.L. Panel Member - Second International Workshop Conference on Gestational Diabetes held by the American Diabetes Association, Chicago, Illinois, October, 1984.

Klebanoff, M.A. Coitus, length of gestation and perinatal mortality. Presented to the American Public Health Association meeting, Anaheim, California, November, 1984.

Rhoads, G.G. How to evaluate chorion villus sampling. Presented at the Johns Hopkins University School of Public Health, Baltimore, Maryland, December 7, 1984.

Klebanoff, M.A. Coitus, length of gestation and perinatal mortality: The role of misclassification. Presented at the University of North Carolina School of Public Health, Chapel Hill, North Carolina, January, 1985.

Klebanoff, M.A. The epidemiology of preterm delivery. Presented at the Johns Hopkins University School of Public Health, Baltimore, Maryland, February, 1985.

Mills, J.L. The endocrinology of premature thelarche. Presented at a Conference on Estrogens in the Environment, sponsored by NIEHS, Raleigh, North Carolina, April, 1985.

Shiono, P.H., Klebanoff, M.A., Graubard, B.I., Berendes, H.W., Rhoads, G.G. Birth weight among women of different ethnic groups. Presented to the Society for Pediatric Research (Poster). Washington, D.C., May 7, 1985.

Kebanoff, M.A. Mother's Birth Weight: Does it Predict Large for Gestational Age Babies? Presented to the Society for Pediatric Research, Washington, DC, May, 1985.

Mills, J.L. Safety of spermicide use around the time of pregnancy. Presented to the American College of Obstetricians and Gynecologists, Public Information Session, Washington, DC, May, 1985.

Willoughby, A. The chloride-deficient infant formulas marketed in the U.S. in 1978 and 1979. Presented at the Association of Official Analytical Chemists, Virginia Beach, Virginia, May, 1985.

Shiono, P.H. Smoking and chromosomally normal spontaneous abortions: the effect of nonresponse and misclassification. Presented to the Society for Epidemiologic Research Annual Meeting, Chapel Hill, N.C., June 20, 1985.

Mills, J.L. Diabetes in pregnancy. Visiting Professor in the Department of Community Medicine, Memorial University of Newfoundland, St. Johns, Newfoundland, Canada, June, 1985.

Mills, J.L. Alcohol effects on birth weight and early fetal losses. Visiting Professor in the Department of Community Medicine, Memorial University of Newfoundland, St. Johns, Newfoundland, Canada, June, 1985.

Mills, J.L. Panel Member - Centers for Disease Control Diabetes in Pregnancy meeting, Philadelphia, Pennsylvania, July 17, 1985.

Mills, J.L. Etiologic implications of changing rates of neural tube defects. Presented at the American College of Epidemiologists meeting, Santa Monica, California, September, 1985.

NICHD ANNUAL REPORT

October 1, 1984 through September 30, 1985

Epidemiology Branch

Publications

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Mills, J.L., Graubard, B.I., Harley, E.E., Rhoads, G.G., and Berendes, H.W.: Maternal alcohol consumption and birth weight. J. Amer. Med. Assoc. 252: 1875-1879, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00318-05 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Prospective Study of the Frequency and Duration of Infant Feeding Practices

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

PI:	George G. Rhoads	Branch Chief	EB/EBRP/NICHD
Others:	Natalie Kurinij	Nutritionist	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Computer Sciences Section, EBRP, NICHD (E.E.Harley); Biometry Branch, EBRP, NICHD (B.I.Graubard); CDC, Atlanta, GA (M.R.Forman); G.W.Univ. Medical Center, Washington, DC (M.Edwards); Univ. of Maryland, College Park, MD (M.L.Axelson).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.45	.05	.40

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

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

Although breastfeeding is generally recognized as the optimal way to feed infants through the first 4-5 months, it is well known that many American women nurse their babies for much more limited periods or not at all. This is a prospective study of factors which affect choice infant feeding methods in the first year of life. Characteristics associated with choice and duration of breast feeding are being investigated. The specific objectives of the study are: (1) to provide detailed information on the change in the infant feeding pattern over time; (2) to investigate the underlying meaning of the milk insufficiency syndrome; (3) to investigate the relation between maternal employment and choice and duration of breast feeding; (4) to determine the sociocultural differences in infant feeding between two ethnic groups. Approximately 1200 women having their first child in one of three hospitals in the Washington, DC, area were interviewed with respect to factors that may have influenced their plans for infant feeding. Follow-up through the first year is in progress with detailed interviews scheduled at 1, 3, and 7 months. Data editing and analysis are underway for the post-partum and one month interviews.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00323-05 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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

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

PI: Heinz W. Berendes Director EBRP/NICHD

COOPERATING UNITS *(if any)*
Epidemiology Branch, EBRP, NICHD (L.C.Cooper); Biometry Branch, EBRP, NICHD (D.W.Denman).

LAB/BRANCH
Office of the Director, EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

The D.C. Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to resident mothers in the District of Columbia. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race matched normal weight infant (= >2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. However, if the hospital medical record did not contain adequate prenatal information arrangements were made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

SRA Technologies, Inc., is presently entering all of the data into the computer. It is expected that they will provide us a preliminary tape July, 1985, and a final clean and edited tape September 30, 1985. Data analysis will begin after NICHD receives the final clean and edited tape with all appropriate documentation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00325-04 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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

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

PI:	James L. Mills	Research Medical Officer	EB/EBRP/NICHD
Others:	George G. Rhoads	Branch Chief	EB/EBRP, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

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

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

The Epidemiology Branch (EBRP) is conducting a case-control study in Illinois and California to determine whether the use periconceptional vitamin supplements can reduce the risk of neural tube defects. Women having either a fetus or an infant with a neural tube defect in either state will be ascertained through perinatal networks, vital records, and other sources and will be matched to two controls on maternal age, race and geographic locale. One control will be a mother with a normal pregnancy, and the other the mother of an infant or fetus with a major health problem. Cases and controls will be interviewed within 3 months of the end of pregnancy to determine whether those having a conceptus with a neural tube defect are less likely to have used vitamins in the periconceptional period. The study design, personnel hiring, and forms development have now been completed and case identification is now underway. Field work has been contracted to the Department of Health, State of California and to Northwestern University in Illinois. About 500 cases and 1000 controls are expected to be enrolled over a 2-1/2 year period beginning in mid 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00326-04 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI: James L. Mills Research Medical Officer EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Birth Defects Branch, Centers for Disease Control, Atlanta, GA (G.Oakley).

LAB/BRANCH
Epidemiology 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.)*

At the request of the Department of Health of the Commonwealth of Puerto Rico, the Centers for Disease Control has investigated an outbreak of premature thelarche on the island. Dr. James Mills has been active as a consultant on this project. The most ambitious part of the CDC investigation, a case-control study of 130 matched pairs, has now been completed and the data have been analyzed. The CDC has now presented the Commonwealth of Puerto Rico Commission on Premature Thelarche with a final report based on the case-control study. Their findings are as follows: 1) they were not able to document that an epidemic has occurred, 2) they found no single agent which could account for all the observed cases, 3) they found suggestive evidence that exposure to soy-based formula, chicken, and family history (ovarian cyst in the mother) were important risk factors in girls under two years of age. Notably, they found no evidence that milk was an important etiologic agent. In summary the investigation failed to support allegations by pediatric endocrinologists that contaminated milk was responsible for the epidemic. They could not exclude the possibility that contaminated chicken may have caused some cases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00329-03 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

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

PI: Heinz W. Berendes Director EBRP/NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP (M.Overpeck); Epidemiology Branch, EBRP (L.C.Cooper); Greater Washington Research Center, Washington, DC (J.Maxwell); Children's Hospital National Medical Center, Washington, DC (A.Barnet).

LAB/BRANCH
Office of the Director, EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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 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 a high risk area, help link them with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

The BBP Service Delivery team began collecting data July, 1984, for the project's mini pilot. As a result of the mini pilot findings a number of revisions have been made in the forms and interventions. These revised forms and interventions are presently being developed and piloted.

Evaluation of the project will be provided by the National Institute of Child Health and Human Development, Epidemiology and Biometry Research Program (EBRP).

NICHD has let out two contracts for the Better Babies Project to assist with the evaluation. The D.C. Department of Human Services, Research and Statistics Division, through a contract with NICHD, will be providing us information on all pregnant women delivering in the District of Columbia during the period of the project. Levin and Associates in Rockville, Maryland, under contract, is providing both data support and manipulation in the evaluation of the impact of the BBP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00331-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diabetes In Early Pregnancy Project (DIEP)

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

PI: James L. Mills Research Medical Officer EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Cornell Univ.Med.Center, NY, NY (L.Jovanovic); Brigham and Womens Hosp. Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ. of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ.of Washington, Seattle,WA (R.Knopp).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.0	0.7

CHECK APPROPRIATE BOX(ES)

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

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

The Diabetes in Early Pregnancy Project has the following objectives: 1) To examine the relationship between maternal diabetic control during organogenesis and malformations in the offspring. To identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state; and 2) To compare early fetal loss rates in women with diabetes and control subjects. The Diabetes in Early Pregnancy Project has now successfully completed the data gathering phase. All pregnancies have terminated. 99.5% of the forms have been completed and entered into the computer system and editing is virtually complete. The final laboratory analysis, forms completion, and editing will be finished by the end of August. It should be noted that the DIEP is the first large scale study to enroll women before or immediately after conception and to examine the risks of fetal loss and congenital malformations in a prospective manner. Data analysis is now beginning; however, the change from in-house to contract computer sciences services will delay examination of the DIEP data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00332-02 EB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Risk of Adverse Pregnancy Outcome Following Cervicitis during Pregnancy

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

PI:	George G. Rhoads	Branch Chief	EB/EBRP/NICHD
Others:	Robert P. Nugent	Epidemiologist	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Johns Hopkins University, Baltimore, MD (B.F.Polk, L.Berlin).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

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

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

This contract was funded in May 1983 for two years. Enrollment of women into the study was completed on January 31, 1985 with follow-up data collection to be completed by July 1985. Preliminary data tapes have been provided by Johns Hopkins and data editing programs have been developed. Analysis is expected to begin in August 1985 with articles to be 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 have their cervix evaluated for signs of inflammation. In addition cultures were taken for a number of aerobic and anaerobic organisms and a sample of cervical mucus was evaluated for the presence of inflammatory cells. The women were interviewed to obtain information on a number of risk factors related to preterm and low birth weight delivery. The women are then 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 00333-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Congenital Anomalies and In Vitro Fertilization (IVF)

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

PI: James L. Mills Research Medical Officer EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Director, EBRP (H.W.Berendes); Computer Sciences Section, EBRP (E.E.Harley).

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.)*

In vitro fertilization has become an increasingly popular method of conception over the past few years. To date no formal study of infants conceived in vitro has been conducted to determine if they are at increased risk for congenital malformations. Dr. Mills and the Epidemiology Branch will conduct a historical prospective study of infants which have been conceived in vitro and matched controls to determine whether in vitro fertilization carries an increased risk for congenital malformations. The Eastern Virginia Medical School, Norfolk, VA, will serve as study center and data center for this project (Dr. Fred Worth, Principal Investigator). Because the number of subjects available for study is relatively modest a thorough examination will be performed on each. Detailed physical examination, intracranial ultrasound, echo cardiography, electrocardiography, and abdominal ultrasound will be done on each in vitro fertilization subject and matched control. This contract will be signed in the near future and work will begin on a study protocol, forms design, and recruiting subjects within the next few months.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00334-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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

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

PI: Mark A. Klebanoff Epidemiology Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP, NICHD (B.I.Graubard); Division of Maternal and Child Health, DHHS (S.S.Kessel); Office of the Director, EBRP, NICHD (H.W.Berendes); University Hospital, Uppsala, Sweden (O.Mierik).

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, was published 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 and women weighing 6 to 7.9 pounds are 1.7 times as likely to have a low birth weight infant than women weighing 8 pounds or more at birth.

The relationship between maternal birth weight and the delivery of a macrosomic infant (birth weight >4000 grams) was investigated. Compared to mothers who weighed 8 pounds or more at birth, mothers who weighed 6 to 7.9 pounds were only half as likely, and mothers who weighed 4 to 5.9 pounds were 15 percent as likely to give birth to a macrosomic infant.

In a related study, birth records of a sample of women who were born in a region of Sweden in the 1950's are being abstracted, and the women's reproductive history will be followed via the birth registry. In this manner, the birth weight and gestational age of a mother will be related to the birth weight and gestational age of her children.

Finally, Concept Clearance is scheduled for July 26, 1985, for a contract involving the establishment and follow-up of a cohort of women whose own intrauterine and perinatal experience has been documented. Reproductive outcomes of these women will then be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00336-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI:	Mark A. Klebanoff	Epidemiology Staff Fellow	EB/EBRP/NICHD
Others:	Robert P. Nugent	Epidemiologist	EB/EBRP/NICHD
	George G. Rhoads	Branch Chief	EB/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	.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 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.

This project was terminated. Results were presented at the Annual Meeting of the American Public Health Association and published in Lancet 1984; 2:914-917.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00337-02 EB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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)*

PI:	Mark A. Klebanoff	Epidemiology Staff Fellow	EB/EBRP/NICHD
Others:	George G. Rhoads	Branch Chief	EB/EBRP/NICHD
	James L. Mills	Research Medical Officer	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*

Epidemiology and Biometry Section, NIAID (P.A.Koslowe, R.Kaslow).

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.)*

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 primigravidas, 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. Adjustment for confounders by multiple logistic regression confirmed these associations.

The effect of vomiting in the absence of use of antiemetic drugs on the incidence of congenital malformations was also examined. This analysis used women who registered during the first 20 weeks of pregnancy. Classification of malformations began with the data of Myianthopoulos and Chung, but was modified to reflect current concepts of the pathogenesis of malformations. Antiemetic drugs were defined as those classified by Heinonen, Sloane and Shapiro as "antinausiants, antihistamines and phenothiazines" and were also listed in the 1965 Physicians Desk Reference as being indicated for nausea, motion sickness, hyperemesis or vomiting. It was found that vomiting was unassociated with either major malformations, deformations, hernias or minor malformations. Adjustment for race, maternal age, gravidity, infant sex, study center and antiemetic use did not substantially alter the odds ratios.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00338-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI: Mark A. Klebanoff Epidemiology Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Columbia University, New York, NY (Z.A.Stein, L.H.Lumey).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

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

Girls born during the Dutch famine of 1944-45 are known to have been growth retarded as a direct result of maternal starvation, although 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 was determined. These cohorts include women who were conceived and born before the famine, conceived before and born during the famine (subdivided into exposed third trimester only and exposed second and third trimester), conceived before and born after, conceived during and born after, and conceived and born after the famine.

Of all singleton deliveries in 1960-1983 at the Wilhelmina Gasthuis in Amsterdam, there were 1808 to primiparous women born between January 1, 1944, and June 30, 1946. Adult height and prepregnant weight did not differ by birth cohort. In spite of this, the infant birth weight varied by birth cohort of the mother. Mean infant birth weight was highest among mothers conceived and born before the famine. It progressively decreased to over 180 grams less among women exposed to famine during their own first and second trimesters. Women conceived and born after the famine had infants whose birth weights were not significantly different from those conceived and born before the famine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00339-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI: Mark A. Klebanoff Epidemiology Staff Fellow EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Office of the Director, EBRP, NICHD (H.W.Berendes); National Academy of Science/Institute of Medicine (S.Brown).

LAB/BRANCH
Epidemiology 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.)*

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.

This project was terminated. Results were published in: Institute of Medicine: Preventing Low Birth Weight. Washington, DC, National Academy Press, 1985, pp. 52-58.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00340-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ethnic Differences in Birth Weight and Length of Gestation

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

PI:	Patricia H. Shiono	Epidemiologist	EB/EBRP/NICHD
Others:	Mark A. Klebanoff	Epidemiology Staff Fellow	EB/EBRP/NICHD
	George G. Rhoads	Branch Chief	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP, NICHD (B.I.Graubard); Office of the Director, EBRP, NICHD (H.W.Berendes).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)
☐ (a) 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 Birth Defects Study are being used to evaluate differences in birth weight and gestational age among four different ethnic groups. The ethnic groups included in the Kaiser study are Whites, Hispanics, Blacks and Asians. In addition to studies of ethnic differences, we have evaluated the effects of smoking and drinking during pregnancy on preterm births.

Univariate and multivariate statistical analyses of the effects of 23 factors on the ethnic differences in birth weight have shown that substantial ethnic differences in birth weight remain after accounting for differences in 23 relevant covariates. Compared to Whites, the relative mean differences in birth weight are estimated as -246 for Blacks, -210 for Asians, and -105 for Hispanics. The low birth weight rates and crude odds ratios are 7.7% (2.17) for Blacks; 5.57% (1.57) for Asians; and 4.00% (1.13) for Hispanics. The study concluded that factors currently used to control for ethnic differences in birth weight are insufficient to explain the observed differences.

Similar analyses are being conducted to evaluate the ethnic differences in mean gestational age and preterm births (those less than 37 weeks gestational age). Preliminary results show that the ethnic group specific rates of preterm birth are 6.1% for Whites, 11.8% for Blacks, 8.7% for Hispanics, and 7.9% for Asians. Controlling for possible confounding did not substantially change the odds ratios.

The effects of cigarette smoking and of alcohol consumption on length of gestation were also examined in this population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00341-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI:	Patricia H. Shiono	Epidemiologist	EB/EBRP/NICHD
Others:	George G. Rhoads	Branch Chief	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
American College of Obstetricians and Gynecologists (ACOG), Washington, DC (W.H.Pearse).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, BETHESDA, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0

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.)*

A survey is being conducted by ACOG to determine the cesarean childbirth rates in the U.S. and current hospital policies regarding cesarean childbirth. Members of the EB staff are acting as consultants to ACOG to assist in the design of the survey and sampling methodology. In the past 10 years, rates of cesarean births have risen dramatically. In 1979, a Consensus Conference on Cesarean Childbirth was sponsored by NICHD. Results from the current survey will help to determine if rates of cesarean births are changing and the reason for the changes. In addition to the survey, NICHD is in the process of obtaining information from the Commission on Professional Hosptal Activities on national rates of cesarean childbirth that will be comparable to information obtained by the institute in 1980.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00342-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI:	Natalie Kurinij	Nutritionist	EB/EBRP/NICHD
Others:	George G. Rhoads	Branch Chief	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP, NICHD (B.I.Graubard).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0	0.2

CHECK APPROPRIATE BOX(ES)

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

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

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 00343-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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)*

PI: H.W. Berendes Director, EBRP NICHD

COOPERATING UNITS *(if any)*
Centers for Disease Control, Nutrition Research Branch, Atlanta, GA. (M.R. Forman); Biometry Branch, EBRP, NICHD, (B. Graubard); Center for Health Sciences, Ben Burion University on the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH
Office of the Director, EBRP

SECTION

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.)*

This study is documenting the rates of breast and bottle feeding among different Bedouin tribes who are residing in the Negev. These tribes are changing at various rates from a nomadic to a seminomadic and to a sedentary life style. Another objective is the evaluation of the effect of differences in infant feeding practices during the first year of life on physical growth of the children and on morbidity especially respiratory and gastrointestinal disease during the first year of life.

Data have been collected on about 5,000 infants. Twenty five hundred were identified at birth with a subsample followed for a period of from 5-8 months. Another sample identified children at 6 months of age and obtained infant feeding histories retrospectively prior to 6 months with a follow-up of a sample of these children to 18 months of age. The information collected include demographic data about the mother and information about the tribe and it's life style, medical and reproductive history as well as information on events sur rounding the period of birth including complications. Information about the children in the study include neonatal conditions and problems, infant feeding practices and changes over time during the first year of life and data on child rearing practices, morbidity and various measures of physical growth of the children.

The data collection is complete and the information collected has been computerized. Considerable problems were countered in cleaning up the tape and involved numerous interactions with the Beer Sheva group. Data analysis is in progress with three papers from these data to be presented at one international and one national meeting in 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00344-02 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Long Term Health Effects of Infant Formulas Deficient in Chloride

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

PI: Heinz W. Berendes Director EBRP/NICHD

COOPERATING UNITS *(if any)*
Epidemiology Branch, EBRP, NICHD (A.Willoughby); Biometry Branch, EBRP, NICHD, (B.I.Graubard); Birth Defects Branch, Centers for Disease Control (J.Cordero).

LAB/BRANCH
Office of the Director, EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

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

This is a Congressionally mandated study to determine whether the children exposed to chloride deficient formula in 1978 and 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 currently receiving support services from SAIC, Inc. (formerly JRB Associates). The activity of the past year has centered on 1) identifying subjects eligible for study as cases, 2) investigating the potential problems exposed children may have, 3) defining the test battery that cases and controls will undergo, 4) planning a pilot study to test study procedures including the selection of controls, and 5) securing the input of an interdisciplinary panel of physicians, psychologists and methodologists. Case finding has consisted of surveys of pediatric nephrologists, pediatricians in several states and a systematic CPHA search. Problem identification has consisted of the careful review of several hundred potential case records, a literature review, discussion with physicians who have treated cases, and a review of problems as described by parents.

In subsequent years arrangements will be made to examine the study children and appropriate comparison children in order to assess the long term effects of exposure to chloride-deficient formula.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00345-01 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Food Supplement and Dietary Intake in Women of Childbearing Age

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

PI:	Natalie Kurinij	Nutritionist	EB/EBRP/NICHD
Others:	Mark A. Klebanoff	Epidemiol. Staff Fellow	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Biometry Branch, EBRP, NICHD (B.I.Graubard)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.01	.04

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 relationship between dietary adequacy and food supplement use was examined in 3,227 nonpregnant women aged 15 to 41 from the first National Health and Nutritional Examination Survey. Twenty-five percent of women used dietary supplements regularly, and 67 percent of these consumed some form of multivitamin. Supplement users were of a higher income and education, were more often white, had a leaner body composition, and were more likely to reside in the Western U.S. as compared to nonusers. Caloric intake between supplement usage groups was similar. However, supplement users consumed significantly more dietary protein, phosphorus, iron, potassium, thiamin, and niacin than did nonusers. A considerable portion of both usage groups had intakes below 50 percent of the Recommended Dietary Allowances for calcium, iron, vitamin A, and vitamin C; however, a significantly greater proportion of supplement nonusers had low intakes of iron and vitamin C. Food supplement users were found to consume a more nutrient dense diet and may be the individuals who least need supplements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00346-01 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Time Trends in the Incidence of Biliary Atresia

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

PI:	Mark A. Klebanoff	Epidemiology Staff Fellow	EB/EBRP/NICHD
Others:	George G. Rhoads	Branch Chief	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

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

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

Extrahepatic biliary atresia is a liver disease presenting in early infancy, manifested by progressive obliteration of the extrahepatic bile ducts. It has been estimated to occur in from one per 8000 to one per 15000 live births, and is the single most common indication for performance of liver transplantation in children. None of the incidence figures is based on a well defined geopolitical region; most estimates of the frequency of this condition are derived from referral centers. Some investigators have suggested a time-space clustering of this condition.

This project will gather information on all cases in a well defined geopolitical area for approximately 10 years, and birth certificates will be obtained. Cases will be compared to the other births in the area for evidence of changes in incidence and clustering. It is anticipated that a site will be selected and field work begin in 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00347-01 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

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

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

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	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 effects of nonresponse and misclassification bias on the odds ratio were evaluated in a case-control study of smoking during pregnancy and chromosomally normal spontaneous abortions. Information from cases (N=650) and controls (chromosomally abnormal spontaneous abortions, N=582) was obtained in two ways--a personal interview soon after the spontaneous abortion and a mail questionnaire 1-3 years thereafter. To evaluate nonresponse, information obtained from women who were interviewed but who did not respond to the mail questionnaire was used to estimate the exposure status of the mail questionnaire nonrespondents. Response to the questionnaire varied by exposure status and case-control status. Exposed cases were the least likely to respond (36% response rate), followed by unexposed cases (48%) and all controls (51%). The crude odds ratio for smoking during pregnancy and chromosomally normal spontaneous abortions was 1.2 (p=0.41). After adjusting for the effects of nonresponse, the odds ratio increased to 1.6. Misclassification bias was evaluated by comparing responses to the interview with the responses to the mail questionnaire. This also showed that errors in reporting caused the odds ratio to be underestimated (crude odds ratio, 1.2; adjusted odds ratio, 1.4).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00348-01 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Use of Oral and Other Contraceptives and Congenital Abnormalities

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

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
Hebrew University of Jerusalem, Jerusalem, Israel (S.Harlap)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

CHECK APPROPRIATE BOX(ES)

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

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

Among 33,545 newborns whose mothers had been questioned during pregnancy about use of contraceptives around the time of conception, there were 597 babies (17.8/1,000) with major malformations and 4,046 (120.6/1,000) with minor ones. The 8,522 offspring of mothers who had used oral contraceptives (OC) prior to conception showed 17.2/1,000 major malformations compared with rates of 15.0 and 20.1/1,000 in the groups who had used other methods or no birth control prior to conception. There was no evidence for an increased risk of malformations in women conceiving within one month of stopping OC. There were 850 babies exposed to OC in utero and the ratio of observed to expected cases of major malformations was 1.24 (n.s.) if the mother was a nonsmoker and 2.98 ($p = .028$) if the mother smoked one pack or more of cigarettes daily. There were no significant changes in malformation rates following failures of intrauterine devices, spermicides or rhythm contraception.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00349-01 EB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromosomal Abnormalities and Contraceptive Use Around the Time of Conception

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

PI: Patricia H. Shiono Epidemiologist EB/EBRP/NICHD

COOPERATING UNITS *(if any)*
The Permanente Group of Northern California (S.Ramcharan, R.Bachman, J.Mann); University of California, San Francisco, and Sacramento (M.Golbus, J.P.Lewis); Hebrew University of Jerusalem, Jerusalem, Israel (S.Harlap).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

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

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

Chromosomal abnormalities were studied in 33,551 abortions and births to women whose contraceptive histories had been recorded at their first antenatal visit in 1975-1977. Chromosome examinations were performed exclusively on clinical grounds. There were 45 de novo abnormalities detected (1.34/1,000); three of them were detected at amniocentesis. Trisomy 21 was observed in 27 cases (0.80/1,000), trisomy 18 in 9 (0.27), other trisomies in three (0.09), and translocations or deletions in 5 (0.15). One case of triploidy and six cases of inherited abnormalities were detected. There were no significant racial variations. No increase in risk for chromosomal abnormalities was found among women who had used oral contraceptives prior to becoming pregnant or among women who experienced oral contraceptive breakthrough pregnancies. Two cases of trisomy 18 were observed among the 814 deliveries following oral contraceptive breakthrough conceptions (2.46/1,000), two cases of trisomy 21 occurred in 338 births following failures of rhythm contraception (5.92/1,000), and no cases of trisomy 21 or 18 among the 1,569 women using spermicides at the time of conception.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00350-01 EB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

A Prospective Study of Congenital Malformations and Maternal Smoking

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

PI:	Patricia H. Shiono	Epidemiologist	EB/EBRP/NICHD
Others:	Mark A. Klebanoff	Epidemiol. Staff Fellow	EB/EBRP/NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The relationship between smoking during pregnancy and congenital malformations was studied in prospective studies of 33,434 births in the Northern California Kaiser-Permanente Birth Defects Study and 55,933 births in the Collaborative Perinatal Project (CPP). 28.4% of women were smokers in the Kaiser population and 47% smoked in the CPP population. The odds ratio for smoking during pregnancy and major malformations in Kaiser was 1.00 (95% C.I. 0.8-1.2) and the odds ratio for minor malformations was 0.9 (0.8-0.9) ($p<.001$). The relationship between smoking and 54 individual malformations was evaluated in the Kaiser population. Statistically significant positive associations were observed for ventral hernias (10.1 (1.1-91)) and 'other major gut abnormalities' (12.6 (1.5-108)). However, for each malformation the estimates were based on only one unexposed case. Significant negative associations were found for ventricular septal defects (0.5 (0.2-1.0)), hydroceles (0.7 (0.6-0.9)), clubfoot (0.7 (0.6-0.9)), pigmented nevi (0.7 (0.6-0.9)), hemangiomas (0.8 (0.7-0.9)), and Down syndrome (0.2 (0.1-0.9)). To determine if the findings noted above were an artifact of multiple comparisons, 7 of these 8 malformations were tabulated by smoking status for women in the CPP. All but one of the associations were not confirmed in the CPP. The association between smoking and hemangiomas in the CPP was 0.8 (0.6-1.0) ($p=0.03$). The prevalence of hemangioma at birth was 3.9% in the Kaiser population and 0.5% in the CPP. Adjustment for ethnicity strengthened this association in both data sets. We conclude that smoking is unlikely to be responsible for an increase in malformations.

Z01 HD 00801-10 Studies Based on the Medical Birth Registries of Norway and Sweden
H. J. Hoffman

Z01 HD 00802-10 Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States
H. J. Hoffman

Z01 HD 00803-01 Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors
H. J. Hoffman

Z01 HD 00811-06 National Collaborative Cysteamine Study Data Center
G. F. Reed

Z01 HD 00813-04 Biostatistical Methods for Laboratory Research Studies
G. F. Reed

Z01 HD 00818-04 Research in Developing Nonparametric Methods for Biomedical Applications
G. F. Reed

Z01 HD 00820-04 Statistical Methods for Epidemiologic Data
D. W. Denman

Z01 HD 00821-03 Development of New Graphical Methods for the Analysis of Biomedical Data
D. W. Denman

Z01 HD 00830-04 Child Health Supplement to the 1981 NCHS Health Interview Survey
M. D. Overpeck

Z01 HD 00832-02 Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities
M. D. Overpeck

Z01 HD 00833-01 Outcomes of Deliveries with IUD Use During Conception
M. D. Overpeck

Z01 HD 00840-04 Statistical Discriminate Methods with Applications to Alcoholism Screening
B. I. Graubard

Z01 HD 00841-04 Methods for Comparing and Analyzing Data from Several Complex Surveys
B. I. Graubard

Z01 HD 00842-03 Development of Statistical Methods to Analyze Cluster Samples
B. I. Graubard

Z01 HD 00843-02 An Investigation of Matched Analysis in Case-Control and Cohort Studies
B. I. Graubard

Z01 HD 00844-02 Analysis of NHANES Anthropometric Measurements on Children
B. I. Graubard

Z01 HD 00850-09 Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia
D. A. Bryla

Z01 HD 00851-04 Trends in Time Relating to Maternal and Child Health and Population Research
D. A. Bryla

Z01 HD 00852-03 1980 National Natality Survey and Fetal Mortality Survey
D. A. Bryla

Z01 HD 00853-01 Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine
D. A. Bryla

Z01 HD 00854-01 Analysis of MCH Data from the National Logitudinal Youth Survey
D. A. Bryla

Z01 HD 00860-05 Analysis of Biomedical Time Series Data
H. J. Hoffman

Z01 HD 00861-03 Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth
H. J. Hoffman

Z01 HD 00870-02 Long-Term Reproductive Effects of Cesarean Section Birth
B. I. Graubard

Biometry Branch

The Biometry Branch research activities are structured along three lines: (1) provision of statistical analysis and consultation to NICHD Intramural and Extramural investigators; (2) pursuit of individual and collaborative research in biometry, including both mathematical and biostatistical theory and applications; and (3) support of clinical trials initiated by the NICHD. The Branch maintains strong ties to both the Intramural and Extramural research programs of the Institute. Also, the Branch has supported a number of cooperative studies, including projects supported solely by NICHD and those receiving joint funding from other agencies within the U.S. Public Health Service.

The following review of Biometry Branch research activities is organized by subject matter, rather than by the statistical or mathematical methods utilized in the planning, design, conduct, or analysis phases of these research efforts.

Perinatal Morbidity and Mortality

Perinatal mortality is the key outcome variable for many of the studies currently being performed by the Biometry Branch. A major effort has been devoted to studies comparing United States data with that of two population-based perinatal data sets from Scandinavia, the Medical Birth Registries of Norway and Sweden.

Several important findings have emerged from these studies. Norwegian and Swedish births have been shown to be heavier and not as preterm as a comparable group of U.S. white births. A marked tendency for repeating low birth weight and/or preterm delivery has been demonstrated for successive pregnancies in this population of women. Studies have explored the relationship of perinatal mortality to birth order and sibship size. As sibship size increases, perinatal mortality rates also increase. However, conditional on size of sibship, perinatal mortality rates decline as birth rank in sibship increases. The rates of low birth weight delivery ($\leq$ 2,500 grams) follow the same pattern as does perinatal mortality. The incidence of preterm delivery (less than 36 weeks gestational age) also follows the same pattern. These associations account, in part, for the decline in perinatal mortality as birth rank increases within groups of the same size sibship. Self-selection of women with respect to future pregnancies, depending upon prior fetal outcomes, is, of course, a methodological concern and an important proviso for the interpretation of these findings.

Risk factors for preterm birth has been the subject of a special inquiry based on data from the Medical Birth Registry of Norway. This study was based on longitudinal data in which the first three consecutive single births, 1967-1976, to Norwegian mothers were analyzed. This approach is unique in the field of perinatal epidemiology, and is possible because of the special

attributes of this data collection system. Many of the previously known risk factors (such as, low maternal age, unwed mother, low social class, previous occurrence of low weight or preterm birth, etc.) were also shown to exist in this data set. Placenta previa/abruptio or bleeding early in pregnancy (threatened abortion) were very strong risk factors for a preterm delivery. However, maternal age greater than 35, previous occurrence of a malformed birth, or toxemia in the current pregnancy were not found to be associated with a significantly increased risk of a preterm delivery.

Other research studies have explored the connection between perinatal mortality and the tendency to repeat birth weight and gestational age in successive pregnancies. For example, if the first birth weighed in excess of 3,000 grams then the perinatal mortality of a subsequent low weight second birth (< 2,500 grams) is twice the perinatal mortality rate of a low weight second birth if the first birth weighed less than 3,000 grams. Thus, the baby of a mother who has a light second birth after having had a normal size first birth is at a much higher risk of perinatal death. Also, this research has revealed the importance of crown-heel length measurements, in addition to birth weight, for predicting adverse perinatal outcomes.

In related studies, the birth weight-specific perinatal mortality rates for U.S. Black, U.S. White, and Norwegian births have been compared. Perinatal mortality was defined as including all fetal deaths from 20 completed weeks of gestation and early neonatal deaths occurring in the first week of life. It has been shown previously that these three population groups differ in the occurrence of low weight and preterm births. U.S. Blacks have the largest number of small, preterm babies and the Norwegian population has the fewest such babies. This study found that birth weight-specific perinatal mortality rates (below 3,000 grams) were affected by the relative incidence of low weight births. Thus, Norwegian births have a higher birth weight-specific perinatal mortality rate among low weight births than do the U.S. Blacks. The interpretation of birth weight-specific perinatal mortality rates, therefore, needs to be altered to reflect this additional requirement for standardization. In spite of the difficulty in comparing birth weight-specific perinatal mortality rates, it was shown that the U.S. White and Norwegian births were almost identical in crude perinatal mortality rates for the years 1972-1973 and 1979-1980.

In general, perinatal mortality rates provide a better indication of the availability, utilization, and effectiveness of health care for the pregnant woman and her fetus than the more traditional index of infant mortality. Waaler and Sterky have recently published a report based on data from four Nordic countries, 1900-1980, which showed that "infant" mortality is a sensitive indicator of changing socio-economic circumstances. However, "perinatal" mortality rates are more responsive to changes in underlying demographic factors--maternal age, parity and spacing between births--and to changes in the prenatal, obstetric, and pediatric care provided. A study currently underway in conjunction with the Office of International Statistics, National Center for Health Statistics is examining recent trends in perinatal mortality rates over the past decade for the six countries participating in the International Collaborative Effort on Perinatal and Infant Mortality (ICE). The birth weight-specific comparisons used in this study provide documentation for the probable impact of such technological developments as neonatal intensive care units since 1970.

One research effort that has emerged out of this general interest in perinatal mortality is a prospective study designed to delineate risk factors for fetal growth retardation. Retarded fetal growth, defined as a birth whose weight is below the tenth percentile for gestational age or otherwise labelled small-for-gestational age (SGA), is associated with increased rates of both perinatal mortality and morbidity. Using the research contract mechanism, this prospective study is being implemented at two locations: the University of Alabama in Birmingham, Alabama and the University of Trondheim, Norway. The latter project also includes subcontracts with the University of Bergen, Norway and University of Uppsala, Sweden to supply additional data based on pregnancies and deliveries in these areas.

The aim of this research project is to determine risk factors which will distinguish mothers who have repeated small-for-gestational age (SGA) births from those mothers who have a single, unexpected SGA birth. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally via diagnostic ultrasound measurements and at delivery with standardized measurements. Since this study has only just begun, there are no findings as yet. The study protocol includes recruitment of pregnant women before 17 weeks gestation and subsequent enrollment of women with high risk pregnancies through 33 weeks of gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.

Funding has been committed for five years in order to complete this research project. In this first year of the project, the final study protocol has been developed, study manuals have been prepared, and a pilot is underway to test out the study procedures. From the second through the fourth years of the study, subjects will be enrolled, tests will be performed, and data collected in accordance with the final study protocol. The fifth year of the study will allow time to complete the assessment of all study infants through their first year of life and, also, time to complete checking and editing of the study data files prior to delivering the computerized data sets to NICHD. Scientific reports based on these data will be coordinated by the principal investigators and NICHD program staff.

Extensive time also has been spent assisting in forms development, protocol, and data management for a collaborative, private and public sector project to evaluate interventions to prevent low birth weight in the District of Columbia (the Better Babies Project). Working together with other members of the Epidemiology and Biometry Research Program, an NICHD contract has been negotiated to provide assistance in the data management and analysis for the project. As a multi-year project, it will not provide complete data to evaluate the impact of interventions for a few years, but Branch staff will be actively involved in data development and analysis.

In another study, a secondary analysis of previously unpublished data obtained from the National Center for Health Statistics has been undertaken to review changes in perinatal and infant mortality by race in selected U.S. cities. Data from the current NICHD study of Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates will also be used in this assessment as the data become available. Preliminary findings based on infant mortality

data from five U.S. cities, 1980-1982, indicate that the decline in these years in infant mortality occurred only for births of white race. There was no decline in infant mortality rates of non-white births during this time period. The data are not yet available to determine whether the infant mortality rate of non-white births fell in 1983 and 1984, although it seems probable based on incomplete reports for 1984. Data from two large cities in Missouri show declining rates for both blacks and whites.

Another study is comparing the outcome of deliveries of women who conceived with an IUD in place to those without an IUD present. Analysis of this data set which is derived from the Kaiser Permanente Birth Defects Study is almost complete.

Perinatal mortality and morbidity data are also being examined in several other statistically diverse projects. Data from a variety of sources are being analyzed in different ways to study birth outcomes such as birth size, prematurity, and mortality. In collaboration with a former Visiting Scientist from Sweden, a matched case/control study has demonstrated that there is no adverse effect of a previous induced abortion on gestation or birth weight in the subsequent pregnancy, unless there had been a medical complication with the abortion. In additional studies, perinatal mortality rates have been analyzed in accordance with different levels (primary, secondary, and tertiary) of antenatal care in Sweden. Also, in collaboration with Norwegian scientists, a data base of extensive longitudinal antenatal measurements, for example, symphysis-fundal heights, hemoglobin, maternal weight-gain, and smoking, have been related to weight and length at birth in a Norwegian cohort. These results have yielded results which may be useful clinically in assessing high risk pregnancies. In another research study conducted jointly with a Visiting Scientist from Norway "precise" gestational age values have been determined for various subgroups within a large Scandinavian population of births. Findings from each of these studies will continue to be reported in scientific presentations and in the medical literature.

The Biometry Branch is also working on research studies based on the 1980 National Natality Survey and 1980 National Fetal Mortality Survey conducted by the National Center for Health Statistics. The available data base is comprised of 9,941 live births and 6,386 fetal deaths. Staff of the Pregnancy and Perinatology Branch, CRMC, are also collaborating on the description and and interpretation of these data. Initial maternal blood pressure readings during pregnancy have been analyzed in relation to a number of variables including birth outcome, maternal race, education, and age. An analysis of these data was presented this past year at the annual meeting of the Southern Regional Demographic Society.

Phototherapy Treatment for Neonatal Hyperbilirubinemia

This study is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing treated with untreated infants under specific conditions. The Biometry Branch has served as the data center for this study, and was the focal point for receipt of 1,339 newborn examinations and approximately 1,000 follow-up examinations per year until the children were six years of age. These forms were checked for accuracy, and precoded at each data collection center. The master files for each year's follow-up have been edited for

keypunch and coding errors, and for internal consistency. The Branch has also been responsible for the analysis of the data.

The data collection is now complete. The one-year follow-up data, which includes physical, neurological, ophthalmological, audiological and laboratory information, are currently being analyzed. These data will be compared with the six-year old findings and reported as a combined analysis. The six-year examination consisted of socio-economic, physical, neurological, audiological and psychological data.

During the past year, the analysis of the newborn data was published as a Supplement to Pediatrics. Phototherapy was effective in preventing hyperbilirubinemia in low birth weight infants (< 2000 g) who were placed under daylight fluorescent lights at 24 ± 12 hours of life for 96 hours. In this weight group, the number of exchange transfusions that were required due to hyperbilirubinemia was significantly lower in infants receiving phototherapy (4.1%) than in control infants (24.4%, $P < .001$). This was true even in infants with hemolysis and may be related to the fact that light treatment was initiated well before the development of hyperbilirubinemia.

Phototherapy was effective in controlling hyperbilirubinemia in infants of birth weight 2000 to 2499 g when initiated after serum bilirubin concentration had risen above 10 mg/dL (mean 12.3 ± 1.9 mg/dL), as well as in infants with birth weight greater than 2,500 g whose serum bilirubin level had risen above 13 mg/dL (mean 15.7 ± 2.5 mg/dL) and in whom there was no hemolytic disorder. However, light therapy instituted at these high levels of serum bilirubin did not effectively control hyperbilirubinemia in these two higher birth weight groups if hemolysis was present.

Sudden Infant Death Syndrome (SIDS) Risk Factors

A major effort of the Branch has been invested in support of the NICHD Cooperative Epidemiological Study of the Sudden Infant Death Syndrome (SIDS) Risk Factors. The data coordinating center for this study completed its final year of operation. All of the data collected for this study have been edited and entered onto computer files. With final results of the pathology second review now available, we can state that SIDS was the final classification for 94.6% of the singleton, non turn-around infants submitted to the Pathology Coordinating Laboratory as having died of SIDS by the local medical examiners or coroners. Another 2.3% were classified as "possible" SIDS cases, while 1.4% were impossible to determine due to missing materials or vital information. Only 1.8% of the eligible SIDS infants were determined by the Pathology Review Panel to have died of "known" causes and were, therefore, non SIDS.

Several reports from the NICHD SIDS Cooperative Study were presented at an International Symposium on SIDS held in Santa Monica, California in February, 1984. This Symposium was jointly sponsored by the Intra-Science Research Foundation and NICHD. Information was presented from the study based on the first half of the data set (i.e., the first 400 singleton SIDS infants and their 800 matched living controls). These papers are now in press and expected to be published by the end of the year.

A special analysis has been targeted on the question of SIDS and DTP vaccination. Results from this analysis are outlined here. Overall, 39.7 percent of SIDS cases were inoculated while 55.0 and 53.2 percent of Controls A and B were inoculated, a significant difference ($p < .05$). A larger percentage of Non-Blacks, 54.6 percent, were inoculated with DTP than Blacks, 43.5 percent. For Non-Blacks there was no difference in the percent with DTP inoculation for low birth weight infants compared to not low birth weight infants. However, among Black infants, only 27.1 percent of low birth weight SIDS cases were inoculated with DTP compared with 37.0 percent of the not low birth weight infants. Among Black control infants, there was a similar size shift in the percentage receiving DTP inoculation among the low birth weight infants. At the moment, there is no completely satisfactory explanation to account for these different rates of inoculation by race and birth weight. However, a more detailed analysis will be performed soon to investigate several possible contributing factors. A tendency among Black infants to die of SIDS at a slightly younger age would contribute to fewer of them attending for regular pediatric care visits and thus beginning the series of inoculations around two months of age. Also, the differing socio-economic status of parents by race and birth weight outcome may account for considerable variation in rates of DTP inoculation. The main point, however, is that exposure to DTP inoculation does not correlate positively with other known SIDS risk factors such as low birth weight ($rr = 4.3$) and Black race ($rr = 2.9$).

A comparison was also performed by different maternal age categories of the percent of SIDS and control infants with DTP inoculation. Although there is a tendency for the infants of mothers less than 20 years old to have lower rates of DTP inoculation, this effect is much more apparent for mothers of control infants than for mothers of SIDS cases. Next, a comparison was performed by mother's educational level of the percent of SIDS and control infants with DTP inoculation. The most striking result was that infants of mothers with more than a high school education do not differ in the percent with DTP inoculation, all are between 59.3 and 60.5 percent. If mothers have not completed high school, then the percent of infants with DTP inoculations were lower for both SIDS cases and control infants. Since education is often used as a surrogate measure of socio-economic status, this suggests that socio-economic status may be responsible for much of the variation in the rates of DTP inoculation for SIDS and control infants.

In a different type of analysis, a comparison was made of the time interval from DTP inoculation to death for the SIDS cases or to interview date for controls. Control infants were matched by age to SIDS cases such that the age at interview for control infants was as close as possible to the age at death of SIDS cases. The mean difference in age distributions for SIDS and control infants was less than two days. The most critical comparison was in the percent of infants dying or interviewed within 24 hours of receiving a DTP inoculation. The percentages observed were 1.8, 5.0, and 2.2 for SIDS cases and Controls A and B, respectively. There is no suggestion of an excess of SIDS deaths within the first 24 hours after DTP inoculation, in fact, the SIDS percentage was the lowest observed. The other conclusion was that more than a two week interval is observed for the majority of infants, regardless of whether or not the infant died of SIDS or was a living control. Thus, 65.6, 62.5 and 72.5 percent of SIDS cases, Controls A and B, respectively, had a

more than two week interval between the last DTP inoculation and either death or interview date.

In general, these results from the NICHD SIDS Cooperative Study do not alter the previous conclusion from the analysis of data based on the first 400 eligible singleton SIDS cases and matched control infants. DTP inoculation does not appear to be a factor in the etiology of SIDS. However, given the importance of this issue to the concerns of parents and their families as well as to health care providers, a more exhaustive analysis of the data from the NICHD Cooperative SIDS Study is underway. This analysis will be published in a pediatric journal.

The future plans for analysis and presentation of findings from the NICHD SIDS Cooperative Study include: (1) preparation of a Supplement for a pediatrics journal with approximately 20 chapters prepared by the principle investigators and other researchers associated with the study; and (2) preparation of a SIDS Histopathological Atlas based on the microscopic slides and related pathology materials collected by this study.

Childhood Diseases or Disabilities

A major commitment of time and attention of the Biometry Branch is the use of the randomized clinical trial and its surrogates in order to advance research goals of the Institute. Concentrated in the Branch is expertise in the design, conduct, and analysis of comparative clinical studies that seek to evaluate the efficacy of therapeutic and preventive interventions. The Branch is nearly always called upon to participate when some group in the Institute contemplates such a study. An example of one of our efforts in the area is the long-standing commitment to evaluating therapies for nephropathic cystinosis. Branch staff have maintained the Data Center for the National Collaborative Cysteamine Study, a clinical investigation of cysteamine as therapy for nephropathic cystinosis. Over the past six years, the Branch has taken a large part of the responsibility in planning the study, monitoring the data collection, and analyzing and reporting the results. A manuscript now in preparation details how cysteamine-treated patients experience slower renal deterioration and faster body growth than patients in a historical control group treated with a placebo agent.

Members of the Branch have also collaborated with the Developmental Endocrinology Branch, NICHD, in the analysis of a survey of physicians who treat patients with congenital adrenal hyperplasia (CAH). The purpose of the survey was to determine current aspects of CAH therapy: the distribution of drugs used, their dosages, and the clinical criteria that determine drug and dose. There will also be an attempt to differentiate type of therapeutic approach by geographic region and experience of physician. A manuscript is in preparation describing the results of the survey. The possibility of launching a clinical trial to evaluate different therapeutic strategies is also being considered.

In another study, Branch staff are participating in the evaluation of the long-term effects to children exposed in infancy to chloride-deficient formula. In addition, staff have provided statistical support for the analysis of a small study consisting of about twenty children, who after being exposed to chloride-deficient formula (Neo-Mulsoy), were evaluated at NIH when they were about two years old and re-evaluated two years later. One

of the statistical questions that arose while designing the Neo-Mulsoy study was what the gain would be by selecting a control from the same physician's practice (i.e., a neighborhood control) as the case. This has led to a research project to study empirically the validity and efficiency of neighborhood matching. A statistician in the Biomathematics Department of the University of California, Los Angeles is collaborating with Branch staff on this study.

Another area of collaborative research has been in the treatment, detection and risks of abusive drinking. Branch staff have continued to work with intramural researchers from the National Institute of Alcoholism and Alcohol Abuse (NIAAA) on the problems of finding biological markers for abusive drinking, and in characterizing patients who will be effectively treated for alcoholism. Statistical questions which have been addressed include the best way to derive a set of biological markers for detecting abusive consumption of alcohol and to select a discriminant function for the screening of heavy drinkers in a population. Research has been conducted into the robustness of quadratic, linear, and nonparametric discriminant functions. Also, research has proceeded into the selection of biological markers that discriminate well between disease groups after controlling for potential confounding variables. Related research efforts include the participation of Branch staff in collaboration with the Epidemiology Branch in studying the risks of small babies and malformed babies which are associated with prenatal consumption of alcohol.

Branch staff have also participated in the design of data collection forms for the Vi Polysaccharide Vaccine Trial in Nepal. Instructions for the forms have been written and a close collaboration has been established with the Laboratory of Developmental and Molecular Immunity, IRP, in implementing this project in Nepal.

Another study, based on the 1981 Child Health Supplement, has included collaborative data development and analysis with the National Center for Health Statistics to produce reliable national descriptions of children's health. Two papers have been drafted for publication as journal articles on "The Health Status of Low Birth Weight Children in the U.S." and, also, "Complications of Childbirth: Self-Reporting from the Child Health Supplement of the National Health Interview Survey Compared to Two Other Surveys." Future analysis plans include a more detailed analysis of the low birth weight children in terms of significant prenatal events and the childrens' later health outcome.

Growth and Development

Biometry Branch staff have been involved with the Epidemiology Branch and Mental Retardation and Developmental Disabilities Branch, CRMC, in the planning and development of the Chorionic Villus Sampling and Amniocentesis Study. This multicenter clinical trial began its pilot phase in March, 1985. Also, Branch staff have worked jointly on the Diabetes in Early Pregnancy Study with the Epidemiology Branch. This study is a prospectively-designed clinical investigation to determine the incidence rates of early fetal loss and congenital anomaly in the conceptuses of juvenile-onset diabetic mothers and non-diabetic control mothers. The last births are due the summer of 1985

and a report on the outcome of these pregnancies will be prepared with the study investigators.

A significant amount of Branch staff effort has been in the nutrition and growth area. These efforts first began with the analysis of infant feeding data from the Pima Indian Reservation and the George Washington University Study, and have continued with the analysis of the Bedouin Arab Infant Feeding Study. The Pima Indian and the Bedouin Arab data sets were cluster samples including data on all the children in the family. The proper analysis of clustered data where binary observations within each cluster may be correlated is a statistical problem that has been investigated by Branch staff. In addition, there has been collaboration with staff of the Epidemiology Branch, which has involved several analyses of the first and second National Health and Nutrition Examination Surveys (NHANES I and II). These analyses have involved developing an age adjusted weight-for-height index for children and the analysis of vitamin usage among women in their child-bearing years. In the process of analyzing the NHANES data it became clear that there were deficiencies in the statistical methodology for the analysis of complex survey data such as NHANES. This has resulted in the development of a research contract to develop new methods for doing regression analysis on NHANES. A contract has been awarded to the Research Triangle Institute in North Carolina to expand and develop regression methodology for complex surveys that can be applied to the analysis of growth and nutrition relationships in NHANES.

Several developmental studies utilizing statistical time series methodology have been performed by Branch staff. These applications have been shown to be valuable for the interpretation of a diverse collection of biomedical data sets that were referred to the Branch for analysis. Digital filtering, spectral analysis, and new graphical display methods have been used to identify 20-42 second rhythms in human fetal heart rate recordings, 30-70 minute rhythms in the secretion of gonadotropins in male monkeys, and seasonal and weekly patterns in a 35-year record of oral temperature and pulse rate from one human subject. Special methods have been developed to accommodate short ($n < 100$) as well as long ($n > 10,000$) multivariate time series. Simulations and Monte Carlo methods have been used to evaluate the properties of these newly-devised techniques. The data findings as well as the statistical methodology have been reported in a variety of talks and papers, and several other reports are in preparation.

Other Professional Activities

Dr. Bergsjø, Visiting Scientist in the Biometry Branch, participated actively in the Inter-Regional Conference on Appropriate Technology for Prenatal Care sponsored jointly by the AMRO/EURO Regions of the World Health Organization in November, 1984. Dr. Bergsjø was one of two obstetricians selected by the planning committee to summarize the discussion after each session and, then, to edit the proceedings volume of the conference.

Dr. Bergsjø and Mr. Hoffman both participated in the teaching of a graduate level course on "Reproductive and Perinatal Health: Epidemiology and Evaluation" offered by The Johns Hopkins University, School of Hygiene and Public Health, Department of Epidemiology.

Both Mrs. Bryla and Mr. Hoffman participated on behalf of NICHD in the United States efforts to assist the World Health Organization (WHO) in the tenth revision of the International Classification of Diseases. The tenth revision is scheduled for use in this country in 1992. The WHO Collaborating Center for Classification of Diseases for North America, a unit of the National Center for Health Statistics, is the focal point for all revisions proposed by interested parties in this country. Mrs. Bryla and Mr. Hoffman attended working meetings called by the collaborating center to discuss proposed changes in the nomenclature relating to Chapter XII--Complications of Pregnancy, Childbirth, and the Puerperium and Chapter XVI--Certain Conditions Originating in Pregnancy and around Birth.

Mr. Denman was invited this past year to join the faculty as Adjunct Assistant Professor in the Department of Preventive Medicine and Biometrics, Uniform Services University of the Health Sciences in Bethesda, Maryland. In this capacity, he has given occasional lectures on statistical computing and graphics to biostatistics courses attended by medical students.

Both Mr. Graubard and Mrs. Overpeck have continued to work collaboratively on research projects with staff of the National Center for Health Statistics (NCHS). Their expertise in the design and analysis of complex surveys has provided a beneficial link between our two agencies.

Both Mr. Hoffman and Dr. Bergsjø continued to serve as members of the Planning Group for the International Collaborative Effort on Perinatal and Infant Mortality (ICE), a committee sponsored by NCHS and other U.S. Public Health Service agencies with additional members representing six other countries. The committee is chaired by Dr. Robert Hartford, Office of International Statistics, NCHS.

Mr. Hoffman also served on behalf of NICHD as a member of the U.S. Public Health Service Work Group on Perinatal, Neonatal, Infant, and Maternal Mortality Rates for the Mid-Course Review of the 1990 Objectives for Pregnancy and Infant Health.

At the request of the Scientific Review Program, NICHD, Dr. Reed and Mr. Hoffman provided statistical expertise for the analysis of priority scores for several of the Institute's review committees. The purpose of this statistical investigation is to assess the efficiency of the review

process, and to identify possible means of improving the process, by providing reliable aggregate data to the Director, NICHD and members of these committees.

Biometry Branch

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Hillman, L., Hoffman, H.J., Hasselmeyer, E.G., Jones, M. and van Belle, G.: Maternal and newborn medical risk factors for the Sudden Infant Death Syndrome. In Harper, R.M. and Hoffman, H.J. (Eds.): Sudden Infant Death Syndrome: Risk Factors and Basic Mechanisms. New York, Spectrum Scientific Publications. (In press).

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Presentations:

Bergsjø, P.B.: Hematologic parameters as indicators of fetal well-being. Guest lecturer at the University of Medicine and Dentistry of New Jersey, Department of Obstetrics and Gynecology. Newark, New Jersey, November, 1984.

Bergsjø, P.B.: Provider perspective on perinatal services and prenatal care. Interregional conference on Appropriate Technology for Prenatal Care. AMRO/EURO Regions of the World Health Organization. Washington, D.C., November, 1984.

Bergsjø, P.B.: International collaboration in perinatal surveillance - the Nordic experience. Interregional conference on Appropriate Technology for Prenatal Care. AMRO/EURO Regions of the World Health Organization. Washington, D.C., November, 1984.

Bergsjø, P.B.: Hematological predictors of birth outcome. Seminar lecture given at The Johns Hopkins University, School of Hygiene and Public Health, Department of Maternal and Child Health. Baltimore, Maryland, January, 1985.

Bergsjø, P.B.: Maternal mortality in the Nordic countries. Seminar given at the National Institute of Child Health and Human Development, National Institutes of Health. Bethesda, Maryland, February, 1985.

Bergsjø, P.B.: Hematologic parameters as indicators of fetal well-being. Invited presentation at the Northern California Obstetric and Gynecologic Society. Sacramento, California, February, 1985.

Bergsjø, P.B.: Maternal mortality in the Nordic countries. Invited grand rounds lecture given at the Department of Obstetrics and Gynecology, University of California, Davis. California, February, 1985.

Bergsjø, P.B.: Hypertension and smoking in pregnancy. Seminar lecture for course on Reproductive and Perinatal Health: Epidemiology and Evaluation at The Johns Hopkins University, School of Hygiene and Public Health, Department of Epidemiology. Baltimore, Maryland, March, 1985.

Bergsjø, P.B.: Pregnancy hematology and birth outcome. Invited staff conference lecture given at the New York Hospital-Cornell Medical Center, Department of Obstetrics and Gynecology. New York City, New York, March, 1985.

Bergsjø, P.B.: Hemoglobin, placental exzymes and maternal smoking related to birth outcome. Invited grand rounds lecture given at the Albert Einstein College of Medicine of Yeshiva University, Montefiore Hospital and Medical Center. New York City, New York, March, 1985.

Bryla, D.A., Hoffman, H.J., McNellis, D., Denman D.W. III, and K. Fetterly: Relationships between maternal socio-demographic characteristics, prenatal blood pressure readings, and risk of adverse pregnancy outcomes. Contributed paper to the Southern Regional Demographic Group Meeting. Orlando, Florida, October, 1984.

Catz, C.S., and Bryla, D.A.: Research aspects to enhance community efforts in child health. Invited presentation for a Symposium on Children of the City: Strategies for Improving the State of their Health at the Annual Meeting of the American Association for the Advancement of Science. Los Angeles, California, May, 1985.

Denman, D.W. III: An introduction to SASGRAPH. Invited presentation at the Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences. Bethesda, Maryland, January, 1985.

Graubard, B.I.: Multiple logistic regression models with intraclass correlation. Contributed paper for the 1985 Joint Meeting of the American Statistical Society. Las Vegas, Nevada, August, 1985.

Graubard, B.I., and Korn, E.L.: An empirical study of neighborhood matching. Contributed paper for the 1985 Joint Meeting of the American Statistical Society. Las Vegas, Nevada, August 1985.

Hoffman, H.J., Denman, D.W. III, Brock, M.A., and van der Vate, J.: Time series analysis of a thirty-three year record of human oral temperature and pulse rate. Contributed paper to the XII International Biometric Conference. Tokyo, Japan, September, 1984.

Hoffman, H.J.: Measures of fetal maturity and the epidemiology of adverse pregnancy outcomes. Invited presentation for the Joint Steering Committee for Revision of the Standard U.S. Vital Registration Certificates sponsored by NCHS. Washington, D.C., October, 1984.

Hoffman, H.J., Bakketeig, L.S., and Bergsjø, P.: Fetal maturity and mortality assessed as a function of low birth weight and low ponderal index. Contributed paper to the annual meeting of the American Public Health Association. Anaheim, California, November, 1984.

Hoffman, H.J.: The epidemiology of SIDS: Findings from the NICHD SIDS Cooperative Study. Seminar lecture at The Johns Hopkins University, School of Hygiene and Public Health, Department of Maternal and Child Health. Baltimore, Maryland, January, 1985.

Hoffman, H.J.: Preterm delivery and low birth weight: Risk factors for fetal growth retardation. Seminar lecture for course on Reproductive and Perinatal Health: Epidemiology and Evaluation at The Johns Hopkins University, School of Hygiene and Public Health, Department of Epidemiology. Baltimore, Maryland, February, 1985.

Hoffman, H.J.: Collaborative research on the etiology of preterm birth and intrauterine growth retardation. Presentation at the meeting of the Planning Group of the International Collaborative Effort on Perinatal and Infant Mortality (ICE) sponsored by NCHS. Bethesda, Maryland, February, 1985.

Hoffman, H.J., Bergsjø, P., Denman, D.W. III, and Meirik, O.: Multi-dimensional analysis of fetal maturity indices derived from medical birth registries. Contributed paper to the Centenary Session of the International Statistical Institute. Amsterdam, The Netherlands, August, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00801-10 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies Based on the Medical Birth Registries of Norway and Sweden

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

PI:	Howard J. Hoffman	Chief	BB EBRP NICHD
Others:	Per Bergsjø	Visiting Scientist	BB EBRP NICHD
	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Ernest E. Harley	Chief	CS EBRP NICHD
	Heinz W. Berendes	Director	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. Fricson).

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)

☐ (a) 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 00802-10 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States

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

PI:	Howard J. Hoffman	Chief	BB EBRP NICHD
Others:	Mary D. Overpeck	Health Statistician	BB EBRP NICHD
	Ernest E. Harley	Chief	CS EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD

COOPERATING UNITS *(if any)*
Departments of Health in the following states: California, Michigan, Minnesota, Missouri, New York State, North Carolina, and Utah; Office of International Statistics, National Center for Health Statistics (R. Hartford).

LAB/BRANCH
Biometry 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
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

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

The objectives are to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. Similar information regarding fetal deaths, based on reports filed for fetuses of at least 20 weeks gestation, will also be studied. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g., birth weight, gestational age, maternal age, race, parity, etc.). The information on fetal or infant death records includes immediate and underlying cause-of-death categories corresponding to the International Classification of Diseases (ICD), based on either the eighth or ninth revision of the ICD codes. Some additional data are available from selected states regarding: smoking during pregnancy, maternal prepregnant weight and height, weight-gain during pregnancy, occupation of parents, and the levels of obstetric and pediatric care available to mother and infant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00803-01 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors

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

PI:	Howard J. Hoffman	Chief	BB EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Karla H. Damus	Consultant	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD
	Eileen G. Hasselmeyer	Director	OSR NICHD
	Jehu C. Hunter	Consultant	OSR NICHD

COOPERATING UNITS *(if any)* U. Washington (D. Peterson; G. van Belle); Loyola U. (J. Goldberg); U. Calif., Davis (J. Kraus); N.Y. Med. Health Res. Assoc. (J. Pakter); N.Y. State Health Dept. (D. Janerich); St. Louis MCH Council (L. Hillman); U. Calif, L.A. (R. Harper) and U. London, U.K. (D. Southall); AFIP, Washington, D.C. (S. Heifetz)

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

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.)*

The NICHD Cooperative SIDS Study was designed to enable identification of risk factors which could differentiate SIDS infants from non-SIDS infants. The design is that of a multicenter, population-based, case-control study with a sample of 840 SIDS cases (800 singleton and 40 multiple birth SIDS cases) ascertained under a common necropsy protocol. There were 1,600 matched living singleton control infants and 40 co-multiple birth control infants recruited into the study. It is the largest detailed epidemiological study of SIDS ever undertaken. Data were collected for babies who died over a 15-month period from October, 1978 through December, 1979. Every infant death was autopsied in accordance with a common necropsy protocol developed specifically for the study. Twenty-six different slides of tissues were preserved for detailed examination by a panel of three SIDS pathology experts. Under an Inter Agency Agreement with the Armed Forces Institute of Pathology (AFIP), technical support is being provided for the preparation of a SIDS Histopathology Atlas and "study sets" to be used for the education of practicing forensic pathologists or pathology students.

In another SIDS risk factor study, techniques of time series analysis are being used to examine potential abnormalities in the development of neuro-physiological and cardio-respiratory control mechanisms in the first three months of life. The study materials consist of computerized data sets from long-term electrophysiological recordings of infants from three earlier SIDS research studies. Comparisons will be made among the following groups of infants: subsequent siblings of SIDS infants, "near-miss" infants, twins, matched controls, and infants who later died of SIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00811-06 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 E. 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 (J. Schneider); Uniform Services Univ. of the Health Sciences (J. Schlesselman); Univ. of Michigan Medical School (J. 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)

☒ (a) 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 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 90 children will eventually be enrolled in the current trial, which is anticipated to last about one more year. 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 00813-04 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biostatistical Methods for the Analysis of Laboratory Research Studies

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

PI:	George F. Reed	Mathematical Statistician	BB EBRP NICHD
Others:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Barry Graubard	Mathematical Statistician	BB EBRP NICHD
	Howard J. Hoffman	Chief	BB EBRP NICHD

COOPERATING UNITS *(if any)*
Phil Skolnick, Chief, NS, LBC, NINCDS

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 laboratory studies on: (1) dose-response relationships, (2) bioassay and potency estimation, (3) time to event, life table analyses, and (4) other investigations of the effects of external stimuli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00818-04 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 00820-04 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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:	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
	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:
.6	.6	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 00821-03 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Development of New Graphical Methods for the Analysis of Biomedical Data

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

PI:	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	George F. Reed	Mathematical Statistician	BB EBRP NICHD

COOPERATING UNITS *(if any)*

Bell Communciations Research, Livingston, N.J. (E. Fowlkes)

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 00830-04 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	Daniel W. Denman	Mathematical Statistician	BB EBRP NICHD
	Barry I. Graubard	Mathematical Statistician	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD

COOPERATING UNITS *(if any)*
Division of Health Interview Survey, National Center for Health Statistics
(G. Hendershot and A. Moss)

LAB/BRANCH
Biometry 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 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 00832-02 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities

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

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. Data sources include previously unpublished data from 1970-81 for fetal deaths from the National Center for Health Statistics, and perinatal and infant mortality from 1975-84 based on states participating in a current study of multinational birth weight-specific perinatal mortality rates supported with research contract funds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00833-01 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Outcomes of Deliveries with IUD Use During Conception

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
	Marsha Reichman	Guest Worker	BB EBRP NICHD
	Bruce Stadel	Medical Officer	CEB CPR NICHD

COOPERATING UNITS *(if any)*

University of North Carolina, Department of Epidemiology (E. Heineman)

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 study compares the outcome of deliveries (live births, fetal deaths, early neonatal deaths, and birth weight) of women who conceived with an IUD in place to deliveries without an IUD present. Data are available for deliveries occurring in 1975-1977 from the Kaiser-Permanente Birth Defects Study. The data base contains extensive information on maternal characteristics, patterns of contraceptive use and fetal outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00840-04 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Statistical Discriminant Methods with Applications to Alcoholism Screening

PRINCIPAL INVESTIGATOR *(List other professionel 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, Md.

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.)*

This study will investigate the statistical properties of a variety of discriminant functions and 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 00841-04 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	Dwight B. Brock	Mathematical Statistician	EDB NIA

COOPERATING UNITS *(if any)*
National Center for Health Statistics, Office for Research and Methodology, Statistical Methods Section (R. Casady); Research Triangle Institute (B.V. Shah).

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 study will compare available statistical methods and develop new methods for conducting data analysis with complex survey data, using data from the first and second National Health and Nutrition Examination Surveys (NHANES I and II).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00842-03 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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.)*

This study will 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, and from the Perinatal Collaborative Project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00843-02 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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, leboratory, 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

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.)*

This study will theoretically and empirically compare matched with unmatched designs of case-control, cross-sectional, and cohort studies. The Family Growth Cycle III Survey and the first and second National Health and Nutrition Examination Surveys (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 00844-02 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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
Other:	Natalie Kurinij	Nutritionist	EB EBRP NICHD

COOPERATING UNITS *(if any)*

School of Public Health, Yale University (P. 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 will 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 second National Health and Nutrition Examination Survey (NHANES II) data set.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00850-09 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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)
- ☒ (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, 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 00851-04 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	Charlotte S. Catz	Chief	PPB CRMC 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.)*

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 00852-03 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

1980 National Natality Survey and Fetal Mortality 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
	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Karen L. Fetterly	Computer Specialist	CS EBRP NICHD
	Donald McNellis	Medical Officer (Obstetrics)	PPB 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:
.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 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 00853-01 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine

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

PI:	Dolores A. Bryla	Statistician	BB EBRP NICHD
Others:	George F. Reed	Mathematical Statistician	BB EBRP NICHD
	Charles V. Lowe	Special Assistant/Director	OD NICHD
	John Robbins	Chief	LBMI NICHD

COOPERATING UNITS *(if any)*

Infectious Disease Hospital, Nepal (I. Acharya); Uniformed Services, University of the Health Sciences (J. Schlesselman).

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 study, which is now being developed, is a cooperative, randomized trial to determine the efficacy of Vi polysaccharide in preventing typhoid fever in Nepal. The Biometry Branch's involvement in this study is to design data collection forms, and assist in the data management and the analysis with the study investigators from NICHD and Nepal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00854-01 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Analysis of MCH Data from the National Longitudinal Youth Survey

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

PI:	Dolores A. Bryla	Statistician	BB EBRP NICHD
Others:	Howard J. Hoffman	Chief	BB EBRP NICHD
	Natalie Kurinij	Nutritionist	EB EBRP NICHD
	Donald McNellis	Medical Officer	PPB CRMC NICHD

COOPERATING UNITS *(if any)*

Ohio State University (F. Mott); Division of Nutrition, Centers for Disease Control (M. Forman); R.W. Johnson Clinical Scholars Program, University of North Carolina (C. Homer).

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 has as its primary objective to analyze and publish data based on a series of annual interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. In addition, a wealth of other data have been collected on the youth cohort sample in relation to their employment on work history, military service, educational attainments, etc. The Biometry Branch has joined in the funding of the data collection effort together with the Demographic and Behavioral Sciences Branch, Center for Population Research, NICHD. The mechanism of support for the field study is through an Inter Agency Agreement with the Department of Labor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00860-05 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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
	Mary Ann Brock	Biologist	CI CP GRC NIA

COOPERATING UNITS *(if any)*
Department of Pediatrics, University of South Florida College of Medicine, St. Petersburg, Florida (B. Bercu); 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:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

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

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

The 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 00861-03 BB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

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

PI:	Howard J. Hoffman	Chief	BB EBRP NICHD
Others:	Per Bergsjø	Visiting Scientist	BB EBRP NICHD
	Daniel W. Denman III	Mathematical Statistician	BB EBRP NICHD
	Heinz W. Berendes	Director	EBRP NICHD

COOPERATING UNITS *(if any)*
Department of Community Medicine, University of Trondheim, Norway (G. Jacobsen and L. Bakketeig); Bell Communications, Livingston, N.J. (G.W. Reed); Department of Obstetrics and Gynecology, University of Alabama in Birmingham (R. Goldenberg).

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.6	.1

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.)*

This project has been expanded to encompass two related research studies. The first study has analyzed data derived from a randomized clinical trial of diagnostic ultrasound use during pregnancy conducted by the team of Norwegian investigators in Trondheim, Norway. The purpose of the analysis is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyseal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound; and (4) maternal hemoglobin level. Regression models have been fit to the serial measurements for each mother. The coefficients of the regressions have been analyzed in relation to various indicators of birth size such as weight, crown-heel length, ponderal index, and birth weight-for-gestational age percentile. Using an analysis of covariance procedure, additional factors (e.g., cigarette smoking, alcohol intake, low maternal prepregnancy weight, etc.) will be tested for significance in modifying intrauterine growth patterns.

In addition to the study described above, a prospective study to determine risk factors for intrauterine growth retardation, or small-for-gestational age birth, was begun in 1984 through the research contract mechanism with both the University of Alabama in Birmingham and University of Trondheim, Norway. The study protocol includes recruitment of pregnant women before 17 weeks gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally and at delivery. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HD 00870-02 BB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Long-Term Reproductive Effects of Cesarean Section Birth

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
	Ntinos Myrianthopoulos	Section Chief	DNB CDNDP NINCDS

COOPERATING UNITS *(if any)*

University of Tampere, Department of Public Health, Finland (K.E. Hemminki); New York State Department of Health (D. Glebatis, D. Janerich and G. Therriault); National Center for Health Statistics, Family Growth Branch (W. Mosher).

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 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. Subsequent studies include linking this data to the hospital discharge register to study problems not related to pregnancies ending in birth.