ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
Fiscal Year 1981

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

ANNUAL REPORT

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

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National Institute of Child Health and Human Development

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October 1, 1980 through September 30, 1981

OFFICE OF THE DIRECTOR

The National Institute of Child Health and Human Development is unique among the National Institutes of Health, in that it concentrates its research programs primarily on the health and well being of the normal adult. Research on the conditions that will assure lifelong health is mainly focused on early events in the life cycle: fertilization, pregnancy, infancy, childhood, and the adolescent years. For it is these events which can set a course either for health or for disease and disorders that may last a lifetime.

In this Institute, the organizational units which lend focus and coherence to these research approaches are the Center for Population Research (CPR), the Center for Research for Mothers and Children (CRMC), the Epidemiology and Biometry Research Program (EBRP), and the Intramural Research Programs (IRP). These organizations have each prepared summaries and/or project descriptions of their research efforts during the fiscal year for this annual report. This record of accomplishments, and the reports of the great variety of activity being pursued by Institute staff or supported through grants and contracts, contains the elements of significant benefit to improved health and well-being of the earth's peoples.

In this report you will find descriptions of research efforts in a range of biomedical and behavioral sciences. In the reproductive sciences, the purpose of the Institute's program is to advance basic knowledge to improve the reproductive health of men and women, to identify and develop new leads for less hazardous and more effective methods of fertility regulation, and to alleviate infertility and prevent diseases and disorders of the human reproductive system.

In the behavioral and social sciences, researchers in the population program continue to investigate the reasons behind high pregnancy rates in adolescents and the consequences of teenage childbearing for the child, the mother, the father, and the family. It was found, in research on the societal costs of early childbearing, that mothers receiving Aid to Families with Dependent Children (AFDC) are more likely to have been teen mothers than were American women in general. Among AFDC mothers under age 30, 64 percent had been teenage mothers, whereas only 24 percent of all American women aged 20 to 30 in 1975 had given birth before age 20. Estimates of the public sector costs related to early childbearing are that in 1975 more than $8.5 billion was expended on AFDC households in which the mother was a teenager at the time she bore her first child.

The Institute's program of research in pregnancy and infancy includes studies on clinical nutrition and early development, human learning and behavior, mental retardation and the developmental disabilities. Current studies have identified the need for additional research related to hypoxia, the lack of oxygen, in the fetus and the newborn infant. Improved methods to detect hypoxia could greatly reduce perinatal death and the number of damaged infants who are born.

Great strides in the management of diabetic pregnancy have reduced the number of babies lost from 20 to between 6 and 10 per 1,000 births. Because pregnancy in diabetic women is still considered a high risk situation, the NICHD will continue research on this problem through its Major Research Programs which are particularly

devoted to studies on pregnancy and birth. Study of normal pregnancy and birth should contribute to reducing infant mortality and morbidity.

One very important activity in the organizational life of this Institute, which is described nowhere else in this report, is the effort expended over approximately the last year and one-half to evaluate and describe the Institute's 10 programs of research activity and develop a 5-year plan from the assessment of this current status. This effort, coordinated by the Office of Planning and Evaluation, will be completed with the presentation of the evaluation and the plan to the Institute's Advisory Council at its first meeting in Fiscal Year 1982. The techniques of evaluation and assessment used have been original and of varied success using a combination of NICHD staff members, disciplinary experts and consultants from academic research endeavors, and the logistic support activities of a contractor. Indications are that this is a cost-effective way to conduct this kind of planning/evaluation operation and beneficial results of these efforts in guiding development of the Institute's program are anticipated.

This report marks the valedictory for Norman Kretchmer as Director after seven years in the position. His departure and return to academe finds the programs of the NICHD in the healthy state described in the progress reports of the projects which comprise this volume. His attention to organization of research program, his requirement for honest and thorough assessment of state-of-the-art, and his creative leadership in development of longer-range planning leave the Institute in a strong position for continued scientific growth and development and, for presentation to the public, a clear expression of the investment potential contained in the Institute's programs.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Center for Population Research
Office of the Director

INTRODUCTION

The Institute's Center for Population Research (CPR) continued to fulfill its responsibility for the primary Federal effort in population research. Significant progress has resulted again this year from the Center's support of research in the population sciences through grants and contracts for:

Fundamental biomedical research in the reproductive sciences relevant to problems of human fertility and infertility.

The development of safe and efficacious methods for fertility regulation.

The evaluation of the medical effects and efficacy of contraceptive methods.

Population dynamics research in the social and behavioral sciences on the causes and consequences of population structure and change.

Research in the reproductive sciences on fertility and infertility is concerned with the reproductive processes involved in the fertility of men, women, and animals with reproductive processes similar to those in human beings. Studies on fertility and infertility are subsumed under the four major disciplines which comprise the reproductive sciences: (1) reproductive endocrinology; (2) reproductive biology; (3) reproductive medicine; and (4) reproductive chemistry. Reproductive endocrinology includes the secretion, action, and metabolism of the reproductive hormones and the endocrine glands which secrete them. Reproductive biology includes the reproductive processes and events such as oocyte maturation, sperm production, ovulation, sperm maturation, sperm capacitation, gamete transport, fertilization, early embryonic development, implantation, reproductive behavior, and nutrition in animals. Reproductive medicine includes human nutrition, human infertility, human reproductive diseases and disorders, andrology, and clinical studies of human reproduction. Reproductive chemistry includes synthesis of reproductive peptides and steroids, isolation and purification of substances involved in reproduction; and chemical modification of substances involved in reproduction for increased efficacy and diminution of undesirable side effects.

The contraceptive development program pursues through clinical trials and laboratory studies, the development of new and improved methods of fertility regulation, for both men and women, which are safe, effective, reversible, and acceptable to various population groups. Research activities included in contraceptive development are: (1) synthesis and biological evaluation of promising new compounds that may affect reproductive processes in the male or female; (2) the development of technology for improved administration of contraceptive drugs; (3) the development of improved vaginal and uterine contraceptives based on chemical or physical methods; (4) clinical trials of sex steroids and peptides for suppression of sperm production and the consequent development of chemical contraceptives for men; (5) clinical and toxicological evaluation of long-acting progestin as a female contraceptive; (6) laboratory studies and clinical trials to develop and evaluate antifertility methods based on periodic abstinence; and (7) studies required to clarify

mechanisms of action of specific contraceptive drugs.

The program of research on contraceptive evaluation deals with the safety of various contraceptive methods which are currently in use and endeavors to clarify and improve their benefit/risk characteristics. Emphasis is placed on detecting and evaluating effects on health that must be weighed with efficacy to assess fully the value of different fertility regulating methods. Research on contraceptive evaluation includes the following: (1) Studies of steroid contraceptives to determine their effects on cancer and other neoplasia, heart disease, hypertension, thromboembolic disease, metabolic, nutritional, and immunologic disorders. Also included are studies of the role of the immune system in mediating adverse effects of oral contraceptives, evaluation of individual variation in pharmacologic response to contraceptive steroids, and evaluation of sequelae, especially the risk of cancer, after exposure to diethylstilbestrol in utero or during pregnancy. (2) Studies of intrauterine devices to determine their effects of the occurrence of serious gynecologic and obstetric disorders such as pelvic inflammatory disease, vaginal hemorrhage, uterine perforation, ectopic pregnancy, fetal loss, abruptio placenta, placenta previa, and infertility. (3) Studies of the medical sequelae of male and female sterilization and research to clarify factors related to return of fertility following anatomic reversal of sterilization in men and in women. (4) Studies of the effects of induced abortion on subsequent reproductive function, such as the occurrence of spontaneous abortion, prematurity, infertility, etc. (5) Studies on the use-effectiveness of inadequately evaluated methods, such as the diaphragm and condom.

Research in the social and behavioral sciences is concerned with the factors governing variations in the growth, distribution, and characteristics of people and the impact of population changes on the health and well-being of individuals, families and society as a whole. This research includes two major categories:

(1) Studies of the causes of population change, including factors affecting the three components of population change: fertility, mortality, and migration. Research has emphasized factors affecting fertility, since this has been the largest and most variable component of population change in the United States. Research on the causes of variations in fertility is concerned primarily with social, economic, and psychological determinants. Also included are such topics as choice of methods of fertility control, attitudes towards such methods, the effectiveness with which methods are commonly used, the number of children wanted and expected, changes in family structure and function, the desired timing of childbearing and the incidence of unwanted childbearing. Research on the causes of migration, deals with factors affecting trends in internal migration, such as the movements between metropolitan and non-metropolitan areas, and immigration. Research in mortality includes studies of mortality differences between various social and economic groups and of social and economic factors affecting trends in mortality.

(2) Studies of the consequences of population change, including research on the personal, familial, and social consequences of changes and differentials in the components of population change (fertility, mortality, and migration) and the resulting characteristics and distribution of the population. Emphasis has been placed on the consequences of childbearing patterns for individuals, families, and society; for example, studies of the effects of maternal age, birth spacing, and birth order on the psychosocial development of the child, the economic well-being of the parents, the participation of women in the labor force and the stability of the family unit. Special attention is given to the consequences of teenage

childbearing for mother and child. Research on the consequences of migration include the social and economic impact of large movements of people into or out of specific areas. Studies of the consequences of mortality include the impact of the size of specific age groups and the effects of family composition. Research on the consequences of population change also includes studies of the social, economic, and other impacts of varying rates of population growth.

RESEARCH RESULTS

The following are some of the recent findings and achievements in population research supported by the Institute:

Reproductive Sciences

1. The development of an *in vitro* cell culture system for chick oviduct tissues has been reported. This technological advance is of significant interest to those researchers working on the mechanism of action of steroid and peptide hormones in reproductive tissues.

2. Investigators have succeeded in stabilizing the steroid receptors by molybdate. This finding allows for the first time precise physical characterization of the androgen as well as progesterone receptors.

3. Significant progress has been made in identifying and characterizing specific surface components of sperm in order to determine their role in reproduction. This research has provided evidence that individual antigenic determinants are restricted to specific areas of the sperm surface and has demonstrated that sperm surface components produced by other cells in the reproductive tract become applied to and associated with specific topographically restricted areas of the sperm surface.

4. The mechanism of the effect on fertility of gossypol, a non-steroidal compound that inhibits sperm production in animals and humans, has been further clarified. Researchers have demonstrated that gossypol interferes with testosterone production by the Leydig cells of the seminiferous tubules of the testis. Serum testosterone and luteinizing hormone (LH) levels were significantly reduced following the administration of gossypol to rats at concentrations which inhibited fertility without an apparent loss of libido. Sperm in the resulting ejaculates were reduced in number and rendered immotile.

5. Considerable progress has been made concerning hypothalamic control of gonadotropin secretion in the rhesus monkey. A model for neuroendocrine control of the menstrual cycle in this species has been developed that can probably be extrapolated substantially to the human female.

6. Recent advances in opiate research have enhanced our understanding of neuroendocrine control of pituitary function. Studies in women during the menstrual cycle suggest that endogenous opiates are involved in the regulation of luteinizing hormone (LH) during the late follicular (high estrogen) and mid-luteal (high estrogen-progesterone) phases but not in the early follicular phase of the cycle. This research provides indirect evidence that endogenous opiates may play an important role in control of gonadotropin releasing hormone (GnRH) secretion during the menstrual cycle.

Contraceptive Development

1. Efforts to synthesize more potent antagonists of luteinizing hormone releasing hormone (LHRH) have been successful. Compounds have been developed that are active in blocking ovulation in the rat at a dosage which represents a four-fold improvement in activity over what was reported last year. At this dosage the LHRH antagonists are sufficiently potent to merit exploration of their safety in animal models and preliminary studies in both men and women.

2. Clinical pharmacology studies of LHRH agonists have been investigating the potential of different administration schedules to block ovulation and to produce luteolysis. Administration of different analogs during the midluteal phase of the cycle resulted in a lowering of serum progesterone levels and to early menstruation. Continuous administration of one of the analogs for ninety days induces anovulation and amenorrhea.

3. The effects on pituitary-gonadal function of a LHRH agonist have been evaluated in normal men who received a daily injection for up to ten weeks. This treatment resulted in a substantial decrease in plasma testosterone by the fourth week in all eight subjects. Sperm density and motility fell in each subject, and in each case sperm density returned to normal levels within 10-14 weeks after the treatment was stopped. Five men developed impotence between the sixth and seventh weeks of treatment, although impotence was reversed within two weeks after discontinuation of the injections.

4. The relation between the estimated time of ovulation and the day of defined post-ovulatory infertility was studied for different Natural Family Planning methodologies. Inherent weaknesses were identified for methods using calendar and basal body temperature (BBT) calculations as the only endpoints. Addition of cervical mucus observations to BBT recording significantly improved precision of the estimate.

5. The synthetic chemical facility synthesized a number of long-acting progestins, unnatural amino acids and other intermediates. The facility is continuing purification and chemical analysis of gossypol samples, which not only provides this drug as starting material for other synthetic programs but also provides the pure compound for biological work by numerous scientists outside of the program.

6. The biological drug testing facility has carried out numerous screening assays and has also conducted intensive evaluation of several drugs that are being considered for clinical evaluation. Pharmacokinetic studies of several long-acting progestins have been completed, and results from this monkey study will permit selection of a norgestrel ester for animal safety evaluation. This aspect of the testing program is part of a collaborative WHO/NIH program to develop improved injectable long-acting contraceptive drugs.

Contraceptive Evaluation

1. A study to evaluate the relationship between oral contraceptive (OC) use and the occurrence of myocardial infarction (MI) has found that long-term past OC use increases the risk of MI. For women 40-49 years of age, five or more years of past OC use appears to increase the risk of MI about two-fold relative to the risk among non-users. This study also confirmed previous reports that current OC use increases the risk of MI, and that current OC use interacts with the effects of cigarette

smoking in a multiplicative manner.

2. A multi-center study of the relationship between intrauterine device (IUD) use and the occurrence of serious gynecologic and obstetric disorders has been completed. It has confirmed that IUD use increases the risk of pelvic inflammatory disease (PID), and has shown that the use of oral contraceptives and barrier methods of contraception (diaphragm and condom) decrease the risk of PID. The study also confirmed that women with an IUD in place at the beginning of the second trimester of pregnancy are at increased risk of septic fetal loss, and found that this increased risk is not present for women with an IUD in place at conception but removed in the first trimester of pregnancy.

3. A study was completed which sought to ascertain whether steroid contraceptive use, which is known to result in changes in glucose tolerance, is associated with an increased risk of overt clinical diabetes mellitus. Data from this study do not demonstrate any association between the frequency or duration of use of contraceptive steroids and subsequent development of diabetes mellitus.

4. A study has revealed no increase in diastolic blood pressure (DBP) among black women who use oral contraceptives (OCs) as compared to black women who use other non-OC forms of contraceptives. However, there does appear to be a slight increase in the risk of elevated DBP for white women using OCs, although this increase in risk is apparent only among white women 22 years of age.

5. A study to determine the effects of prenatal exposure to diethylstilbestrol (DES) on the health of male and female offspring, especially on reproduction and the risk of cancer, was based on a previous controlled clinical trial. Male offspring exposed in utero to DES revealed a greater proportion with minor abnormalities of the external genitalia including epididymal cysts and also low sperm counts and decreased sperm mobility. Among female offspring a high proportion reveal vaginal and cervical adenosis, but no case of clear-cell adenocarcinoma of the vagina or cervix has been identified. The data also suggest the possiblity of increased primary infertility among DES-exposed daughters and a higher rate of unfavorable outcome among those becoming pregnant.

6. A study is pursuing preliminary findings which indicate that vasectomy may significantly increase the severity of atherosclerosis in monkeys. Researchers who previously demonstrated that vasectomy considerably escalated the extent of atherosclerosis in a small group of cynomolgus monkeys fed a high cholesterol diet, recently have demonstrated markedly increased atherosclerosis in rhesus monkeys maintained for 9-14 years after vasectomy on a diet free of cholesterol.

Social and Behavioral Sciences

1. A study of the trends of family formation and family dissolution involving a comparison among the United States, Sweden, England, and Belgium over the time period 1910-1975 has concluded that there has been a substantial movement toward earlier and more universal marriage, although there has been some reversal of that trend during the last 10-15 years. A significant finding is the rise in the proportion of marriages ending in divorce which has accelerated since 1965. For instance, about 12 percent of American women married in 1915 will have their marriages end in divorce; this probability grows to 32 percent for those women married in 1945, falls to 25 percent for those married in 1955, and accelerates sharply thereafter to an estimated 42 percent of American women who married in 1975.

2. Research has been carried out on the extent to which children are involved in changing marital patterns. A study of women and children in remarriages from 1960 to 1975 indicates that one-third of the children in recent remarriages were under the age of five and one-sixth were teenagers aged 14-17. Most of the children involved in remarriage had siblings and half had two or more. For many of the children studied, remarriage followed very soon after the divorce, but for almost a fifth, their mothers waited five or more years before remarrying. The study estimates that the number of children experiencing a second marital disruption has doubled in recent years.

3. Research on the determinants of fertility in the United States involving analyses of the contraceptive behavior of a national sample, indicate that between 1970 and 1975 there has been a sizeable decline in the rate of intended conceptions. During this period there was also a large increase in the delay of intended conceptions, an unprecedented decrease in failure rates by couples attempting to delay or to terminate fertility, and widespread use of sterilization to end reproduction.

4. Recent changes in fertility patterns and in the orientation of people toward family size in the U.S., have been reported from a longitudinal survey of mothers and their "Baby Boom" offspring. While mothers in the sample had an average of 3.9 children in 1980, the number of children they now preferred averaged 3.3. In 1962, 84 percent of the mothers said that they believed that all married couples who can ought to have children, while in 1980 only 43 percent held this view. The sons and daughters of these women stated that they would like to have, on average, 2.9 children. A comparable proportion of children as their mothers, 39 percent, said they thought all married couples ought to have children.

5. Research on the consequences of family size concerning studies of only children, show no adverse effects of being an only child. Only children were found to be more like firstborns in larger families, and were shown to be slightly superior on cognitive abilities and achievement to children with siblings. They have not been shown to differ from children with siblings on a wide range of factors including physical development, use of medical care, behavioral and psychological factors, marital status, number of children they had, divorce rates, occupational choice, or levels of income.

6. Research has been carried out on the societal costs of early childbearing. It was found that mothers receiving Aid to Families with Dependent Children (AFDC) are more likely to have been teen mothers than were American women in general. Among AFDC mothers under age 30, 64 percent had been teenage mothers, whereas only 24 percent of all American women aged 20 to 30 in 1975 had given birth before age 20. Initial estimates of the public sector costs related to early childbearing indicate that in 1975 more than $8.5 billion was expended on AFDC households in which the mother was a teenager at the time she bore her first child.

7. Research on the determinants of teenage pregnancy and childbearing has included studies of individual, couple, familial, and societal level factors affecting adolescent behavior. In 1979, data for metropolitan areas show about half of women aged 15-19 have experienced premarital intercourse. There was no lowering of the age at first intercourse as noted earlier in the decade, but the porportional increases have been greatest at ages 15 and 16. The probability of intercourse increases with age, exceeding 50 percent only for 18- and 19-year-olds.

8. A study of internal migration within the U.S. has identified three aspects of

population change that will shape the future demands for health services and the provision of health care. These factors are: (1) shifts in the age distribution which will give greater prominence to the health care needs of the elderly; (2) changing settlement patterns which will alter the geographical demand for health care, shifting some of it away from large population centers to places where specialty medicine is less readily accessible; and (3) increasing concentration of population in large central cities whose needs will tend to strain health care delivery systems.

9. Several studies have shown that housing and neighborhood characteristics are more important than employment opportunities in determining trends in residential location. The increase of households having members with jobs in the suburbs has encouraged both the growth of suburban housing and suburban employment, but primarily the former. It was found that there is a tendency for suburban employment to follow the growth of suburban populations. Also, one study reports that household characteristics have an important role in residential mobility, such as the gain or loss of family members, or simply children's growth.

COORDINATION AND COMMUNICATION

The activities of the Center to enhance the coordination of Federal population research programs and to foster the communication of biomedical and behavioral research information in the population sciences, are presented in this portion of the report since they are coordinated in the Office of the Director.

Interagency Committee on Population Research (ICPR), which is chaired by the Director of CPR, coordinates the population research activities supported by Federal agencies and facilitates the exchange of information and ideas among Federal programs involved with population research. The ICPR, established by the Secretary of HEW on October 5, 1970, has been extended by the Secretary of HHS through June 30, 1982. It is comprised of representatives from the various Federal agencies concerned with research related to human population problems. The major products of the ICPR are the following two publications produced annually with the assistance of NICHD's Office of Planning and Evaluation.

Inventory and Analysis of Federal Population Research for fiscal year 1980 was issued by the ICPR. This report contains (1) a description of the background and role of the ICPR; (2) a statistical analysis with fiscal tables by research areas and supporting agencies; (3) a detailed inventory of all Federally supported population research projects classified according to the major research category; (4) a listing of projects started in FY 1980; and (5) a membership list of the ICPR.

Inventory of Private Agency Population Research for 1979 was published by the ICPR. This report provides information on the population research projects sponsored by the major U.S. private organizations in this field. The four principal private agencies involved with research in the population sciences, the Ford, Rockefeller, and Mellon Foundations and the Population Council, have provided the data on their research projects in the same way as the Federal agencies and the information has been published in the identical format as the Federal Inventory.

Population Research Monographs published by the Center through the Government Printing Office furthers the dissemination of information in the population sciences. These books provide a review of the state-of-the-art or report on progress in specialized areas of population research in the biomedical or social sciences, and

indicate future research directions to obtain needed knowledge. The Center's conferences and workshops also continue to result in publications which constitute a valuable addition to the population research literature.

The Walnut Creek Contraceptive Drug Study - Volume III is the third Population Research Monograph to report on this study, which was among the initial research projects developed by our Contraceptive Evaluation Branch. The study, directed by Dr. Savitri Ramcharan of the Kaiser-Permanente Medical Center in Walnut Creek, California, is one of only a few prospective projects world-wide designed to detect and quantitate adverse and beneficial effects of oral contraceptive drugs in a large population of health women. This volume summarizes the hospitalization and mortality experience of oral contraceptive users as compared to non-users in this population.

The Center enters progress and final reports of grants and contracts selected by our Branches into the National Technical Information Service (NTIS) in order to facilitate the dissemination of information on research findings and thus further the transfer of knowledge. NTIS, part of the U.S. Department of Commerce, is being utilized since they are the central source for the public sale of scientific and technical reports of U.S. Government sponsored research and development, and they also comprise the largest documentation center in the world for the public distribution of scientific publications. The following are the most recent reports entered by the Center into NTIS:

Accession No.	Title and Author(s)
PB81-206914	Family Formation and Fertility Control in the Early Years of Marriage by Arthur G. Neal, H. Theodore Groat, Jerry W. Wicks
PB81-188260	The Personal Meanings of Voluntary and Involuntary Childlessness by Warren B. Miller
PB81-183303	Development and Testing of New Biodegradable Delivery Systems by Robert V. Petersen, Sung Wan Kim, James M. Anderson, Sen Maw Fang, Dennis L. Coleman, Jan Feijen
PB81-171993	An Empirical Investigation into the Intellectual, Physical, Psychological, and Social Consequences of Being Reared an Only Child by John T. Doby, Martin L. Levin, S. Mitra
PB81-140709	Studies on the One-Child Family by Bea J. Van den Berg, Frank W. Oechsli
PB81-132136	Development and Evaluation of a Biodegradable Drug Delivery System by K.R. Sidman, A.D. Schwope, W.D. Steber, S.E. Rudolph. S.B. Poulin, G.K. Schaper
PB81-100927	The One-Parent/One-Child Family: Social and Psychological Consequences by Denise Polit

PB81-102857 Differential Consequences of Having Been an Only Versus a Sibling Child
by H. Theodore Groat, Jerry W. Wicks, Arthur G. Neal

PB81-102865 The Consequences of Being an Only Child: An Analysis of Project Talent Data
by John Claudy, William Farrell, Charles Dayton

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Contraceptive Development Branch
Contract and Collaborative Research

Research sponsored in fiscal year 1981 has produced considerable clinical evidence to suggest new approaches to fertility regulation. Equally important, several new clinical projects in barrier contraception have been initiated and in time are likely to provide the public with improved methods of contraception. Additionally, at the laboratory level we continue to support a broad program in several areas that is continuing to yield important information on possible future methods of fertility regulation.

A. Product Development

Drug Development Program

Continued efforts to synthesize more potent antagonists of LHRH have been successful in developing compounds that are active in blocking ovulation in the rat at dose levels as low as the 2.5 μg. This represents a fourfold improvement in activity over what was reported last year. At this dosage the compounds are sufficiently potent to merit exploration of their safety in animal models and preliminary studies in both men and women. Plans for the safety and clinical studies of antagonists are being implemented.

In the area of LHRH agonists in-depth evaluation of the safety of two compounds is being carried out. Additional animal safety data will permit a broader evaluation of these drugs for their contraceptive potential.

In the area of male antifertility drugs, attempts are in progress to modify gossypol. Gossypol has been extensively evaluated in clinical studies in China and in a variety of animal experiments all over the world. Modification of the molecule is required in order to make this drug safer. Research is currently in progress to evaluate whether separation of antifertility and toxic properties is possible.

Development of drugs that can interfere with the production of progesterone and induce luteolysis has been stymied by the lack of an adequate animal model for the testing of such compounds. During the past year a nonhuman primate model has been developed in our primate facility and utilized for the evaluation of several prostaglandin analogs. Among several of the compounds tested, 13 dehydro PGF_2 appears capable of depressing progesterone production. Another category of drugs that may prove useful for fertility regulation are antiprogestins. Development of these drugs has been very slow for the lack of adequate leads. Intensification of our efforts in this area of research has been implemented in FY 1981.

Synthetic Chemical Facility

The facility continues to play an important role in the overall synthesis program. During FY 1981 it synthesized a number of long-acting progestins, unnatural amino acids and other intermediates. Purification and chemical analysis of gossypol samples continues. The latter activity not only provides this drug as starting material for other synthetic programs but also provides the pure compound for biological work by numerous scientists outside of our program.

Biological Drug Testing Facility

During the past fiscal year the facility has not only conducted a large number of screening assays but has also conducted intensive evaluation of several drugs that are being considered for clinical evaluation. Pharmacokinetic studies of several long-acting progestins have been completed. Results from this monkey study will permit a selection of a norgestrel ester for animal safety evaluation. This aspect of the testing program is part of a collaborative WHO/NIH program to develop improved injectable long-acting drugs. In another phase of this collaborative effort, evaluation of an implantable drug delivery system for local irritation has also been undertaken.

Drug Delivery Systems and Oral Formulations

Many activities discussed in this section of last year's progress report have shifted from laboratory and animal studies to clinical investigation and will be reviewed in the clinical trials section.

Development of an injectable microcapsular system has been implemented. Preliminary in vitro studies of several batches of microcapsules reveal acceptable release rates of norethindrone. Microencapsulation of levonorgestrel has required a slight modification in the encapsulation technology because of physical characteristics of the micronized drug. This has now been accomplished and batches of levonorgestrel microcapsules should be available for *in vivo* evaluation in the near future.

In the bioerodable drug delivery systems area research has been conducted along two main fronts. The advanced Alzamer™ (formerly Chronomer™) system has undergone intensive evaluation in cynomolgus monkeys in order to resolve the issue of tissue irritability at the implant site. Results of this study did not confirm that complexes produced by irradiation for sterilization cause irritation at the implant site. Based on the results of this study WHO plans to conduct additional clinical studies of these devices. The alternate bioerodible system requires additional formulation studies in order to improve its drug release characteristics.

Device Development

Developments in this area of research will be discussed under the topic of clinical trials.

Clinical Trials (Female)

Plans for continuation of clinical evaluation of norethindrone enanthate as a long-acting injectable contraceptive have been finalized. Clinical trials will involve an initial period of three injections at 8 week intervals with all subsequent injections taking place at twelve week intervals. Acceptable efficacy can be anticipated from this dosing schedule based on results from WHO supported trials. In conjunction with the efficacy trials, additional animal safety studies have been implemented.

Clinical pharmacology studies of LHRH agonists has been in progress for the past 18 months. The basic aim of these investigations is to focus on the potential of different administration schedules to block ovulation and to produce luteolysis. Administration of different analogs during the midluteal phase of the cycle resulted in a lowering of serum progesterone levels and to early menstruation. Continuous administration of one of the analogs for 90 days induces anovulation and amenorrhea.

Successful induction of short luteal phase was achieved by the administration of the analog during the early (D1-3) follicular phase. Cycles were extended due to a longer follicular phase. The short or inadequate luteal phase is clinically associated with infertility and may represent an appropriate contraceptive approach. Results of current investigations strongly suggest that ovulation inhibition can be routinely accomplished with the agonists and that more extensive clinical investigations are in order.

Initial clinical evaluation of a new oral dosage form for norethindrone and ethynyl estradiol has been completed. The aim of this new dosage form is to reduce the initial drug absorption peak and to prolong effective drug blood levels. Determination of blood levels of norethindrone clearly indicated that the formulation did produce the desired pharmacokinetic profile. The results with ethynyl estradiol are equivocal and additional formulation work must be carried out. It is of interest that in the baboon model kinetics of both drugs exhibited the desired profile.

Phase I thirty day clinical study of the implantable Capronor™ drug delivery system has been completed. The devices did not produce irritation at the implant site and the release of levonorgestrel over the 30 day period was relatively stable. These highly encouraging results suggest that more extensive clinical studies are warranted. In order to undertake these studies a more extensive animal safety evaluation is required. Plans for animal safety studies are being finalized.

During the past two years the contraceptive development program has intensified its efforts to develop and test new approaches to barrier methods of contraception. Extensive clinical trials aimed at efficacy assessment of a polyurethane sponge diaphragm and of the cervical cap have been initiated. The trials have been in progress for too short a time to be able to draw any conclusions. A much smaller clinical trial involving a cervical cap that is custom fitted and has a one-way valve allowing evacuation of cervical and uterine secretions has been initiated. This type of cervical cap can be left in place over extended periods of time. An additional clinical effort is underway to assess the dimensions and properties of the cervix that are pertinent to cap design and to see how these factors change during the menstrual cycle and in response to other biological events. Increasing the number of available sizes of cervical caps may lead to improved acceptability of this method of contraception.

It has been recognized for some time that the currently available spermicidal preparations could be improved from both the standpoint of efficacy as well as from the standpoint of user acceptability. This can be achieved either through the use of new spermicidal compounds or through reformulation of existing spermicides. The CDB program is in the process of negotiating new contracts that will address both of these issues.

Clinical Studies (Male)

The effects on pituitary-gonadal function of a LHRH agonist have been evaluated in normal men who received a subcutaneous injection daily for up to 10 weeks. This treatment with the drug resulted in a substantial decrease in plasma testosterone by the fourth week in all 8 subjects. Five men developed impotence between the sixth and seventh treatment weeks. Impotence was reversed within two weeks of discontinuation of treatment. Sperm density and motility fell in each subject, and in 6 subjects it fell to an average of 6×10^6 sperm/ml. In each case sperm density returned

to normal levels within 10-14 weeks after the treatment was stopped. These data suggest that the compound does influence sperm production. However, a dosing regimen will have to be established which consistently reduces sperm density and motility. It appears that testosterone supplementation will be necessary to correct the impotence created by the LHRH agonist.

Distribution Program

Distribution of reagents continues to represent an important aspect of the program. Availability of a variety of reagents facilitates not only the applied research sponsored by the program, but also is of great importance to scientists conducting basic biomedical research. Additionally, reagents to detect pregnancy in nonhuman primates have been of benefit to breeding programs all over the world.

During the past year the CDB has distributed 50 liters of porcine follicular fluid. This fluid is used by scientists for exploration of its effects on reproductive function as well as for the isolation of several bioactive peptides contained therein. One of these peptides has been termed folliculostatin and has the ability to preferentially inhibit FSH secretion.

B. Directed Fundamental Research

RIA Development

Evaluation of a radioimmunoassay for monkey LH has been completed. The reagents appear to have both high specificity and affinity. They should be available for distribution to scientists in the very near future. In addition to the LH-RIA a new reference preparation for monkey FSH has been secured.

The development of a RIA for rat androgen binding protein (ABP) has proceeded on schedule. Sufficient ABP has been generated for immunization of rabbits and high titers of ABP antibodies have been obtained from several rabbits. Their specificity is currently being tested. Large uniform batches of ABP are being prepared to be used as a radiolabelled standard. Approximately 9 mg of this material are on hand.

Folliculogenesis

Dynamics of follicular development during the menstrual cycle in the rhesus monkey were studied in ovaries removed during various phases of the cycle. Preantral follicles were measured and classified according to size. Results show that the number of primordial follicles in a pair of ovaries are similar but vary greatly among monkeys. The mean percent of follicles of the same size is similar between ovaries from the same animal. When ovaries were grouped into the major stages of the cycle according to the status of the antral follicles or the corpus luteum, a significant increase in the largest size of follicles occurred during the periovulatory period. During this period atresia was also minimal. These data suggest that: 1. development of preantral follicles is symmetrical between ovaries regardless of stage of the cycle, 2. uniformly the larger follicles increase in size during the periovulatory period.

Gonadocrinin

Gonadocrinin is a newly discovered ovarian polypeptide that has biological activity similar to LHRH, but is of different chemical structure. It very well may represent

an internal regulator of ovarian function, as well as participating in overall hypothalamic-pituitary-ovarian interrelation. Its isolation, purification and characterization is an important but very difficult task. Initial attempts at isolation of this material used ovaries stimulated with a specific batch of PMSG. Subsequent batches of ovaries came from rats stimulated with a different batch of PMSG. The latter ovaries had little if any gonadocrinin activity. This observation represents a biological puzzle which needs to be resolved since the ovaries stimulated by different batches of PMSG appear to be morphologically and functionally similar.

Natural Family Planning (NFP)

Analysis of data relating menstrual cycle phenomena to the fertile period and presumptive ovulation has been continued. The data suggest that intermenstrual pain may not be due to any one specific cause but, rather to several related factors. However, intermenstrual pain is a better sign of ovulation than abdominal bloating, low backache or intermenstrual bleeding.

The relation between the estimated time of ovulation and the day of defined postovulatory infertility was studied for different NFP methodologies. Inherent weaknesses were identified for methods using calendar and basal body temperature (BBT) calculations as the only endpoints. Addition of cervical mucus observations to BBT recording significantly improved precision of the estimate.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Intrauterine Release of Estriol for Contraception
Contract No. : N01-HD-3-2729
Contractor : Michael Reese Hospital
Money Allocated: $133,233 (FY 74); $172,289 (FY 75); $107,760 (FY 76); $143,154 (FY 78); $47,419 (FY 79); $42,210 (FY 80)

Objectives: The objectives of this project are: (1) evaluation of the effects of an IUD releasing 12.5 µg/day of estriol in women; (2) evaluation of the long-term effect of intrauterine estriol on endometrial proliferation in baboons.

Major Findings: Intrauterine devices releasing estriol or control placebo devices were inserted in women for a three month period. Patients were evaluated by pre-insertion and periodic endometrial biopsies, preinsertion and final pap smears, histological examination of the hysterectomy specimen three months post IUD insertion and hormonal evaluation (follicle stimulating hormone, luteinizing hormone, estrone, estradiol and progesterone) in the third post-insertion cycle. Patients were also instructed to keep basal body temperature charts during the study period.

The results of the studies so far have indicated that the length and bleeding pattern of the menstrual cycles in the three-month study period have been similar to the pre-IUD-insertion cycles and except for spotting following endometrial biopsy, no intermenstrual bleeding has been noted. Biphasic basal body temperatures have been noted in most of the study cycles and hormonal evaluation in the third study cycles have been generally in agreement with the basal body temperatures and indicative of ovulatory cycles. A comparison of the preinsertion and follow-up Pap smears has failed to reveal any significant changes in the histological interpretation and grading. No significant differences have been noted between the histological evaluation of the preinsertion and follow-up endometrial biopsies and the microscopic examination of the hysterectomy specimen, except in one patient, who showed cervical dysplasia. However, the uterine specimen failed to reveal any hyperplastic changes in the endometrium.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: Information desired on the pharmacodynamics of continuous administration of estriol should be available by the end of the current contract period. Further development is not projected at this time.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : A Study of the Use of Biodegradable Polymers for the Sustained Delivery of Contraceptive Drugs
Contract No. : NO1-HD-3-2741
Contractor : Research Triangle Institute
Money Allocated: $298,347 (FY 73 for two years); $185,262 (FY 75); $224,082 (FY 76); $211,983 (FY 77); $210,081 (FY 78); $306,740 (FY 79); $258,688 (FY 80); $213,383 (FY 81)

Objectives: To develop a biodegradable polymer reservoir for subdermal delivery of a contraceptive steroid, e.g. norgestrel, for periods of one year or longer. The device is designed to provide constant release rate by diffusion control and to biodegrade after the drug is exhausted.

Major Findings: A one month clinical trial of Capronor™, a biodegradable subdermal delivery system for levonorgestrel based on poly(ε-caprolactone), was undertaken in 7 women. The preparation and characterization of Capronor™ and its constituents were documented. The metabolism of poly(ε-caprolactone) in rat was shown to involve extensive carbon skeleton cleavage as well as hydrolysis to ε-hydroxycaproic acid. The rate of ester hydrolysis in the bulk polymer decreased when the M_n was approximately 3500. Ethyl oleate was the most effective dispersing agent for levonorgestrel, as judged by a high and constant levonorgestrel release rate. A one year study in rabbits demonstrated efficacious release rates for this time period, while an *in vitro* study was continued for 1.5 years.

Random linear and star block copolymers of caprolactone were prepared and their degradation *in vitro* and *in vivo* was characterized by measurement of viscosity, molecular weight, crystallinity, and weight loss. While their rate of bioabsorption was greater relative to poly(ε-caprolactone), the period prior to loss of capsule strength was less. The permeability of the copolymers to levonorgestrel was greater. Chemically crosslinked homo- and copolymers of ε-caprolactone were also prepared and their degradation *in vitro* and *in vivo* characterized by the same techniques.

Significance to Biomedical Research and Program of the Institute: Development of drug delivery systems for contraceptive drugs is one of the stated goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of a Long-Acting Injectable Contraceptive
Contract No. : N01-HD-5-2817
Contractor : Schering AG
Money Allocated: $1,211,168 (FY 75) 3 years; $120,000 (FY 78); $142,477 (FY 79) $88,782 (FY 80); $280,917 (FY 81)

Objectives: The purpose of this contract program is to acquire sufficient data to support the filing of a New Drug Application (NDA) for norethindrone enanthate, a long-acting progestational agent. This requires support of preclinical, clinical and long-term drug safety studies.

Major Findings: Representatives of the sponsor and the Contraceptive Development Branch met with the Food and Drug Administration to discuss the Agency's request for a two year rat toxicity study comparing norethindrone enanthate and a combination oral contraceptive containing norethindrone. Following this meeting and after numerous communications with the Agency, a protocol design was established which was mutually acceptable to all parties. After soliciting bids for the study from U.S. laboratories, it was determined that the sponsor could undertake the work, including all of the pharmacokinetic portion, for the least cost. The study is scheduled to commence in September.

Long-term dog and monkey studies continue in their sixth year with no unexpected findings.

A bidders conference was held with prospective offers for expanded Phase II clinical studies scheduled to be initiated early in 1982. The conference was most useful in that it permitted the sponsor to detail the clinical protocol and receive comments and recommendations for alterations in study design by clinicians active in the field.

Significance to Biomedical Research and Program of the Institute: The development of a long-acting injectable contraceptive is directly related to the goals of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort encompassing completion of all preclinical and clinical requirements for the filing of a New Drug Application (NDA) and long-term drug safety studies in dogs and monkeys.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Biological Testing of Luteolytic Prostaglandin Analogs
Contract No. : N01-HD-7-2816
Contractor : University of Chicago
Money Allocated: $137,214 (FY 77); $134,539 (FY 78); $142,000 (FY 80); $54,700 (FY 81)

Objectives: The major objectives of this project are: 1) To synthesize 10, 10-difluoro-prostaglandin analogs for evaluation as luteolytic agents in the monkey; 2) Evaluate previously synthesized 13-dehydroprostaglandin analogs for luteolytic activity and smooth muscle activity in the monkey.

Major Findings: 16-Fluoro-13-dehydro-$PGF_{2\alpha}$ had been tested as a mixture of (15R, 16S) and (15S,16R)-isomers, the first-named being the one of natural configuration at C-15. Two general approaches offered themselves to obtain the two compounds in pure form: 1) To resolve the eight carbon ω-chain utilized in the synthesis or 2) to re-examine the separation of 15-epimers which in the case of the 13,14-acetylenic prostaglandins had in the past proved impossible. The second route, if successful, would avoid a good deal of difficulty. Attempts at separation by a variety of chromatographic systems of the substances themselves or after acylation proved completely unsuccessful. However, when the acetylenic moiety was complexed with dicobalt octacarbonyl the resulting stable dicobalt hexacarbonyl adducts could now be separated chromatographically with ease and the resulting dicobalt hexacarbonyl adducts reconverted into the acetylenic prostaglandins by oxidation with ferric or ceric ion. Thus, for the first time optically active pure natural 16-fluoro-13-dehydro-$PGF_{2\alpha}$ (erythro form) was obtained. The unusually high biological activity of the natural 16-fluoro compound (43-84 x $PGF_{2\alpha}$ in the hamster antifertility assay and 2-3 x $PGF_{2\alpha}$ in the oxytocic and gerbil colon assay) is in line with the activity previously obtained on the mixture which consists of the 15R,16S and 15S,16R-isomers in a 1:2 ratio. Separation of the corresponding ent mixture produced uninteresting biological data.

X-ray crystallographic studies confirm the absolute configuration of the previously prepared 10,10-difluoro-13-dehydro-$PGF_{2\alpha}$. The compound showed four times the activity of $PGF_{2\alpha}$ in the hamster antifertility assay and 0.25-0.5 x $PGF_{2\alpha}$ in the oxytocic and gerbil colon assay.

Intravenous infusion of 13-dehydro-$PGF_{2\alpha}$ at a dose of 2 mg/kg/hr for six hours to hCG treated monkeys (10,20, and 40 i.u. hCG on cycle days 20, 21 and 22) resulted in complete and permanent suppression of progesterone levels.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: Termination. Most of the workscope objectives have been completed.

Contract Title : New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2825
Contractor : ALZA Corporation
Money Allocated: $206,289 (FY 77); $204,284 (FY 78); $455,411 (FY 79); $356,612 (FY 80); $35,463 (FY 81)

Objectives: The goal of this project is the development of an erodible, subcutaneous implantable delivery system that will deliver predictable, contraceptive amounts amounts of synthetic progestins over a four to six month period.

Major Findings: This project is funded jointly by the National Institute of Child Health and Human Development (NICHD), the World Health Organization (WHO) and ALZA Research. An implant has been developed based on ALZAMER™, which is a poly(ortho ester), steroid, and a rate-controlling excipient.

During this reporting period, the project has established U.S. facilities for manufacturing and packaging clinical and toxicological units and completed an excretion/distribution study in rats which indicates that the breakdown products of the matrix polymer are not sequestered in any tissue, but are excreted rapidly, in a manner similar to the way the ultimate hydrolysis products are excreted following subdermal injection. In addition, the project has completed formulation studies on a formulation of polymer/norgestrel which provides a stable serum level in baboons for six months; demonstrated two-year storage capability for a polymer/norethindrone formulation (previously seen to function for five months in baboons) and prepared norethindrone and norgestrel units for a study in cynomologous monkeys to evaluate the effect of irradiation of the systems on local irritation which had been observed in a small clinical study performed in Sweden under the auspices of WHO. Because a complex between steroid and polymer is known to be formed during the sterilization procedure which uses 2.5 megarad of γ-irradiation, the local irritation study was designed to obtain further information before proceeding with clinical studies.

Significance to Biomedical Research and Program of the Institute: Development of new drug delivery systems for contraceptive drugs is one of the goals of the Contraceptive Development Branch.

Proposed Course: This project is currently in a holding status pending results of the local irritation study in monkeys. If this study and additional safety studies show the systems to be clinically acceptable, the project will continue to be supported.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of New Biodegradable Drug Delivery Systems
Contract No. : NO1-HD-7-2826
Contractor : SRI International
Money Allocated: $149,786 (FY 77); $237,555 (FY 78); $270,465 (FY 79) $238,768 (FY 81)

Objectives: It is proposed to develop non-toxic biodegradable polymers capable of delivering a contraceptive agent with zero order kinetics for at least a six-month period. Additionally, drug release and polymer solubilization should take place concomitantly so that rate of drug release is controlled by the rate of polymer dissolution.

Major Findings: Drug release and erosion studies from poly(ortho ester) devices containing 10 and 20 wt% norethindrone and 10 wt% of water soluble salts have continued.

Linear polymers were prepared by the addition of diols to a diketene acetal. Even though the polymerization reaction proceeds readily, purification of the diketene acetal to the required 99+% purity is very difficult and becomes virtually impossible with batches larger than about 50 g. For this reason a new monomer has been developed which is considerably more stable and can be prepared in large batches in virtually quantitative yield by a one step rearrangement of an inexpensive commercially available compound. Polymerization with diols using conventional acidic catalysis proceeds readily with no competing cationic homopolymerization.

Drug release studies show linear release for 80 to 105 days after which release rate accelerates. The acceleration is accompanied by a significant increase in size of the devices and is caused by osmotic imbibing of water and consequent device expansion. Erosion takes place within the water inclusions and is physically evident by SEM examination of the devices. As found previously, high molecular weight poly(ortho esters) with Na_2CO_3 release the drug at constant rate, swell but do not erode.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of a Radioimmunoassay for Rhesus Monkey Luteinizing Hormone
Contract No. : NO1-HD-7-2829
Contractor : University of Pittsburg
Money Allocated: $66,331 (FY 77); $65,160 (FY 78); $91,664 (FY 79); $108,160 (FY 80)

Objectives: The aim of the program is to develop a radioimmunoassay for rhesus monkey luteinizing hormone (LH) and to provide sufficient reagents to prepare 200 kits (15,000 tubes per kit) for distribution to the scientific community.

Major Findings:

1. An antiserum, R13, was selected, on the basis of titer, specificity, and binding affinity, from over one hundred other antisera produced in rabbits which had been immunized with hCG, ovine LH, or bovine LH. This antiserum, when used in conjunction with a tracer obtained by radioiodination of highly purified cynomolgus LH and with a well-characterized rhesus LH reference preparation as standard, provides a sensitive, precise, and specific radioimmunoassay for macaque LH. The quantities of these these three reagents are sufficient to provide over two hundred RIA kits, if a kit contains reagents for 20 to 25 assays of 500 to 1000 tubes each.

2. Another antiserum has been identified, R132, which possesses characteristics quite similar to those of R13, i.e., high titer, good specificity, and strong binding affinity for LH from various macaques, including rhesus, cynomolgus, and cercopithecus monkeys. In addition to the R132 antiserum, a quantity of cynomolgus LH suitable for a reference preparation is available, and, if a sufficient amount of highly purified LH can be obtained from some eight hundred cercopithecus pituitary glands which were previously provided by Dr. R. Lequin, then an additional batch of several hundred RIA kits should be forthcoming.

Significance to Biomedical Research and Program of the Institute: The work to be undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to acquire specific substances concerned with aspects of reproduction processes that are subject to experimental interventions.

Proposed Course: The contract has been extended an additional year, without funds, to develop the additional batch of RIA kits, using the R131 anti-sera and the cercopithecus LH.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Orally Active Dosage Forms for Steroids
Contract No. : N01-HD-7-2831
Contractor : Southwest Research Institute
Money Allocated: $95,027 (FY 77); $104,104 (FY 78); $323,500 (FY 79); $280,883 (FY 80)

Objectives: The aim of this program is to prepare sustained-release oral formulations of contraceptive steroids by incorporating these products in a matrix of microcapsule and to determine whether this dosage form will minimize daily peaks of the drugs and reduce body burdens while maintaining contraceptive efficacy and clinical acceptability.

Major Findings: Studies have continued over the past year on the development and evaluation of sustained-release microspheres containing ethynylestradiol (EE), norethindrone (NET) and norethindrone acetate. Aging stability at room temperature and 37°C of glyceride microspheres containing EE and NET has been extensively investigated. The EE-containing microspheres show good aging stability for up to one year but the NET-containing microspheres show a reduced *in vitro* release rate after aging at 37°. This appears to be due to crystallization of the NET. Other microspheres using excipients such as fatty alcohols show good aging stability with NET at 37°C but the *in vitro* release rate was undesirable. NET acetate was therefore investigated as a substitute for the NET. Glyceride microspheres of NET acetate give the desirable *in vitro* release and also have shown good aging stability at room temperature and 37°C for up to 4 months, the extent of testing to date.

During the past year, glyceride microspheres containing EE and NET (Formulation FD-1) were prepared under GMP procedures for clinical evaluation.

Using the baboon as model, and comparing it continuously with *in vitro* methods for assessing dissolution rates, oral preparations (aqueous solutions, commercial tablets and the microsphere formulations) have been administered and plasma kinetics of norethindrone, norethindrone acetate and ethynylestradiol have been studied. By this means, it was possible to confirm the slow-release characteristics of certain microsphere formulations. Intravenous/oral administration comparisons were carried out in a 4-subject human study with ethynylestradiol and the appropriate pharmacokinetic parameters calculated. Subsequently, a 6-subject crossover study in human subjects was carried out, comparing 2 tablets of Brevicon (0.07 mg ethynylestradiol and 1.0 mg norethindrone) with a similar amount of these steroids encapsulated in formulation FD-1. The desired advantages in kinetics were apparent with norethindrone, but the results to date with the ethynylestradiol comparison are equivocal.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the published purpose of the Contraceptive Development Branch to support research directed towards development of new methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Folliculogenesis in the Rhesus Monkey During the Menstrual Cycle
Contract No. : N01-HD-7-2835
Contractor : George Washington University
Money Allocated: $45,999 (FY 77), $22,204 (FY 78), $57,818 (FY 80), $4,822 (FY 81)

Objectives: Preantral follicles are the initial pool from which the dominant follicle is recruited and little is known as to how their development relates to the hormonal changes that occur during the 28-day menstrual/ovarian cycle. This study was undertaken to determine the status of preantral follicles at various stages during this cycle.

Major Findings: Both ovaries were removed from 18 regularly cycling rhesus monkeys, serially sectioned and mounted. On every 20th section primordial follicles and the primary follicles with a single layer of flattened granulosa cells were counted; the largest cross diameter of all remaining preantral follicles were measured on the section that contained the nucleolus. The morphological assessment of all other ovarian components was previously determined. The measured follicles were classified into size categories: I-40-59 μm, II - 60-79 μm, III - 80-99 μm, IV - 100-119 μm, V - 120-150 μm. Atresia was recognized by the distortion of the oocyte and/or pyknosis of the granulosa cells. Final results were obtained by utilizing correction factors that gave an estimated count for all preantral follicles that were present in each ovary.

Results show that the number of primordial follicles in a pair of ovaries are similar but vary greatly between monkeys with the ovary of one monkey having 11,000 and another having 125,000. However, the mean percent of follicles in each size category is similar between a pair of ovaries. When ovaries were grouped into the major stages of the cycle according to the status of the antral follicles or the corpus luteum, a significant increase ($p < 0.05$ occurred) in the mean percent of follicles in size categories IV-V occurred during the periovulatory period. Atresia was minimal (<4%). These data suggest: 1. development of preantral follicles is symmetrical between ovaries regardless of time in cycle. 2. uniformly, follicles between 100-150 μm in diameter increase in size during the periovulatory interval which correlates with a unique microenvironment rich in estrogen and pituitary gonadotrophins.

Significance to Biomedical Research and Program of the Institute: Since the ovarian follicle is the target for contraceptives, the knowledge of its natural history is important for appreciation of the possible mechanisms of action of contraceptives.

Proposed Course: The contract was extended to permit additional observations. Research was completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Contraceptive Efficacy of Androgen-Estrogen Polydimethylsiloxane Implants in Rhesus Monkeys
Contract No. : N01-HD-8-2805
Contractor : The John Hopkins University
Money Allocated: $70,937 (FY 78), $163,604 (FY 79), $96,217 (FY 80) $31,930 (FY 81)

Objectives: The overall goal of this study is to design a reversible contraceptive technique for the human male which does not alter sexual behavior, accessory sex organ function or result in untoward side effects. The immediate objects of this proposal are twofold: (1) To define the most efficacious contraceptive formulation of testosterone and estradiol delivered via PDS subdermal implants to rhesus monkeys; and (2) to test the effect of this contraceptive formulation on fertility and erythropoietic system, blood clotting mechanisms and major organ function in rhesus monkeys.

Major Findings: Twenty adult male rhesus monkeys were randomly assigned to control and treatment groups containing 10 monkeys each. The treated monkeys received subdermal "silastic" implants designed to release 100 g testosterone/kg/day and 0.5 µg estradiol/kg/day, respectively. Semen was collected at bi-weekly intervals for 12 months. Sperm numbers were enumerated and seminal volume, seminal fructose concentration measured. Blood samples were collected via femoral vena puncture at monthly intervals for subsequent blood cell counts, serum chemistry and measurement of blood clotting characteristics. Testosterone and estradiol metabolic clearance rate was determined by measuring the disappearance of ^{3}H testosterone and estradiol from blood plasma. Autopsies were performed during the spring of 1981.

Nine of 10 monkeys were azoospermic or severely oligospermic as a result of the contraceptive treatment. One monkey's response was anomalous. He was azoospermic at the beginning and at the end of the year-long experimental period but produced enormous quantities of sperm during the middle portion of the year. Although serum testosterone and estradiol were elevated in the treated animals, there was no significant difference in 24-hour production of testosterone and estradiol due to the treatment. Ejaculate volume was identical in control and treated monkeys. However, seminal fructose and prostate weight were elevated in contracepted monkeys. In contrast, there was no significant difference in seminal vesicle weight between the two groups of animals. There was no clinically significant difference between the two groups in blood clotting time, prothrombin time, partial thromboplastin time, red blood cell count, total neutrophils, lymphocytes, monophils, eosinophils, white blood cells, hematocrit, hemoglobin, total protein, MCV, MCH, MCHC, reticulocyte, phosphorus, BUN, glucose, albumin, total bilirubin, SGOT, SGPT, alkaline phosphatase, cholesterol, ion, magnesium, sodium, potassium, gamma glutamyl transpeptidase, triglycerides, calcium, uric acid, LDH and chloride.

Significance to Biomedical Research and Program of the Institute: Development of new techniques for the control of fertility in the male is one of the stated goals of the program.

Proposed Course: These studies are expected to be completed at the end of the contract year.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Progesterone Analogues of Novel Structures with Potential Antifertility Activities
Contract No. : NO1-HD-8-2812
Contractor : Laval University
Money Allocated: $74,715 (FY 78); $64,78 (FY 79); $16,500 (FY 81)

Objectives: The objective of this contract is to synthesize derivatives of 17α-hydroxymethylprogesterone and 11α-substituted progesterone analogues in view of the potentiating effect of 17-alpha substituents and the influence of substitution at C-11 on progestational activity. These compounds will be tested for antifertility activity.

Major Findings: Sufficient quantities of 17-acetoxymethylprogesterone and 17-methoxymethylprogesterone were obtained for biological testing. The in vitro binding activity of 17-methoxymethylprogesterone showed approximately one fourth that of progesterone towards a progesterone receptor. In rats, it possesses deciduogenic activity somewhat superior to progesterone and no anti-deciduogenic activity. One of the target compounds, 11α-methylprogesterone was synthesized from 3α,20β-diacetoxy-11α-methyl-5β-pregnan-12-one. It was found that 11α-methylprogesterone showed approximately the same binding activity towards a progesterone receptor as progesterone itself and possesses stronger deciduogenic activity than the natural hormone, but no anti-deciduogenic activity in rats. It did not possess antiprogestational activity in anti-clauberg assay at a total dose of 50 mg subcutaneously in the rabbit. In the course of the synthesis of 11α-methylprogesterone sufficient quantities of 3α,20β-diacetoxy-11β-methylpregnan-12-one were obtained and could be used for the synthesis of 11β-methylprogesterone. 3α,20β-Diacetoxy-11α-tosyloxymethyl-5β-pregnane was obtained from the 3,20-diacetate of 11α-hydroxymethyl-5β-pregnane-3α,20β-diol. In a preliminary experiment it was established that this tosyloxy substituent be replaced by a fluorine atom.

Significance to Biomedical Research of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: Termination in the absence of antiprogestational activity for these compounds.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Biological Testing Facility
Contract No. : N01-HD-8-2813 continued as N01-HD-0-2846
Contractor : Mason Research
Money Allocated: $700,623 (FY 78); $845,501 (FY 79); $156,490 (FY 80); $839,980 (FY 80 under N01-HD-0-2846); $1,085,506 (FY 81) $1,178,0832 (FY 82); $1,278,131 (FY 83); $1,386,882 (FY 84)

Objectives: The objective of this contract program is the establishment of a biological testing facility capable of broad spectrum evaluation of new drugs and devices and rapid exploitation of potential leads in the control of fertility. Sources of compounds for testing include contract synthetic programs supported by the CDB and numerous private, public and governmental laboratories throughout the world. The staff of the CDB reviews all compounds submitted for evaluation to determine the precise manner in which each drug will be studied.

Major Findings: The testing facility continues to evaluate new drugs, formulations and delivery systems in an efficient manner and has extended its capabilities to include radioimmunoassay of natural and synthetic steroids.

Emphasis continues to be placed on the screening of potent new analogs of LHRH. Both antagonists and agonists have been identified which exhibit biological activity with as little as a few micrograms.

A substantial number of long-acting androgens and estrus-suppressing steroids continue to be evaluated for potency and longevity of action. Several compounds have been identified which exhibit sufficient activity to warrant extended assessment.

Several long-acting analogs of norethindrone are being studied as potential long-acting injectable contraceptives.

The radioimmunoassay laboratory is currently assessing serum levels of estrone, estradiol, progesterone, testosterone, LH, FSH, norethindrone and norgestrel on a routine basis.

Data generated at the facility are entered into a computerized data analysis and storage system and are usually available to CDB staff through their inhouse terminal within 24 hours of experiment termination. Data from long term studies are entered weekly so that the progress of the study may be monitored on a regular basis.

Significance to Biomedical Research and Program of the Institute: The heart of any drug development program is the rapid identification of biological activity and exploitation of promising leads. The ability of the CDB to evaluate compounds and devices in a uniform manner is of great importance to our contract synthetic program and the stated goals of the Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Novel Steroids with Potential Male Contraceptive Activity
Contract No. : N01-HD-8-2815
Contractor : Laval University
Money Allocated: $33,812 (FY 78); $37,178 (FY 79)

Objectives: The objective of this contract is to synthesize 11-aza-5α-dihydrotestosterone analogs which will lead to the study of a distinctly different 11-hetero steroid. The importance of the 11-position in governing both estrogenicity and effects on gonadotrophin is now apparent. These compounds will be tested for the male contraceptive activity.

Major Findings: The 3,3-mono dimethyl ketal derivative has been resynthesized from 11-aza-5α-pregnane-3,20-dione in excellent yield, and the corresponding N-fluoroacetyl derivative was obtained in > 60% yield. However, Barton's procedure via the hydroperoxide could not hydroxylate this product in the 17α-position. As shown in the case of piperidine, the fluoroacetyl protection is indeed hydrolyzed under the reaction conditions, which explains the lack of success of the 17-hydroxylation since it was previously established that the secondary amine, constituted by the 11-aza structure, does not tolerate treatment under the conditions of the Barton reaction. This pathway has been definitely abandoned. New attempts were made to degrade the methyl ketone side chain in position 17 prior to the development of the 11-aza structure. Finally, 2 kg of hecogenin became available which is in the process of being degraded by Ayerst Laboratories in Montreal in their pilot plant so that the experiments foreseen may be carried out with sufficient material.

On the other hand, while waiting for availability of commercial hecogenin, preliminary investigations have been undertaken in view of the synthesis of 11-aza 3,17-dioxygenated 5α-androstanes by a pathway quite different from the one originally planned; it involves 9,11-seco-9-oxo-12-androstanoic acid, as a key intermediate, oxygenated in positions 3 and 17. Therefore, 4-androstene-3,11,17-trione was reduced with lithium in liquid ammonia and the crude product reoxidized with Jones' reagent. 5α-androstane-3,11,17-trione thus obtained was selectively ketalized in positions 3 and 17 in > 88% yield by the formation of the 3,17-bisethylenedioxy derivative. Lithium aluminum hydride reduction gave the 11β-alcohol in 95% yield which was dehydrated in ∿ 90% yield with thionyl chloride to 3,17-bisethylenedioxy-5α-pregn-9(11)-ene. These experiments will be pursued in the near future and the experiments as briefly outlined above will be undertaken as soon as the degradation product of hecogenin will have been received from Ayerst Laboratories.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Propose Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthetic Chemical Facility
Contract No. : NO1-HD-8-2823
Contractor : Southwest Foundation for Research and Education
Money Allocated: $147,323 (FY 78); $156,620 (FY 79); $166,014 (FY 80); $102,215 (FY 81)

Objectives: The purpose of this project is to have this synthetic chemical facility synthesize rapidly laboratory scale as well as larger quantities of specific compounds required by the Contraceptive Development Branch for contraceptive investigation.

Major Findings: During the third year of the contract a total of twenty-two compounds were synthesized. (D,L)-3,4-dehydroproline was prepared on a large scale from pyrrole-2-carboxylic acid (2.5 mol.). Preparation of the d-(+)-tartaric acid salt and purification of the desired optical isomer followed by hydrolysis of the salt gave optically pure L-3,4-dehydroproline which was distributed to the peptide chemists for the preparation of LHRH antagonists. A number of long-acting esters of levonorgestrel were prepared including cyclopropyl and cyclobutyl carboxylates, their oxime derivatives, acetate, and propionate. Also, 7α-methylnorethindrone pentanoate, hexanoate, octanoate, nonanoate and 2-heptynoate were prepared. Previously, thallous ethoxide had been used for the preparation of esters of hindered alcohols. Since it is a highly toxic material and does not give good yields on a large scale, a new procedure was adopted for the preparation of esters of hindered alcohols employing the acid and trifluoroacetic anhydride either with or without tosic acid as a catalyst. Attempts to prepare norethindrone glycolate by published procedures were unsuccessful. An efficient synthesis of this ester was developed by first preparing the t-butyldimethylsilyl ether derivative of glycolic acid chloride and then reacting it with norethindrone to give the desired ester. Norgestrel glycolate was obtained in low yield by means of Schering's procedure. The optically pure methyl ester of 6-bromo-7-carboxybicyclo[2.2.1]heptane was prepared on a large scale starting with 2 kg norbornadiene. An efficient resolution was accomplished and after three additional steps, 143 g of the desired ester was obtained which is an intermediate for the preparation of 12-fluoro $PGF_{2\alpha}$. The synthesis of another prostaglandin intermediate, 2-fluorohexanal was completed and will be used for the preparation of erythro-16-fluoro-13-dehydro $PGF_{2\alpha}$ in the future. 3,4-Bis[(3-hydroxyphenyl)methyl]dihydro-2(3H)-furanone isolated originally from the female urine was prepared, following the known procedure and tested. Gossypol acetic acid 360g, 99+% pure) became available for testing as an oral antifertility agent for males. The crude product obtained from Southern Regional Research Laboratories was recrystallized three to four times from a mixture of methyl ethyl ketone and acetic acid (3:1).

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis of specific steroidal and nonsteroidal compounds for evaluation for contraceptive utility.

Proposed Course: The objective of the contract were completed. It will be continued in a new contract.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Analysis of Data from the Study of Correlation of Cervical Mucus Changes with Serum Hormone Levels
Contract No. : N01-HD-8-2825
Contractor : Creighton University
Money Allocated: $22,564 (FY 78), $22,093 (FY 79)

Objectives: Under a previous contract (N01-HD-6-2823) numerous data were acquired relating menstrual cycle phenomena to the fertile period and presumptive ovulation time. The objective of the present contract is to analyze the data and to discover whether objective practical determinants of the fertile period can be derived from the data.

Major Findings: Graphic display of 73 cycles has been completed. Several signs of ovulation are correlated with the estimated time of ovulation in 23 subjects and 64 hormonally normal menstrual cycles. The data suggests that intermenstrual pain may not be due to any one specific cause but rather several related factors. As a sign of ovulation, intermenstrual pain was more specific than low backache, abdominal bloating and intermenstrual bleeding but, nonetheless, it had a broad periovulatory association. The most reproducible and predictable sign of those covered in this report appeared to be the postovulatory appearance of breast tenderness. In a limited study of autopalpation of the cervix, the broad association of this finding with ovulation is confirmed but a lack of precision in its relation to ovulation is identified.

The correlation of the estimated time of ovulation (ETO) with the day of defined postovulatory infertility has been carried out for each of 15 different Natural Family Planning methodologies. Inherent weaknesses were identified in methods based upon calendar calculations or basal body temperatures only. These weaknesses could be removed for the BBT-only methods if symptoms, especially the Peak mucus symptom, were added to the BBT records. However, the Peak mucus symptom alone had the greatest precision of all methods studied. No advantage could be identified in combining the BBT with the Peak symptom. To do so increases the number of days in which genital contact must be avoided without improving the ability of the Peak symptom to identify post-ovulation infertility.

Significance to Biomedical Research and Program of the Institute: Utilization of the mucus cycle as a method for fertility regulation is a subject of current investigations. Research relating the mucus cycle to hormonal status is directly related to investigations supported by the Contraceptive Development Branch.

Proposed Course: Project was completed in this fiscal year.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Pharmacokinetics of Testosterone Enanthate in the Human Male
Contract No. : NO1-HD-8-2830
Contractor : Professional Staff Association of Los Angeles
Money Allocated: $66,875 (FY 78), $58,283 (FY 79)

Objectives: Testosterone enanthate, a 7 carbon ester at the C17 position of the testosterone molecule, has been extensively utilized in investigations as a male contraceptive agent. Although it has been considered to be a long acting androgen preparation, inadequate information was available on the kinetics of its delivery and disappearance. Contract No. 1-HD-8-2830 was directed toward this end.

Major Findings: Blood samples from 30 normal male volunteers were collected on a schedule designed to maximize the information content of the data, yet minimize the amount of blood drawn. Using celite chromatography, labeled and cold steroids present in serum samples were separated into four steroid fractions: TE*, DHT*, T* and E_2*. Radiolabeled and unlabeled steroid concentrations were corrected for recovery and quantified. The data obtained were used to develop a linear 2 and 3 pool model model for T and TE kinetics after intravenous T and TE injections. A nonlinear 2-3 compartmental, 2-3 pool model was designed to depict the kinetics of the intramuscular injection of TE*. The model structure suggests that injected TE diffuses out of the TE-sesame oil suspension and enters a small intramuscular TE pool, from which TE has three possible fates: 1) de-esterification of the enanthate side chain to form T and transport into the circulation; 2) transport from the muscle and into the plasma as TE; or 3) irreversible metabolism (or degradation) in the tissue. Further, the model parameters indicate that the ratio of these three processes is 3:1:0.01. TE conversion to T mainly occurs at or near the injection site, although additional conversion of TE to T occurs after entry of TE into the plasma pool. Circulating T concentrations predominately reflect the absorption into the plasma of T derived from TE.

A similar model was developed for the kinetics observed following the i.m. injection of TE* + 200 mg TE. The effects of the co-injection of 200 mg unlabeled TE on the absorption and metabolism of labeled TE* were as follows: 1) a decreased rate of diffusion of labeled TE out of the sesame oil; 2) an inhibition of the absorption rate of labeled T derived from labeled TE; 3) an increase in the absorption rate of labeled TE from the muscle pool. These alterations in the absorption subsystem account for inhibition of the computed absorption rate for T and the experimentally observed decrease of T and slight increase of TE plasma radioactivity. The ratio of computed absorption rates for TE/T increased from 0.3 for the i.m. tracer alone to 1.7 for 1.m. tracer + unlabeled TE. This finding further suggests that unlabeled TE shifts the processing of TE toward direct absorption. This result is consistent with saturation of the side chain cleavage enzyme system by unlabeled TE in the muscle pool.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new male methods of contraception.

Proposed Course: Completed.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Contraceptives

Contract No. : N01-HD-8-2856
Contractor : Cedars-Sinai Medical Center
Money Allocated: $124,338 (FY 78), $132,069 (FY 79), $60,741 (FY 80)

Objectives: The objective of the proposed study project is to determine the use-effectiveness and the acceptability of an intravaginal contraceptive, Encare Oval. The use-effectiveness and acceptability rates of the Encare Oval will be compared to those of a control group using the diaphragm with spermicidal gel or cream as a contraceptive method.

Major Findings : The final phase of the Barrier Method Contraceptive Study will be completed in fiscal year 1980-81.

During this year, the 18 months of enrollment was completed. 630 Barrier Method users and 280 Encare users were enrolled. The six-month follow up period ended in January, 1981. The clinic enrollment sites were closed and data collection completed. Presently, data analysis is 95% completed and the final report is in preparation.

Some of the pertinent findings of the study are:

1. The overall survival of both groups does not differ greatly.

2. The probability of pregnancy within 12 months is not discernably different from one group to the other.

3. Encare users are more likely to terminate for medical reasons than are diaphragm users.

4. The probability of termination for personal reasons is the same for both groups.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: Studies should be completed by the end of the contract period.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Investigation of a Polyurethane Contraceptive Vaginal Sponge Containing a Spermicidal Agent
Contract No. : N01-HD-8-2857
Contractor : University of Southern California School of Medicine
Money Allocated: $31,081 (FY 78); $11,829 (FY 79)

Objectives: The purpose of this project is to evaluate the ability of a polyurethane contraceptive vaginal sponge containing nonyl-9 as a spermicidal agent to prevent the penetration of spermatozoa into and the migration of sperm through human cervical mucosa after seven days use will also be determined.

Major Findings: Studies indicate that the medicated sponge is more effective than the non-medicated sponge in inhibiting sperm transport, but not as effective as Delfon foam, except when the sponge has been left in place 24 to 48 hours prior to coitus. In terms of fertility potential, there is less difference between the medicated sponge and the aerosal foam.

Extensive clinical laboratory work performed on each subject (chemistry, CBC and urinalysis) showed no significant changes after using either a medicated or non-medicated sponge.

Smears for cytology were taken from the exocervix and the vaginal walls adjacent to the cervix before the sponge was inserted and after it was removed. Conversions from a Class I to a Class II smear occurred in three subjects involving four wear cycles. In each case the cytology reverted to Class I when smears were repeated.

Sponges worn for seven days (but no single sponge left in place for longer than 48 hours) caused symptoms of irritation in half of the subjects. All symptons resolved upon removal of the sponge.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: These studies have been completed this year, but final analysis of the data is forthcoming.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Primate Testing Facility
Contract No. : NO1-HD-9-2838
Contractor : Hazleton Laboratories America, Inc.
Money Allocated: $906,286 (FY 79) 3 years

Objectives: The objective of this contract program is the establishment of a primate testing facility for evaluation of the clinical potential of new contraceptive drugs, formulations and delivery systems. The present colony consists of 120 female and 31 male Cynomolgus monkeys (Macaca fascicularis). Initially they will be used to study potent analogs of LHRH and prostaglandins. The data derived from studies at this facility will determine, to a large degree, whether the institution of clinical trials is warranted.

Major Findings: Two studies have been completed demonstrating the ability of various regimens of human chorionic gonadotrophin to prolong the functional integrity of the corpus luteum.

A study to evaluate local irritation following subcutaneous implantation of several types of drug delivery systems has been completed. Analysis of results including circulating levels of the drugs involved is in progress.

A superagonist of luteinizing hormone releasing hormone (LHRH) appears to cause suppression of testosterone levels and sperm production in some animals following treatment with 10 or 25 micrograms/kg/day for 4 weeks.

A colony of very old ovariectomized rhesus monkeys has been transferred to the contractor's laboratories for evaluation of continued usefulness in the estrogen withdrawal bleeding test.

Significance to Biomedical Research and Program of the Institute: Evaluation in subhuman primates is absolutely essential to establish the clinical potential of new contraceptive modalities before undertaking the extensive and costly safety studies required for clinical trials. Additionally, certain drugs appear to exhibit substantially different pharmacological properties in primates and in small laboratory animals thus limiting the utility of the latter. The ability of the CDB to evaluate compounds in subhuman primates is of great importance to our contract synthetic program and the stated goals of the Branch.

Proposed Course: This is expected to be a continuing contractural effort leading to the development of new contraceptive modalities and is an integral part of the contraceptive development program.

Contract Title : Development of Techniques for the Prediction and Detection of Ovulation in Humans
Contract No. : NO1-HD-9-2841
Contractor : Northwestern University Medical School
Money Allocated: $40,290 (FY 79)

Objectives: The proposed study is designed to develop a simple semi-quantitative detection system for urinary pregnanediol-3-glucuronide to be used by women at home for detecting the time of ovulation. The test would employ the specific and semi-quantitative visualization on a "dip stick" of rhodamine-labeled antibody following the addition of excess rhodamine-labeled anti-body to the urine sample.

Major Findings: Antiserum to pregnanediol glucuronide (APD) was purified by DEAE cellulose chromatography; the IgG fraction was tested for purity, specificity, and total binding activity. Colored derivatives of both purified APD and a steroid have been conjugated to glass fiber paper through 3-aminopropyltriethoxysilane for the solid-phase separation component of two different visual assays. The most sensitive visual assay system is composed of an analog of pregnanediol (20α-hydroxy-4-pregnen-3-one) conjugated to the glass fiber paper; after mixing 0.1 ml of urine with a measured amount of APD, the uptake of APD not already combined with pregnanediol in the urine is taken up by the steroid on the solid phase. This APD is detected as it rapidly dissociates from the paper in a second solution of acidic Coomassie blue; the intensity of the blue color that develops in solution is proportional to the amount of APD taken up which, in turn, is inversely proportional to the concentration of pregnanediol glucuronide in urine.

The reliability of assessing ovulation by measurements of pregnanediol glucuronide in morning urine specimens was investigated using a radioimmunoassay developed from the same antiserum as was used for the work described above. Basal body temperature and urine specimens were collected daily for 2 months by 26 volunteers. In half of the women blood samples obtained for 10 consecutive days starting on the tenth day of the cycle were also analyzed for progesterone. Excellent agreement between estimates of ovulation from urinary pregnanediol measurements, BBT, and plasma progesterone was obtained.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the the new female methods of contraception.

Proposed Course: Termination.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract No. : NO1-HD-9-2842
Contractor : University of California
Money Allocated: $142,863 (FY 79), $149,860 (FY 80)

Objectives: To investigate the clinical pharmacology of D-Trp^6-, Pro^{9}Net-LRF in normal women.

Major Findings: To date, data show that 1) the luteolytic effect of the LRF-ag-[(D-Trp^6,PRO^9, NEt)-LRF] following one or two sc injections (50 μg dose) is reproducible and reversible, and a window of maximal sensitivity occurs on days 5 to 8 of the luteal phase (n=43 cycles); 2) induction of anovulation and amenorrhea has been achieved by continuous daily sc administration (2.5 μg dose) for 90 days in 5 of 6 subjects studied. Pituitary gonadotropin desensitization appears to have occurred and normal follicular phase of E_2 is maintained (50 pg/ml). Return of normal menses occurred at 5.8 ± 1.1 weeks; 3) successful induction of short luteal phase (from 14 to 9 days) was achieved by the administration of 50 μg sc dose of LRF-ag during early follicular phase (D1-3) in 5 normal cycling women. The cycle length was prolonged, due entirely to the longer follicular phase. A significant lower FSH level was found in this mode of treatment, and in all probability, accounts for the genesis of short luteal phase; and 4) we found intravaginal application of 500 μg dose of LRF-agonist a satisfactory alternative to sc administration as judged by the magnitude and duration of gonadotropin-ovarian steroid changes.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive drugs is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Majority of the studies should be completed in FY 1981.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract No. : NO1-HD-9-2843
Contractor : Vanderbilt University
Money Allocated: $152,975 (FY 79), $146,343 (FY 80)

Objectives: To investigate the potential of D-Trp^6-Pro^9-Net-LRF as an antifertility drug in men.

Major Findings: The effects on pituitary-gonadal function of the potent gonadotropin-releasing hormone agonist D-Trp^6-Pro^9-N-Ethylamide-LHRH (LRF_A) have been evaluated in normal men who received 50 µg of the analog subcutaneously either daily or every fourth day for up to 10 weeks. Daily treatment with LRF_A resulted in a substantial decrease in plasma testosterone to 1.3 ng/ml or less by the fourth treatment week in all of 8 subjects. Plasma 17-hydroxyprogesterone and serum estradiol levels decreased concordant with plasma testosterone. Five men developed impotence between the sixth and seventh treatment weeks, with resolution in each case within 2 weeks of discontinuation of LRF_A. Serum gonadotropin levels also fell during the treatment period, rebounding briefly to levels exceeding basal when therapy was stopped. Sperm density and motility fell dramatically in each subject, and sperm density fell to 6 x 10^6/ml or less in six subjects. In each, sperm density recovered within 10-14 weeks of cessation of treatment. Mean plasma testosterone levels remained at 3.1 ng/ml or above during every fourth day administration of LRF_A. Mean sperm density fell to a nadir of 40 x 10^6/ml during treatment, but no consistent pattern was observed in any subject, with values varying between 4 x 10^6/ml and 368 x 10^6/ml. During every fourth day treatment, an agonist effect on LH, FSH, testosterone and estradiol was observed following each LRF_A injection.

The results of daily treatment are consistent with LRF_A-induced pituitary "desensitization," but do not exclude a direct inhibitory effect of LRF_A on testicular steroidogenesis and spermatogenesis. Treatment every fourth day with LRF_A does not appear to be a promising regimen to induce consistent suppression of the pituitary-gonadal axis in man.

Significance to Biomedical Research and Program of the Institute: Development of antifertility drugs for males is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Initial projection called for completion of these studies in FY 1981. However, delays in obtaining of the IND exemption may extend these studies to FY 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract No. : NO1-HD-0-2800
Contractor : Laval University
Money Allocated: $280,300 (FY 80), $427,615 (FY 81)

Objectives: The objective of this contract is to investigate the potential of intranasal administration of D-Ser(TBU)^{6}-desGly-NH_2^{10}-LHRH-EA as an antiovulatory and luteolytic drug in women.

Major Findings: Animal studies have clearly shown that the potency of LHRH agonists as antifertility agents is proportional to their gonadotropin-releasing activity. It is thus expected that the optimal dose of LHRH agonists for induction of luteolysis in women will be the one which leads to a maximal stimulation of serum gonadotropin levels. A dose-response study of the potent LHRH agonist [D-Ser(TBU)^{6},des-Gly-NH_2^{10}] LHRH ethylamide (Buserelin) administered by nasal spray was thus performed in the mid-luteal phase in normal women. When a single dose (50, 100, 200, 500, 1000 or 1500 μg) of Buserelin was administered intranasally, a near maximal stimulation of plasma LH and FSH levels was observed at 4 h. With the highest doses used, serum LH levels were still elevated at 24 h. A transient 2-3 fold elevation of serum progesterone and 17β-extradiol concentration was also observed on the day of Buserelin administration. At doses of 500 μg or higher administered on day 6 after the LH peak, single intranasal administration of the LHRH agonist led to an early fall of serum progesterone and estradiol concentration as well as early menstruation, thus indicating luteolysis. No secondary effect was observed and control post-treatment menstrual cycles were normal. These studies demonstrate that near maximal stimulation of gonadotropin secretion is observed after administration of a 200 μg dose. While the present data show a luteolytic effect at mid luteal phase, the subsequent study will determine the dose and schedule of administration of the peptide leading to similar luteolytic effects at less sensitive periods in the menstrual cycle.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive drugs is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Research should be completed in FY 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Clinical Pharmacology of LH/FSH-RH Analog
Contract No. : N01-HD-0-2811
Contractor : Syntex Research
Money Allocated: $778,226 (FY 80), $1,152,437 (FY 81)

Objectives: The objectives of this contract are to assess: 1) safety of the Syntex agonist; 2) to ascertain its potential for fertility regulation in men and women.

Major Findings: D-Nal(2)^{6}-LHRH is an analog of lutenizing hormone releasing hormone prepared by substitution of the glycine at position 6 with the unnatural, novel amino acid 3-(2-napthyl)-D-alamine. Adequate quantities of this compound have been synthesized for projected studies and a satisfactory injectable formulation has been made. Toxicological and pharmacological studies in support of an IND have been completed. The toxicity studies have failed to reveal significant findings beyond focal testicular infarctions in rats which were not observed in monkeys or dogs. Pharmacological studies have supported and extended earlier work showing suppression of steroidogenic and gametogenic activitites in treated animals. Separate IND's were filed for male and female studies on November 6, 1980.

Clinical trials are ready to begin at two sites, males at Harbor General Hospital (UCLA) and females at UCSF Medical Center. Phase I studies in males will characterize the effects of short-term dose and frequency regimens of the analog on gonadotrophin and androgen production. Safety parameters will be evaluated simultaneously. Testosterone supplementation will be evaluated secondarily and spermatogenesis in Phase II. During Phase I in women, dose-response curves will be established for the analog during various phases of the menstrual cycle; half-life of the analog will be established by RIA methods currently being developed; luteolytic effects will be evaluated as will the impact of HCG treatment; antiovulatory action will be assessed. Phase II trials will further evaluate luteolytic and anti-ovulatory activities.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive drugs is one of the major goals of the Contraceptive Development Branch.

Proposed Course: Research should be completed in FY 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Luteolytic Agents
Contract No. : N01-HD-0-2813
Contractor : Indiana University Foundation
Money Allocated: $82,074 (FY 80); $87,841 (FY 81)

Objectives: The objective of this project is to synthesize 12-fluoro-and 12-hydroxy-prostaglandin analogs related to natural $PGF_{2\alpha}$ which might prove to be better luteolytic agents.

Major Findings: The total syntheses of (+)-12-fluoro-13,14-dihydro$PGF_{2\alpha}$ methyl ester (1), (+)-15-epi-12-fluoro-13,14-dihydro$PGF_{2\alpha}$ methyl ester (2), (+)-12,18,19,20-heptafluoro-20-trifluoromethyl$PGF_{2\alpha}$ methyl ester (3), (+)-15-epi-12,18,19,20-heptafluoro-20-trifluoromethyl$PGF_{2\alpha}$ methyl ester (4), and (+)-17,18,19,20-tetranor-12-fluoro-16-m-trifluoromethylphenoxy$PGF_{2\alpha}$ methyl ester [(+)-12-fluoro-ICI 81,008 methyl ester] (5) have been completed. To date all samples with the exception of 3 and 4 have been evaluated for pregnancy interruption in the hamster and smooth muscle stimulating effects on gerbil colon and hamster uterine strips. An additional 475 mg of (+)-12-fluoro$PGF_{2\alpha}$ methyl ester (6) was synthesized for biological evaluation in the monkey; a total of 604 mg of 6 is now available.

Results with (+)-12-fluoro-13,14-dihydro$PGF_{2\alpha}$ methyl ester indicated that it was two times more potent than natural $PGF_{2\alpha}$ in the hamster antifertility assay. In contrast (+)-15-epi-12-fluoro-13,14-dihydro$PGF_{2\alpha}$ methyl ester was approximately one half as potent as $PGF_{2\alpha}$. These results are intriguing in view of the well known fact that the Δ^{13}-olefin and the C(15) hydroxyl group play an important role in the biological properties of prostaglandins. Both 1 and 2 were evaluated for smooth muscle (in vitro) stimulating effects on gerbil colon and hamster uterine strips. Analog 1 possessed 1.4-1.6% the potency of natural $PGF_{2\alpha}$ in the hamster assay and only 0.4% the potency of $PGF_{2\alpha}$ in the isolated gerbil colon assay. Compound 2 was even more encouraging, possessing only 0.5% the activity of $PGF_{2\alpha}$ in the hamster uterine strip assay and only 0.1% the activity of PGF_2 in the gerbil colon assay. Clearly the most dramatic effects observed with 1 and 2 were their substantially lowered smooth muscle stimulating properties relative to $PGF_{2\alpha}$. Neither 1 nor 2 were substrates for the placental 15-hydroxyprostaglandin dehydrogenase or inhibitors of the enzyme.

At a dose level of 0.125 µg, analog 5 is completely effective. Work is in progress to determine the MED. The data reveal that 5 is at least one hundred times more potent than $PGF_{2\alpha}$. Most disappointing was the finding that 5 possessed 500% the potency of $PGF_{2\alpha}$ in the hamster smooth muscle assay.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and related analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Postovulatory Prostanoid Antifertility Agents
Contract No. : N01-HD-0-2814
Contractor : University of Washington
Money Allocated: $64,692 (FY 80)

Objectives: The objective of this project is to synthesize bistrifluoromethylated prostaglandins having the CF_3 groups at C-16, 17 and 18 in order to test the effect of these trifluoromethyl groups on biological activity, particularly luteolytic and smooth muscle activity.

Major Findings: During the past year methods for the construction of prostaglandin analogs bearing trifluoromethyl groups at the following positions on the prostaglandin skeleton (C-15,16,17 and 18) have been developed. These positions were chosen for study since it was previously known that both methyl and fluorine substituents have beneficial effects on either potency or selectivity at these positions. The working hypothesis was that trifluoromethyl substitution would combine these beneficial effects.

At least one trifluoromethylated prostaglandin of each positional type for antifertility screening was submitted. Gem-disubstitution at C-16→C-18 reduced potency to such an extent (at least 20-fold) that any gains in selectivity associated with this structure variation would not be practical. In the case of substitution at C-15, initial tests indicate that substantial antifertility potency is retained.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandins and related analogs for contraceptive utility.

Proposed Course: This project will terminate since all of the workscope objectives have been accomplished.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Non-Steroidal Antifertility Agents
Contract No. : NO1-HD-0-2815
Contractor : Research Triangle Institute
Money Allocated: $93,071 (FY 80); $48,843 (FY 81)

Objectives: The goal of this contract is to synthesize simpler analogs of the 3, 6-dioxido derivative of Zoapatanol which has been shown to induce menses and to produce a significant reduction in blood progesterone. These compounds will be tested for antifertility activity.

Major Findings: During the present contract year a six-step sequence leading to the synthesis of 2α-methyl-2β-(4-carbomethoxypentyl)5α-hydroxymethyl-1,4-dioxane and 2β-methyl-2α-(4-carbomethoxypentyl)5α-hydroxymethyl-1,4-dioxane was developed. The carboxymethoxy group of the dioxane was hydrolyzed to the acid and the latter was reacted with γ,γ-dimethylallyllithium to give the corresponding 2α-methyl-2β-(4,8-dimethyl-5-oxo-7-nonenyl)5α-hydroxymethyl-1,4-dioxane. It was determined that the protection of the hydroxyl group in the side chain at C-5 as a tetrahydropyranyl ether and preparation of the lithium salt of the acid prior to its reaction with the organolithium reagent led to the ketone in better yields. The ketone function in the side chain at C-2 was protected as a ketal and the transformation of the hydroxymethyl group at C-2 to the corresponding carboxymethyl group was accomplished by a standard three-step sequence consisting of tosylation, displacement with cyanide and basic hydrolysis. Removal of the ketal function gave 2α-methyl-2β-(4,8-dimethyl-5-oxo-7-nonenyl)5α-carboxymethyl-1,4-dioxane.

The two compounds mentioned above at a dose of 50 or 75 mg/animal/day were administered sc to guinea pigs on days 12-14 or 22-24 of pregnancy to determine whether these analogs possessed luteolytic and/or postcoital antifertility activity. Fetuses were examined and counted 10 days after the final injection. Pre- and post-treatment progesterone levels were monitored to assess luteolytic activity. At the doses tested neither compound exhibited luteolytic or postcoital antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis of specific nonsteroidal compounds for evaluation for contraceptive utility.

Proposed Course: Termination in the absence of significant biological activity for the analogs of 3,6-dioxido derivatives of zoapatanol.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : New Antiprogestational Agents

Contract No. : N01-HD-0-2816
Contractor : SRI International
Money Allocated: $125,000 (FY 80); $135,000 (FY 81)

Objectives: The purpose of this projet is to synthesize the 19-nor-5α- dihydrosteroids related to 5α-dihydronortestosterone, 5α-dihydronorprogesterone, and steroids having the Ring-A inverted half-chair conformation to maximize receptor binding and test as antiprogestational agents. These compounds will be tested for antifertility activity.

Major Findings: Based on structure activity studies and computer generated model studies, ten steroidal compounds were selected for synthesis as potential antiprogestational agents useful in the regulation of female fertility. Three target compounds have been prepared during the first contract year; 11β-methoxy-pregn-4-ene-3, 20-dione, 5α-dihydro-19-norprogesterone and 17α-ethynyl-19-nor-5α-androst-1-en-17β-ol-3-one. The common intermediate 3-methoxy-17β-acetyl-1,3,5-estratrien-11-one-20-ethyleneketale has been prepared on a small scale. This key intermediate is now being used for the preparation of target compounds 11β-ethyl-4,9-diene-19-norprogesterone and 11β-methoxymethyl-4,9-diene-19-norprogesterone. The preparation of an additional amount of the key intermediate which is necessary for the preparation of required amount of these target compounds is in progress. Preparation of target compound, 2-methylene-17α-ethynyl-5α-19-noradrost-17-ol failed apparently due to dimerization. Preparation has also started on the preparation of 11β-chloro-pregn-4-ene-3,20-dione. The prepared target compounds have been or are in the process of being evaluated for binding affinity to the progestin, estrogen, and androgen cytosol receptor.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : C-18 Functionalized Steroids
Contract No. : NO1-HD-0-2817
Contractor : State University of New York at Stony Brook
Money Allocated: $86,968 (FY 80)

Objectives: The purpose of this project is to synthesize a series of antagonists for the progestins as possible antifertility agents. By transfering the C-17 substituents to the C-18 methyl group, the compounds are hoped to be still bound to the receptors but unable to effect the subsequent biological events. These compounds will be tested for the antifertility activity.

Major Findings: Cyclization of the ring-C seco compound with boron trifluoride etherate in bezene afforded 17-deoxy-13β-carbomethoxyestra-1,3,5,8,14 pentadiene 3-methyl ether in 50% overall yield. Conventional catalytic hydrogenation on this pentadiene followed by $LiAlH_4$ reduction gave 17-deoxy-13β-hydroxymethylestra-1,3,5,8(9)-tetraene 3-methyl ether in satisfactory yields. Reduction of the tetraene compound with Li in ammonia afforded the C-18 functionalized steroid lacking functionality at C-17. Birch reduction of this steroid, ketalization of 1,4-dihydro compound, and oxidation of 13-alcohol with pyridinium chlorochromate in CH_2Cl_2 produced the aldehyde in very high yield (>90%). Treatment of very unstable aldehyde immediately with acetylene magnesium bromide in ether followed by deketalization by methanolic hydrochloric acid gave one of the diastereoisomers. No stereochemical characterization of this compound has been carried out. No antiprogestational activity was shown when tested subcutaneously at a total dose of 50 mg against 0.8 mg of progesterone in the rabbit. (Anti-clauberg assay). An attempt to introduce the C-13 hydroxyacetone group from the above aldehyde by the action of 1-lithio-1-methoxyethene failed. The synthesis of the 18-acetylmethyl compound has proved somewhat more difficult than expected.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of Sulfur Analogs of Melatonin Derivatives
Contract No. : N01-HD-0-2818
Contractor : Indiana University
Money Allocated: $28,877 (FY 80); $28,477 (FY 81)

Objectives: Two sulfur analogs of 6-substituted melatonin will be synthesized as an antiovulatory agent useful as a contraceptive agent.

Major Findings: A systemic study of the decarboxylation of 5-hydroxy-6-chloro-3-methylbenzo[b]thiophene-2-carboxylic acid has been made, and the phenolic product characterized and derivatized. The radical bromination of esters of 5-hydroxy-6-chloro-3-methylbenzo[b]thiophene has been studied, in an effort to maximize monobromination. The crude bromomethyl ester has been converted in two steps to 5-methoxy-6-chloro-3-cyanomethylbenzo[b]thiophene, a precursor of the two target compounds, 6-chloro-5-methoxy-3β-acetamidoethylbenzo[b]thiophene and 6-fluoro-5-methoxy-3β-acetamidoethylbenzo[b]thiophene as an antiovulatory agent.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel heterocyclic compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Functionalized Polymers as Potential Contraceptive Agents
Contract No. : N01-HD-0-2819
Contractor : SRI International
Money Allocated: $59,645 (FY 80)

Objectives: The objective of this project is to synthesize water soluble functionalized polymers that can augment the barrier method of contraception. The hydrodynamic volume of the polymers would be such that the polymers would be non-absorbable across mucosal membranes and therefore systemically non-toxic. The polymers would consist of polymer conjugates of progestagens which would serve to inhibit spermatozoal action by interfering with plasma membrane function of the sperm.

Major Findings: Significant progress has been made on the synthesis of 7α-(5-carboxypentyl)norgestrel (1). The 5-carboxy-pentyl tether group will be used to conjugatively link the steroid to poly(vinyl amine vinyl sulfonate sodium salt). The 7α-stereochemistry of this group was selected to minimize tether and polymer backbone interactions with the progestogen receptor site to facilitate binding.

The starting material for this synthesis was norgestrel, which was converted to the corresponding 17β-acetoxy-4-en-3-one by de-ethynylation, reduction, selective oxidation, and acetylation. After conversion to the dienol ether, bromination-dehydrobromination was used to introduce the 6,7-double bond. A 1,6-conjugate addition and hydrolysis afforded the 7α-(6-hydroxyhexyl) steroid, the 3-keto group of which was protected as the ketal. Oxidation gave the 7α-(5-carboxylpentyl)-17-keto steroid. Introduction of the ethynylcarbinol group and deketalization should afford target compound (1), which will then be attached to the polymer through a carboxamide bond.

Significance to Biomedical Research and the Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new intravaginal contraceptives.

Proposed Course: Termination. By the end of the contract period all of the workscope objectives will have been completed.

Contract Title : Synthesis and Testing of Antiovulatory and Postovulatory Agents
Contract No. : N01-HD-0-2820
Contractor : Research Triangle Institute
Money Allocated: $149,060 (FY 80); $21,338 (FY 81)

Objectives: The goal of this contract is to synthesize analogs of 5α- and 5β-dihydronorethindrone which may be antiprogestational agents. These compounds will be tested for antifertility activity.

Major Findings: Eight compounds, 5α-dihydronorethindrone, a mixture of syn-and anti-isomers of 5α-dihydronorethindrone 3-oxime, 2α-methyl-5α-dihydronorethindrone, 2α-methyl-5β-dihydronorethindrone, 11β-methyl-5α-dihydronorethindrone, 7α-methyl-5α-dihydronorethindrone, 5α-dihydronorgestrel, and the 17α-vinyl analog of 5α-dihydronorgestrel, have been synthesized and tested for estrogen, progesterone, and androgen receptor binding (in vitro), estrogenic and androgenic activity and postcoital antifertility activity. 5α-Dihydronorethindrone, 5α-dihydronorethindrone 3-oxime, 7α-methyl-5α-dihydronorethindrone possessed significant estrogenic activity as shown by increased uterine weights and peroxidose activity in immature rat uteri, whereas 2α-methyl-5-dihydronorethindrone, 11β-methyl-5α-dihydronorethindrone, 5α-dihydronorgestrel and the 17α-vinyl analog of 5α-dihydronorgestrel had very weak estrogenic activity. 2α-Methyl-5β-dihydronorethindrone, which has a 5β-H configuration was devoid of estrogenic activity. 11β-Methyl-5α-dihydronorethindrone showed androgenic and anabolic potencies slightly greater than that of testosterone in the castrated immature male rat (Hershberger Assay), whereas 7α-methyl-5α-dihydronorethindrone and 5α-dihydronorgestrel showed minimal androgenicity. 5α-Dihydronorethindrone and its corresponding oxime were highly effective in the rat postcoital antifertility assay, perhaps owing to their estrcgenicity; 2α-Methyl-5α-dihydronorethindrone and 2α-methyl-5β-dihydronorethindrone were inactive in this assay. The synthesis of 3-thia-S-oxide and 3-aza-$\Delta^3(\Delta^2)$-N-oxide analogs will be completed within a few months and be tested for antiprogestational activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: The objectives of the contract were completed.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis of New Potent Oral Antigonadotropic Agents For Male Fertility
Contract No. : N01-HD-0-2828
Contractor : SRI International
Money Allocated: $119,048 (FY 80); $128,383 (FY 81)

Objectives: The goal of this contract is to synthesize new potent oral antigonadotropic androgenic agents for male fertility control. These compounds will be tested for the male contraceptive activity.

Major Findings: It was expected that several molecular modifications of the androstane and 19-norandrostane nuclei might yield new potent oral androgenic antigonadotropic agents for male fertility control. Previous studies to incorporate both potent androgenic and antigonadotropic activity into the same molecule have not, to our knowledge, been undertaken. The new male antifertility agents would act by suppressing pituitary release of the luteinizing hormone necessary for the intratesticular production of the testosterone required for spermatogenesis. The potent androgenic activity of these new agents would maintain libido and secondary sex characteristics.

During the first year of the contract the 6α-fluoro-16α-propyl-testosterone derivative and the 6β-fluoro epimer were synthesized and tested in subcutaneous Hershberger assay. These compounds were devoid of any androgenic activity. The synthesis of 16β 17β-dihydroxy-2-cyano-16α-propyl-estra-2-en, 16β, 17β-dihydroxy-2-methyl-16α-propyl-estra-2-en-3-one and 16-acetoxymethylene-$\Delta^{14, 16}$-D-homo-testosterone will be completed shortly and will be tested for biological assays. The other target compounds are scheduled for synthesis during year two of the contract.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Male Contraceptive Agents: Gossypol Derivatives
Contract No. : N01-HD-0-2829
Contractor : SISA
Money Allocated: $138,000 (FY 80); $175,000 (FY 81)

Objectives: The purpose of this project is to investigate the structure-antifertility activity relationship in gossypol. The ultimate goal is the discovery of an effective male contraceptive which does not have the toxic properties of gossypol.

Major Findings: A program of functional group modification has been outlined to accomplish the above goals. Five derivatives of gossypol have been prepared in quantities sufficient for testing in male hamsters. All of these are modified at the six hydroxyl groups (ether or esters) and at the carbonyl group. Twelve other compounds have been prepared in quantities sufficient for testing in an *in vitro* spermacidal assay. These vary in structure from simple imines of gossypol to intermediates in the preparation of hemigossypol. One of the latter compounds is equipotent to gossypol in the *in vitro* spermacidal assay. No *in vivo* tests have been completed. In preparing these derivatives, very valuable information about the chemistry and properties of gossypol and related compounds were obtained.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards the development of male contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Inhibitors of LHRH as Ovulation Inhibitors
Contract No. : N01-HD-0-2830
Contractor : University of Louisville Foundation
Money Allocated: $104,261 (FY 80); $46,714 (FY 81)

Objectives: The objective of this project is to synthesize LHRH analogs that contain modified peptide backbone structures by replacement of amide bonds with thiomethylene [CH_2S] and its sulfoxide [CH_2SO], alkylthiomethylene [CHRS] and its sulfoxide [$CHCH_3SO$] and aminomethylene [CH_2NH]. These amide bond replacements might provide LHRH analogs with increased half lives of circulation and increased lipophilicity while maintaining conformational features necessary for receptor interaction.

Major Findings: Peptides containing the amide bond surrogates ψ[$CHCH_3S$], ψ[$CHCH_3SO$] and ψ[$CHCH_3SO_2$] have been synthesized in addition to further tests with ψ[CH_2S]- and ψ[CH_2SO]-containing peptides. While not all of the above new peptides have yet been biologically evaluated, preliminary evidence indicates that the somewhat less flexible amide bond replacement ψ[$CHCH_3S$] may emerge as the best such replacement. *In vitro* tests demonstrate that N-terminal 1-2 modified antagonists are comparable in activity to their amide counterparts, and the most active *in vitro* compounds, [AcGlyψ[$CHCH_3S$]D-pClPhe$^{1-2}$,D-Trp^{3,6}]LHRH (2 diastereomers) have shown partial *in vivo* antiovulatory activity at the 250 μg/rat level. Most of the pseudopeptide analogues that have been prepared have been based on the highly potent parent compound, [Ac-Gly^1,D-pClPhe^2,D-Trp^{3,6}]LHRH. Other N-terminal modified antagonists *not* containing amide replacements have been prepared in the search for even more potent model structures, but none have yet been found to be potent as the AcGly^1 compound. The latter structure is also an ideal vehicle for introduction of additional amide bond replacements in view of its 1) high potency; 2) relatively accessible N-terminal position for ease of introducing modified 1-2 position replacements, and 3) lack of a chiral center at the 1-position, further simplifying the synthetic protocol for new glycine-based surrogates.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: Termination. By the end of the contract period all of the workscope objectives will have been accomplished.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : N01-HD-0-2831
Contractor : Tulane University School of Medicine
Money Allocated: $171,238 (FY 80), $183,225 (FY 81)

Objectives: The objective of this project is to prepare LHRH analogs modified in positions 1,2,3,5 and 10 to obtain more potent inhibitory activity. Fluorinated analogs of LHRH will also be examined. The analogs will be tested for in vivo and in vitro activity and receptor binding experiments will be conducted.

Major Findings: The most interesting and recent observation was that Ac-D-p-Cl-Phe^{1,2},D-Trp^{3},D-Lys^{6},D-Ala^{10}-LHRH retains very high anti-ovulatory activity (100%, 15 µg) despite the absence of the usually obligatory bulky position 6 aromatic side-chain. This analog has the advantage of being considerably more water soluble due to the extra amino group. It is possible that modifications at the N- and C- termini have made it no longer necessary to rely on aromatic D-amino acids in position 6 and a whole series of new analogs will be made to test this hypothesis.

In physiological studies, the long-acting nature of N-Ac-D-p-Cl-Phe^{1,2}, D-Trp^{3},D-Phe^{6},D-Ala^{10}-LHRH was assessed. A single injection of 15 or 150 µg of the antagonist in 40% propylene glycol/saline was given on different days of the estrous cycle. The 15 µg dose was effective in blocking ovulation only when given on proestrus (P) and then only for one day since the animals began ovulating on diestrus I (DI). Importantly, 150 µg of the antagonist inhibited ovulation if given two or three days before ovulation and blocked ovulation for three days if given just prior to the pre-ovulatory surge of LH. There were few or no changes in LH, FSH, or estradiol after antagonist treatment and progesterone levels were altered on the morning of sacrifice only when the antagonist was given on P. In spite of the fact that 15 µg caused a blockade of ovulation when injected on P, there was no difference in the number of pituitary binding sites for LHRH between the control and 15 µg groups on estrus (E) and DI. The injection of 150 µg induced an extended inhibition of ovulation and reduced the number of pituitary binding sites for LHRH. The affinity constants were similar for all three groups indicating that the antagonist did not alter receptor affinity. The results strongly suggest that a decrease in available pituitary LHRH receptors is associated with the prolonged inhibition of ovulation by the antagonist and may be one of the mechanisms by which the analog exerts long-term contraceptive action.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : NO1-HD-0-2832
Contractor : University of Colorado Health Sciences Center
Money Allocated: $176,746 (FY 80); $165,864 (FY 81)

Objectives: The objective of this project is to design and synthesize antagonists of LHRH modified in such a manner as to increase receptor binding and improve resistance to enzymatic degradation. The research will focus on modifications to [N-Ac-Ala^{1},D-p-Cl-Phe^{2},D-Trp^{3,6}]LHRH, one of the most potent analogs known. *In vivo* and *in vitro* assays will be performed.

Major Findings: During this work period 16 new analogs of LHRH have been synthesized and submitted for bioassay. All these proved to be inhibitors of LH release, although no large increases in potency were obtained over those of compounds previously synthesized. A major new direction in synthesis was incorporation of 2,3-unsaturated amino acids into LHRH inhibitor analogs. Since these are unstable as single amino acids, they are being incorporated as dipeptide derivatives; these are being synthesized by Dr. Charles Stammer, at the University of Georgia. Both the synthesis of these dipeptide derivatives and their incorporation into LHRH analogs have presented unexpected and major difficulties. Efforts to develop new methods of achieving these goals are continuing. A number of cyclic LHRH analogs, synthesized by incorporating two cysteine residues into the peptide, have significant inhibitory activity. It is not clear that these are acting at the receptor in the cyclic form, however.

A LHRH pituitary radioreceptor assay was performed using a synthetic agonist radiolabel, [D-Leu^{6}desGly NH$_2^{10}$]LHRH which was also the reference compound in this assay system. This compound is 15 times as potent as LHRH in releasing gonadotropins and has 10-fold greater affinity for the LHRH receptor than LHRH. Two antagonists ([Ac-Ala^{1},D-p-Cl-Phe^{2},D-Trp^{3,6}]LHRH and [Ac-DHP^{1},D-p-Cl-Phe^{2},D-Trp^{3,6}]LHRH) which block ovulation in the female rat completely at 25 micrograms had 3.4 and 5.5 times the binding affinity of the index compound in the radioreceptor assay. Compounds with less binding affinity than the index compound were invariably inactive at 25 micrograms in the antiovulatory assay. The dispersed pituitary cell assay results were calculated as ICR_{50}, the concentration of antagonist which would inhibit LH secretion *in vitro* by 50% in the presence of 10^{-9} molar LHRH. Those compounds active at 25 micrograms in the antiovulatory assay had an ICR_{50} of less than 2×10^{-10} molar. Compounds with an ICR_{50} greater than 10^{-9} molar were invariably inactive in the antiovulatory assay at 25 micrograms. There was an excellent correlation among the results obtained in the three biological assay systems used for the current series of compounds.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors
Contract No. : N01-HD-0-2833
Contractor : University of Texas at Austin
Money Allocated: $474,634 (FY 80)

Objectives: The objective of this project is to synthesize analogs of LHRH to produce antagonists with increased potency and duration of action. The most potent antagonist known to date will be modified extensively at position 1. This will be followed by a search for new flexible positions to be substituted to further increase potency. In vivo and in vitro testing will be conducted.

Major Findings: Based on designs for greater metabolic stability and higher potency of antagonists of the luteinizing hormone releasing hormone (LHRH), a new approach appeared from the synthesis and assay of a large number of new analogs. Introduction of an increasing number of D-amino acids resulted in the discovery that [N-Ac-Thr^1, D-Phe^2,D-Trp^3,D-Ser^4,D-Tyr^5,D-Trp^6,D-Arg^8]-LHRH has an advanced level of potency for a new structural type. Levels of 10 and 30 ng, in vitro, were inhibitory; a level of 25 µg in the antiovulatory assay was 71% effective. This analog may be more resistant to biological degradation and therefore could have improved oral activity. Azgly^{10}-analogs provide C-terminal protection and retain potency, exemplified by [N-Ac-Ala^1,DpClPhe^2,D-Trp^{3,6},Azgly^{10}]-LHRH which inhibited at 0.03 nM in vitro and in vivo by 50% at 6 µg, and by [N-Ac-D-Thr^1,DpClPhe^2,D-Trp^{3,6},Azgly^{10}]-LHRH which also inhibited ovulation by 50% at 6 µg. Other analogs based upon multi-D substitutions and position-10 protection have been synthesized and are under assay.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Peptide Antagonists of LHRH as Ovulation Inhibitors

Contract No. : N01-HD-0-2836
Contractor : Salk Institute
Money Allocated: $401,538 (FY 80)

Objectives: The objective of this project is to improve the potency of presently available LHRH antagonists by designing and synthesizing analogs based upon Hansch analysis as modified by Topliss. The premise is that improved potency of existing antagonists can be achieved if the predominant factors (hydrophobicity, electronic distribution or steric factors) affecting the antagonist's efficacy can be identified for loci which contain aromatic side chains.

Major Findings: Systematic modifications at positions one, two, three, five, six and seven were introducted. Emphasis was put on substitutions at the two positions where D-phenylalanine analogs were introduced. Those phenylalanine analogs (approximately 15 of them) designed following the guidelines of Topliss were introduced in the basic structure [Ac-dehydro Pro^1,X,$DTrp^{3,6}$]-LRF. Four peptides were obtained that were particularly potent: these had X = pCl-DPhe, pFDPhe, pNO_2-DPhe and 3,4-Cl_2 DPhe (total blockade of ovulation at 7.5 μg/rat). Other less active analogs gave an appreciation of the receptor requirements for high biological potency. Both (2-Naphthyl)DAla and β (1-Naphthyl)DAla were introduced at the 3,6 or 3 and 6 position of [Ac-dehydro-Pro^1pFDPhe^2,X^3,Y^6]-LRF where DTrp was the other amino acid in the mono (Naphthyl) substituted analogs. Those peptides were found to be 2 to 3 times more potent than earlier analogs and the disubstituted (Naphthyl) analog completely blocked ovulation at 2.5 μg/rat.

The most powerful antagonists *in vivo* are also potent in *in vitro* and radio-receptor assays, although there are differences in the rank orders of peptides in the two tests. Peptides such as [Ac$\Delta^3$$Pro^1$,$pNO_2$DPhe^2,$DTrp^{3,6}$]-LRF, which are more potent than the corresponding analog with pClDPhe^2 in the radio-receptor and *in vitro* tests but not in the *in vivo* assay, perhaps differ in their resistance to degradation.

[Ac-dehydro Pro^1,pFDPhe^2,$DTrp^{3,6}$]-LRF, one of the most potent analogs, was synthesized in gram quantities for *in vivo* studies and a master file was submitted to the FDA so that preliminary clinical evaluation could be carried out by clinicians with that compound.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Isolation and Purification of Rat Androgen Binding Protein
Contract No. : N01-HD-0-2845
Contractor : Population Council
Money Allocated: $106,671 (FY 80); $91,912 (FY 81); $48,460 (FY 82)

Objectives: To produce 100 radioimmunoassay (RIA) kits for the quantification of rat androgen binding protein (ABP). This goal is to be implemented by the purification of ABP and generation of antibodies against it, and development of an RIA using these reagents. The goals for the first year include the preparation of the immunogen, immunization of rabbits, collection of antisera and preparation of the tracer ABP.

Major Findings: The first quarter of the contract period was devoted to procurement of a large quantity of tissue for starting material and the preparation of the androgen affinity matrix. This was followed by the utilization of these materials to initiate the purification of sufficient ABP to be used as an immunogen. The procedure used involved, primarily, androgen affinity chromatography. Procedures for streamlining the isolation of ABP were investigated. By use of an optimal ratio of affinity resin to epididymal cytosol (which enhanced the effectiveness of the purification step) as well as the use of procedures which both concentrate and purify, the number of steps were reduced and the yield enhanced. The material was used to immunize rabbits. At present, high titers of ABP antibodies have been obtained from several rabbits. These antisera have been characterized with regard to titer and are currently being assessed for their specificity. To date, over 200 ml of what appears as good quality antisera have been accumulated. Given that this antibody is satisfactory in terms of specificity, there is more than enough to meet the contract obligation.

Large uniform batches of ABP are being prepared to be used as a radiolabelled standard for the RIA. Approximately 9 mg. of material are on hand. It is this preparation which is destined for distribution with the RIA kits and will be used in testing of ABP stability under shipping conditions.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new male methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Microcapsules Containing a Contraceptive Progestagen
Contract No. : NO1-HD-0-2847
Contractor : Biotek, Inc.
Money Allocated: $166,711 (FY 80); $109,228 (FY 81)

Objectives: The objective of this project is to develop and test an injectable microcapsule system for the delivery of the contraceptive synthetic progestagen, levonorgestrel. The drug will be encapsulated in a biodegradable polylactide polymer and the microcapsules will be spherical reservoirs which deliver the drug over a period of six months.

Major Findings: During the reporting period, three kilograms of the encapsulating polymer were synthesized. The reduced specific viscosity (R.S.V.) of this polymer which is an index of its molecular weight, was 0.93 $\pm$ 0.02 dl/g and meets the contract specification of 1.0 $\pm$ 0.2 dl/g. The polymer is now being fully characterized by determining its molecular weight distribution and the concentration of residual monomer, residual solvent, and leachable tin catalyst. The polymer is stored in special containers under argon gas at 0°C.

Because of the high cost of levonorgestrel, preliminary microencapsulation experiments were conducted with norethindrone as a model drug. This progestagen possesses physical, chemical and biological properties which are very similar to levonorgestrel. Another objective of the model studies is to prepare drug particles which flow easily and which do not break into smaller particles during coating in the Wurster air suspension coating chamber. Several model microencapsulation runs have been completed using a variety of starting norethindrone particles. The microcapsules were characterized by measuring their size distribution, assay of hormone content, physical morphology by scanning electron microscopy and rate of drug release release *in vitro*. The results indicate that microcapsules prepared using a composite starting core particle made from 90% drug and 10% polymer gave zero-order release of the hormone with little or no burst effect. Microcapsules of the 106-125 μm range have delivered between 4 to 5 μg of the drug per day per mg of encapsulated drug over a period of 3 months. At this rate the system is expected to deliver a constant dose of the drug for a total period of 6-8 months.

Microencapsulation of levonorgestrel using the techniques developed above has recently started.

In preparation for clinical studies operating procedures were established for meeting the requirements for good manufacturing practice (GMP).

Significance to Biomedical Research and the Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development of Hydrogel Materials as Vaginal Barrier Contraceptives
Contract No. : N01-HD-0-2854
Contractor : Southern Research Institute
Money Allocated: $546,338 for 3 years (FY 80)

Objectives: The objective of the project is the development of spermicide-releasing hydrogel materials for use as disposable (single use) vaginal barrier diaphragms.

Major Findings: During the reporting period commercially available latex vaginal diaphragms were evaluated for mechanical properties. The latex materials employed in diaphragms for long-term use had tensile strengths in the range of 1300-2800 psi, ultimate elongations of 600-800% and Shore A hardness values in the range of 45-75.

Hydrogel films for the disposable vaginal diaphragm were prepared. The use of monomers with trace amounts of impurities gave polymers with undesirable levels of crosslinking and resultant poor film mechanical properties. Purified monomers with known concentrations of crosslinking agent yielded polymers that exhibited improved mechanical properties when processed into films. In general, the fully hydrated polymer films had better properties than the dry or partially hydrated materials with HPMA polymers giving tensile strengths of 400-700 psi and ultimate elongation values of 60-247%.

Films of crosslinked nylon polymers had tensile strengths of 2100-3800 psi and ultimate elongations of about 250% when dry, but these values decreased significantly when the films were hydrated. Modifications in polymer molecular weight and degree of crosslinking are expected to improve the hydrated film properties.

Nonoxynol-9, a commercial spermicidal agent, was incorporated into films of both hydrogel materials by absorption, and preliminary diffusional parameters were determined. Optimization of release rates and film properties is currently being explored.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

Contract Title : Development and Testing of a New Cervical Cap and Inserter
Contract No. : NO1-HD-0-2855
Contractor : Brigham and Women's Hospital
Money Allocated: $428,649 (FY 80 for 2 years)

Objectives: The goal of this project is to develop an improved contraceptive cervical cap.

Major Findings: The first efforts have been aimed at determining the properties and dimensions of the cervix that are pertinent to cap design and how these might change under various circumstances. Initial findings are that both the surface of the cervix and the underlying stroma are far more mobile, compliant and changeable than anticipated or previously described. The nature of these changes and their relevance to cap design is discussed below.

A prime requirement of any cervical cap is to stay on the cervix under actual use conditions, especially during intercourse. The kinds of changes in shape and size of the cervix that have been documented would make this requirement seem nearly impossible to fulfill. Yet there are both soft and firm prefabricated caps that now exist and are known to stay in place in a significant, if unacceptably small, proportion of users. Furthermore, there is recent evidence that soft custom-made contact caps can stay in place over long periods of time. Thus, it is clear that achieving stability is not impossible. The mechanical forces and interactions between cervix and cap that make this stability possible include the following:

1. Gas pressure differences (suction or potential suction)
2. Hydraulic flow (of spermicidal creams and jellies)
3. Mechanical grasping or forced compliance ("dog collar" effect)
4. Surface tension phenomenon or capillary attraction (contact lens effect)

So far it appears that all of the above forces may play a role with different types of caps depending predominantly on different stabilizing forces. Mechanical grasping seems to be more important in the cavity rim caps than originally supposed.

Significance to Biomedical Research Program of the Institute: Development of new contraceptive technology is a stated goal of the Contraceptive Development Branch.

Proposed Course: This project is expected to be a continuing contractual effort leading to the development of improved contraceptive technology.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Isolation, Characterization and Synthesis of Gonadocrinin
Contract No. : N01-HD-0-2860
Contractor : The Salk Institute
Money Allocated: $402,719 (FY 80)

Objectives: A new polypeptide of ovarian origin that possesses the characteristics of LHRH, although of different chemical structure, has been discovered. It very well may represent an internal regulator of ovarian function, as well as participating in the overall hypothalamic-pituitary-ovarian interrelation. The objective of the proposal is to isolate, purify and characterize the LRF-like material, gonadocrinin, from rat ovaries, establish the primary molecular structure of the substance, reproduce it by total synthesis, develop a RIA for the peptide, and demonstrate that the native and synthetic materials are biologically active. RIA reagents will be provided to NIH for distribution to other investigators.

Major Findings: During the past six months, 10,000 immature rats have been treated with pregnant mare's serum gonadotropin and primed ovaries were collected in liquid nitrogen and stored in deep freezer. When conventional methods were used to extract gonadocrinin from these ovaries, however, very little activity other than gonadostatin activities were extracted. Further, pilot purification of ovarian gonadocrinin from randomly selected batches of these ovaries showed far less gonadocrinin activity than expected. Subsequently, all batches of ovaries as well as ovaries from other sources have been screened. Gonadocrinin activity was found to vary considerably from batch to batch, and even be totally absent in some. Even in the batches showing gonadocrinin activity, these ovaries showed far less gonadocrinin activity than earlier experiments in which a fresh preparation of PMSG was used. Different sources of PMSG preparations will be used to test this possibility.

Since other investigators have observed similar LRF-like factor in the male rats which can be detected by an antiserum against an LRF agonist, D-Ser $(TBu)^6$LRF-EA, but not read by antiserum against synthetic LRF, samples of gonadocrinin were sent for assaying in the D-Ser $(TBU)^6$ LRFEA-RIA. The gonadocrinin showed little cross-reaction with this antiserum, but showed 4 and 20% crossreactivity with two antisera to LRF. This may prove to be an efficient method to separate gonadocrinin from rat ovarian extract by affinity chromatography.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive drugs.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Contraceptives
Contract No. : N01-HD-1-2800
Contractor : International Fertility Research Program
Money Allocated: $220,534 (FY 80), $330,881 (FY 81), $91,124 (FY 82)

Objectives: It is proposed to carry out a multi-clinic Phase III clinical trial of a newly developed non-prescription vaginal contraceptive method, the Secure Sponge, in comparison with the diaphragm with spermicide. The incidence of adverse side effects and complaints associated with the use of the Secure sponge compared with the diaphragm will be evaluated. Further, the rates of user compliance for the Secure sponge will be determined. The sponge affords several significant advantages over the diaphragm. First, a single size can be used by all women; second, it is intended to be a non-prescription contraceptive; third, it is less messy than other contraceptives and can be inserted up to 24 hours in advance of coitus.

Major Findings: As of 30 April 1981, 365 women had been recruited into the comparative evaluation of the Secure and diaphragm being conducted in 14 clinics. No pregnancies have been reported for women using either contraceptive method. During the first three months of the contract, the protocol and forms were finalized and subcontracts negotiated with the individual clinics. The clinical trial was initiated in late February. It is too early to report any definitive results. All 1600 women are expected to be recruited by 1 March 1982.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Development and Testing of Vaginal Barrier Contraceptives

Contract No. : N01-HD-1-2801
Contractor : International Fertility Research Program
Money Allocated: $171,333 (FY 81 for 18 months)

Objectives: A custom-fit cervical cap with a one-way valve which allows discharge from the cervix will be tested for up to six months in 300 women in order to determine preliminary estimates of safety, efficacy and acceptability.

Major Findings: This project has just begun and, therefore, there are no results to report. A brief description of the work to be done is included here for informational purposes. The cervical cap to be evaluated is known as the Contracap and is designed for continuous wearing. It is individually fitted to the wearer by first making an alginate impression of the cervix uteri and then forming a plaster cast. The synthetic elastomer Kraton is then formed into a cap which has a thin membrane covering an exit orifice. The making of the impressions and ultimately the manufacture of the cap is facilitated by the availability of kits from the developer.

The caps will be evaluated at three centers, each of which is expected to recruit 100 women who will use the device for up to six months. The trial is expected to take 18 months to complete and will evaluate ease of fitting, use and removal of the caps, as well as their safety and efficacy.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive methods is a stated goal of the Contraceptive Development Branch.

Proposed Course: This project should provide sufficient data to interest the private sector in further testing of this device.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Use-Effectiveness Study of Cervical Caps
Contract No. : N01-HD-1-2804
Contractor : Los Angeles Regional Family Planning Council, Inc.
Money Allocated: $477,303 (FY 81); $650,385 (FY 82); $599,267 (FY 83); $489,040 (FY 84)

Objectives: The purpose of the proposed research is to evaluate the contraceptive effectiveness of the cervical cap, its side effects, and how well it will be accepted by different segments of the population. This will be a multi-center, prospective clinical study in which the cap will be compared to the vaginal diaphragm. It will be conducted under the auspices of a group of investigators associated with the Los Angeles Regional Family Planning Council, which will coordinate and monitor the project as well as provide for the analysis and interpretation of the data collected. The data should be sufficient to predict whether or not the cap will be a useful addition of the contraceptive methods presently in use throughout the country.

Major Findings: None - contract just negotiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of the new female methods of contraception.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

Contract Title : Synthesis and Testing of Novel Steroids as Antiprogestational Agents
Contract No. : N01-HD-1-2807
Contractor : Research Triangle Institute
Money Allocated: $114,554 (FY 81); $152,039 (FY 82)

Objectives: The purpose of this project is to synthesize retrotestosterone derivatives and 16-ethynyl derivatives of testosterone as antiprogestational agents. These compounds will be tested for antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

Contract Title : Synthesis and Testing of Antiprogestational Agents
Contract No. : N01-HD-1-2808
Contractor : Research Triangle Institute
Money Allocated: $107,421 (FY 81)

Objectives: The purpose of this project is to synthesize progesterone derivatives such as 17α-substituted 19-nor-5α-pregnane-3, 20-dione, 17α-substituted-5α-pregnane-dione, 17α-substituted 19-norprogesterone and 17α-substituted progesterone. The structural features exhibited by these compounds may be expected to impart affinity for uterine progesterone receptor sites, and to possess antiprogestational activity. These compounds will be tested for antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

Contract Title : Synthesis and Testing of Antiprogestational Agents
Contract No. : N01-HD-1-2809
Contractor : SRI International
Money Allocated: $164,747 (FY 81)

Objectives: The objective of this project is to synthesize 15,16-seco analogs possessing a ring A inverted half chain form that promotes optimal receptor binding in accord with a ring A-initiated binding model and/or that are conformationally similar to 5α-dihydro-norethindrone, a reported *in vivo* antiprogestational agent.

Major Findings: This project has only recently begun and there are no results to report at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new contraceptive agents.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthesis and Testing of Antiprogestational Agents
Contract No. : N01-HD-1-2810
Contractor : The Research Foundation of the State University of New York
Money Allocated: $55,698 (FY 81); $22,465 (FY 82)

Objectives: The purpose of this project is to synthesize tetracyclic compounds possessing a displaced C ring steroids (when compared to natural steroids) to maximize binding to the progesterone receptor as well as to maintain the compound's stereochemical integrity of the major functions of the B and C rings of natural steroids. These compounds will be tested for antiprogestational activity as well as for antifertility activity.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Synthetic Chemical Facility
Contract No. : N01-HD-1-2811
Contractor : Southwest Foundation for Research and Education
Money Allocated: $240,716 (FY 81); $259,973 (FY 82); $280,770 (FY 83); $303,230 (FY 84); $327,490 (FY 85)

Objectives: The purpose of this project is to have this synthetic chemical facility synthesize rapidly laboratory scale as well as larger quantities of specific compounds required by the Contraceptive Development Branch for contraceptive investigation.

Major Findings: None. The project has just been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis of specific steroidal and nonsteroidal compounds for evaluation for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Center for Population Research

The Contraceptive Evaluation Branch is responsible for a comprehensive research contracts program on the evaluation of the safety and effectiveness of methods of contraception and fertility regulation which are in use. Major program components deal with hormonal contraceptives, intrauterine devices, male sterilization and effects of prior induced abortion on subsequent reproduction.

Steroid Contraceptives and Cardiovascular Disease

A large case-control study of the relationship between oral contraceptive use and the occurrence of myocardial infarction has been completed. This study has confirmed previous reports that the overall risk of myocardial infarction for current oral contraceptive users, relative to the risk for non-users, is about 3-4. For women who both smoke cigarettes heavily and are current oral contraceptive users, relative to women who neither smoke nor are current oral contraceptive users, the risk estimate is 39.

A major new finding from this study is that long-term past oral contraceptive use was found to increase the risk of myocardial infarction: for women 40-49 years of age, 5 or more years of past oral contraceptive use appears to increase the risk of myocardial infarction about 2-fold (relative to the risk among non-users). This finding supports the concept that some oral contraceptive formulations may accelerate the process of atherogenesis, possibly by altering lipoprotein patterns and carbohydrate metabolism. To investigate this issue further, a prospective study of the effects of three currently marketed oral contraceptive formulations on lipoprotein patterns, glucose tolerance, and insulin secretion in women has recently been initiated.

Steroid Contraceptives and Cancer

After a 2-year development phase, a comprehensive case-control study of the relationship between oral contraceptive use and the occurrence of breast, endometrial, and ovarian cancer is now in progress. Data collection began in December 1980, and it is anticipated that over the next two years 6000 incident cases of breast cancer, 1500 of endometrial cancer, and 900 of ovarian cancer in women less than 55 years of age will be compared to 6000 controls. The cases are being ascertained from a network of U.S. Cancer Registry; the controls are being identified by random digit dialing. The large size of this study will provide for rigorous assessment of the overall effect of oral contraceptive use on the occurrence of breast, endometrial and ovarian cancer, and for evaluation of possible interactions between oral contraceptive use and other risk factors for these forms of cancer (e.g. family history).

Intrauterine Devices

A multi-center case control study of the relationship between the use of intrauterine devices and the occurrence of serious gynecologic and obstetric disorders has been completed. This study has: 1) confirmed that the use of

intrauterine devices increases the risk of pelvic inflammatory disease, but also shown that the use of oral contraceptives and barrier methods of contraception (diaphragm and condom) decrease the risk of pelvic inflammatory disease, 2) confirmed that women with an intrauterine device in place at the beginning of the second trimester of pregnancy are at increased risk of septic fetal loss, 3) provided evidence that the use of intrauterine devices was not a major factor in the tripling of ectopic pregnancies that occurred in the United States during the years 1965-1977, 4) provided evidence that the use of intrauterine devices does not in general increase the risk of vaginal hemorrhage that is serious enough to lead to hospitalization, 5) shown that the risk of uterine perforation following intrauterine device insertion is greatest for post-partum insertion in women who are lactating, 6) shown that the use of intrauterine devices does not increase the risk of abruptio placenta or placenta previa.

Two case-control studies designed to evaluate the relationship between birth control practices and the occurrence of subsequent undesired infertility are now underway. In these studies, women identified as having female-factor infertility are being compared to women from infertile couples whose infertility is due to male factors, and to recently delivered women. The cases (women with female-factor infertility) and controls are interviewed to obtain information concerning birth control practices and other relevant data. Analysis will focus upon estimating the risk of female-factor infertility in women who have used intrauterine devices, undergone induced abortion, and so forth relative to the risk for women who have not used these methods of birth control.

Male Sterilization

A glycolipid antigen has been identified from guinea pig sperm and its chemical structure, made up of carbohydrate and lipid, has important implications. Similar sperm antigens apparently occur in other species, including man, and the glycolipid structure offers an important new potential mechanism for the development of atherosclerosis after vasectomy.

The cohort study of vasectomized males continued to make satisfactory progress with data collection expected to be complete in 1 to 1-1/2 years. Three additional epidemiologic studies were funded to evaluate the possible risk of accelerated atherosclerosis after vasectomy, as suggested by published monkey studies. One will re-examine an established data file of coronary angiographs in relation to vasectomy history. A second will compare vasectomy history in men with coronary symptoms or with documented heart attacks with healthy control subjects. The final study is a classic case-control study of men under 55 with first myocardial infarction in 75 hospitals.

Effects of Induced Abortion on Subsequent Pregnancies

Studies of the effect of induced abortion on subsequent reproductive function pregnancy outcome continue to provide generally reassuring information. In one recently completed study of 520 women who underwent one or more induced abortions prior to their first live birth, the risk of adverse pregnancy outcomes (low birth weight, gestation less than 37 weeks, spontaneous mid-trimester fetal loss, reduced Apgar score, pre-clampsia, premature rupture of membranes, or late bleeding) was found to be no greater than among women with no previous pregnancies.

The findings from this study suggest that termination of one or more pregnancies by induced abortion prior to actually giving birth has the effect of delaying the risks of adverse pregnancy outcome that are normally associated with first pregnancies.

In a second study that previously reported women with a history of induced abortion to have a somewhat (about 23%) higher risk of subsequent first trimester spontaneous abortion than women with no history of induced abortion, recent analyses have indicated that this finding is an artifact that can be explained on the basis of a higher probability of repeat abortion among women with a history of induced abortion than among women with no history of induced abortion. However, this study has found that when induced abortion is accompanied by infection or retained placenta, the risk of subsequent ectopic pregnancy is increased about 5-fold (relative to when induced abortion is not accompanied by infection or retained placenta).

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Secondary Amenorrhea, Pituitary Adenoma and Oral Contraceptives

Contract Number	Contractor	Money Allocated
NO1-HD-7-2834	Johns Hopkins University	$117,000
NO1-HD-9-2810	University of Southern California	----
NO1-HD-9-2811	Johns Hopkins University	----
NO1-HD-9-2812	University of California, San Francisco	----

Objectives: These contracts support a collaborative case-control study to determine relationships between secondary amenorrhea, pituitary adenoma and previous use of oral contraceptives. Patients meeting specified criteria for secondary amenorrhea and/or galactorrhea, prolactin-secreting pituitary adenoma, or elevated prolactin levels, are interviewed to determine their contraceptive history. Data for comparison are obtained from matched normal controls. The data are being analyzed to determine whether oral contraceptive use was more common or of longer duration in patients in one or more of the disease categories than in the controls.

Major Findings: No findings are available as yet, but the study is progressing well. A detailed protocol, questionnaire, and manual of procedures were developed, data collection has been completed, and analyses are underway. It is anticipated that the study will provide information on approximately 235 women with confirmed pituitary adenoma, 150 equivocal cases, 170 with secondary amenorrhea without evidence of pituitary tumor, and one normal control subject for each case.

Significance to Biomedical Research and the Program of the Institute: Pituitary adenomas are being diagnosed in increasing numbers of women with secondary amenorrhea. This may be the result of improved radiographic and radioimmunoassay techniques which can now identify very small tumors, or it may be that these tumors are increasing in frequency. Many of these tumors have been reported in women who have discontinued use of oral contraceptives. This study should determine whether there is a correlation between the occurrence of pituitary adenomas and previous pill use, and should distinguish a possible causal relationship from the reappearance of a tumor present before pill use but masked by the steroid medication.

Proposed Course: Analysis of the data will be completed early in FY 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Interagency Agreement Title: Oral Contraceptive Use and the Risk of Breast, Endometrial, and Ovarian Cancer
Interagency Agreement Number: YO1-HD-8-1037
Agency: Center for Disease Control, Atlanta, Georgia
Money Allocated: $1,069,775

Objectives: To evaluate the relationship between oral contraceptive (OC) use and the occurrence of breast, endometrial, and ovarian cancer by means of a population-based case-control study of incident cases of breast, endometrial, and ovarian cancer.

Major Findings: During the developmental phase (May 1, 1978 - June 30, 1980) of this project, the Centers for Disease Control (CDC) developed and field-tested the study design, data instruments, and operational procedures for this population-based, case-control study of the relationship between OC use and the occurrence of breast, endometrial, and ovarian cancer. The implementation phase of the study commenced June 30, 1980. In preparation for this, CDC solicited, received, and reviewed contract proposals for: 1) data processing and random digit dialing; 2) case-ascertainment and interviewing. Contracts for these functions were negotiated and made effective during the summer of 1980. The field staff for the study were trained in the fall of 1980, and data collection began December 1, 1980. In March, 1981 CDC began analysis of the first month of data collection. This analysis has shown that case and control interviews are being carried out in a timely fashion, and that the error rate in the information obtained in these interviews is in general low.

Significance to Biomedical Research and Program of the Institute: The relationship between OC use and the occurrence of breast, endometrial, and ovarian cancer is an issue of major public health importance. This study is designed to rigorously access this issue.

Proposed Course: The data collection phase of this study will proceed over a two-year period. During this time, it is anticipated that approximately 6000 incident cases of breast cancer, 1500 of endometrial cancer, and 900 of ovarian cancer that occur among women less than 55 years of age will be studied. In addition, approximately 6000 controls selected via random digit dialing procedure will be studied. Data analysis will begin near the end of the first year of data collection. It is anticipated that this will lead to the first publication from the project. Subsequent analyses will build upon this first analysis, and detailed final analyses will be carried out after data collection is complete. These analyses will examine the overall relationship between OC use and the occurrence of breast, endometrial, and ovarian cancer, and will also examine in detail the possibility of interactions between OC use and other risk factors (such as family history) for these forms of cancer.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Case-Control Study of the Relative Risk of Malignant Melanoma of the Skin Among Women on Contraceptive Steroids
Contract Number: NO1-HD-8-2803
Contractor: University of California, Los Angeles, California
Money Allocated: $350,234 from FY 78, Fy 79, FY 80 and FY 81 Funds

Objectives: These are to estimate the relative risk of malignant melanoma in women exposed to steroid contraceptives taking confounding variables of sunlight exposure and sun sensitivity into account. One hundred fifty incident cases are to be ascertained from the two population-based tumor registries in Los Angeles and the five county San Francisco Bay area. Incidence cases are preferred to prevalence cases since the effect of steroid contraceptives on survival time is not known and to avoid errors in recall and other biases in the interpretation and generalizability of results. For each case, two controls are to be selected, one of which is to be a longstanding friend, the second a neighbor. Both are to be matched for age and skin color. They will serve as partial controls for life-style, sun exposure and sun sensitivity, judged from complexion and skin color. Variables not matched in the design are to be analyzed as covariates. Both matched pair and covariate analysis are to be used.

Major Findings: None have yet been made; but the operational phase of the project has been completed. 202 cases and 348 neighborhood and friend controls have been interviewed. The pathology review is nearly completed, except for 15 cases, which will be finished by the middle of July 1981. Coding and editing of all interviews has been completed also. The investigators are in the midst of their final data analysis, to be completed in September 1981. So far a preliminary analysis on a subsample of 50 cases and their matched controls has been performed and reported in the Progress Report, submitted to NICHD on June 30, 1981. In this preliminary work the confounding effect of varying solar exposures emerged as a major analytical difficulty to be overcome. In the preliminary work, limited by time of analytical effort and small sample size, this could not yet be done. It is for these reasons that the Principal Investigator (Dr. Elashoff) declined to cast the results of his preliminary analysis into anticipating whether oral contraceptive use is causally associated with the incidence of malignant melanoma.

Significance to Biomedical Research and Program of the Institute: A major concern with safety of oral contraceptives is their potential causal association with malignant diseases. Malignant melanoma of the skin is one of several clinical entities for which such an association has been suggested but not established.

Proposed Course: The final analysis of all collected data, including the logistic regression program is to be completed in September 1980.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Case-Control Study of the Relative Risk of Malignant Melanoma of the Skin Among Women on Contraceptive Steroids
Contract Number: NO1-HD-8-2804
Contractor: University of Sydney, Sydney, Australia
Money Allocated: $109,319 from FY 78, FY 79, FY 80 and FY 81 Funds

Objectives: These are to characterize the association between the use of oral contraceptives and occurrence of malignant melanoma, the determination of relatived risk, causative and confounding factors. The most recent 300 prevalent cases in women aged 15-49 years, diagnosed since 1970 were to be ascertained from the files of the Sydney Hospital, Melanoma Unit. Further, 100 incident cases were to be ascertained as they are diagnosed in Sydney Hospital during the first 18 months of this contract. For each case selected, one neighborhood and one hospital control matched by years of birth was to be ascertained. Cases and controls were to be interviewed using a questionnaire to obtain contraceptive reproductive history and sunlight exposure. The presence of confounding factors will be sought by stratification of data according to age, parity, marital status, sunlight exposure, skin color or sun sensitivity. It is projected to be able to detect a relative risk of 1.8 with 90% certainty at a 1% significance level.

Major Findings: The operational phase of the project, carried out in Australia, has been completed. All cases with malignant melanoma and their corresponding controls have been ascertained and interviewed. These data are on computer tape in London, England, where the analytical phase of the project is carried out. So far, preliminary analyses have been performed on 274 cases and their corresponding 548 controls. This analysis suggests that use of oral contraceptives increases women's risk to develop malignant melanoma and that this risk further increases with increasing duration of contraceptive use. Most marked was this increased risk among women who had used these drugs for ten or more years. However, it has not yet been determined whether this relationship is confounded by some other factors. This knowledge is essential because melanoma risk also was found to be determined by skin or hair color and a tendency to burn, blister or freckle, when exposed to sunlight. Therefore, it is still necessary to examine all findings in detail and to exclude the possibility that the observed relationship is not secondary to some confounding factors.

Significance to Biomedical Research and Program of the Institute: This is marked since a major concern with safety of oral contraceptives is their potential, causal association with malignant diseases.

Proposed Course: The final analytical phase of the project, to be completed on September 30, 1981, is being performed in London, England. Participation of the Australian investigators, who conducted the past operations of the project, in the still necessary final analyses, is limited to consultation by Dr. H. Shaw. Purpose of this contemplated consultation is to provide answers to questions, regarding operational aspects of the study, which might arise in the course of its final analysis.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Decreased Risk of Breast Disease Among Oral Contraceptive Users: Histopathologic Evaluation of Epithelial Atypia
Contract Number: NO1-HD-9-2813
Contractor: Stanford University, Stanford, California
Money Allocated: 0

Objectives: To evaluate whether the reduction in risk of benign breast disease that is associated with oral contraceptive use of about two to four years duration or longer varies for different histopathological forms of benign breast disease.

Major Findings: 444 women with benign breast disease, hospitalized during 1970-1977 in the San Francisco Bay area, were diagnosed histopathologically with regard to degree of ductal epithelial atypia and other histological characteristics of breast disease. Established and suspected risk factors for benign breast disease were studied for relationship to pathological findings in breast tissue. Relative risks of benign breast disease with specified degrees of atypia were estimated for oral contraceptive users as compared to non-users.

In this study, the degree of ductal atypia in benign breast disease was found to be unrelated to race, level of education, body mass index, age at menarche, nulliparity, age at first childbirth, number of parities, menopausal status, age at menopause, prior benign breast disease, maternal history of breast cancer, or ever use of oral contraceptives or conjugated estrogens. The risk of benign breast disease with any degree of ductal atypia was estimated to be about 33% less among oral contraceptive users than among non-users. No important variation in this reduced risk of benign breast disease among oral contraceptive users was found for benign breast disease of differing histopathological characteristics.

Significance to Biomedical Research and Program of the Institute: One published study has suggested that oral contraceptive use of about two to four years duration or longer decreases the risk of those histopathological forms of fibrocystic breast disease (the most common form of benign breast disease) that are not, or are only weakly, associated with an increased risk of subsequent breast cancer. That study has suggested that oral contraceptive use does not decrease, and may increase, the risk of those histopathological forms of fibrocystic breast disease that are more strongly associated with increased risk of subsequent breast cancer. The study supported by this contract does not confirm these previous findings.

Proposed Course: This project is complete.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-5-2830
Contractor: Evans County Health Department, Claxton, Georgia
Money Allocated: $453,227 from FY 75 - FY 80 Funds

Objectives: This is a prospective cohort study to determine change in blood pressure before and subsequent to initiation of contraceptive steroids, in a biracial population residing in the southeastern hypertension stroke belt in southeastern Georgia.

Major Findings: 22,097 patient clinic visits to seven Family Planning Clinics located in six counties of southeastern Georgia were screened to determine and enroll 1,001 black and white women meeting appropriate eligibility criteria. The induction period started in June 1976 and extended through October 1978; follow-up contacts were completed through June 1980 at which time over 90% of the women were eligible for the 24-month follow-up. The analysis of the study is based on complete follow-up data on 505 black and 485 white women. At induction, the ages of the subjects ranged from 13 to 41; the median age was 21, the mode, 18. The analysis consisted of comparing the frequencies of all OCA-users and non-users who became hypertensive (DBP 90 mm HG) after 6, 12 and 24 months of medical follow-up. The odds ratio (Mantel-Haenszel summary odds ratios, adjusted for the effects of age and initial diastolic blood pressure) was selected as measure of risk because deemed less perturbated by possible biases peculiar to this study and independent of study design. The analysis revealed no increase in diastolic blood pressure (DBP) among black women who use oral contraceptives (OC) as compared to black women who use other non-OC forms of contraceptives. However, there does appear to be a slight increase in the risk of elevated DBP in white women using OCs. This increase in risk is apparent only among white women aged 22 years. Nevertheless, the odds ratios are not statistically different from unity, and, in fact, within most age/initial DBP strata, the point estimates are less than unity. The slight increases of mean blood pressures, remaining within the normotensive range, associated with current OCA-use, as reported from contract -HD-75-32 were also noted, but not considered important (slight shift in the distribution of normal pressures).

Significance to Biomedical Research and the Program of the Institute: This contract differs from the other two in NICHD's recent program on oral contraceptives and hypertension in several features: a) induction of all subjects into the cohort occurred prior to initiation of contraceptive therapy; b) subsequent medical examinations were conducted after 6, 12 and 24 months of therapy; c) the study estimates of the risk ratios suggest that there may be a hypertensive effect of OCAs among white women of older age. Such effects had been reported from all studies which led to the initiation of contract program -HD-75-30, -31, -32. The Evans County Health Department is nationally known for the quality of its medical follow-up procedures, which may be related to some discrepancies of its conclusion from others.

Proposed Course: The contract has terminated. The remaining problem of oral contraceptives and hypertension has been addressed in NICHD's Contract Plan for FY 82 and will require workshops and further analysis for a solution.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Contraceptive Steroids on Blood Pressure
Contract Number: NO1-HD-75-2832
Contractor: Harvard Medical School, Boston, Massachusetts
Money Allocated: $507,462 from FY 1975-FY 1979 Funds

Objectives: In 1973 a total community survey on all 5,000 white females, ages 16-49 years, residing in East Boston was conducted. In this survey blood pressure, contraceptive use and demographic characteristics were ascertained. This provides the basis for the objective of this project: Determine incidence of blood pressure changes associated with continued, discontinued, or initiated contraceptive therapy since 1973.

Major Findings: According to the variable of contraceptive use the cohort comprises 4 subcohorts: (a) 1973 No/1976 No = continuous OCA-non-user, (b) 1973 Yes/1976 Yes = continuous OCA-users, (c) 1973 Yes/1976 No = discontinuing OCA-users, (d) 1973 No/1976 Yes = OCA-use initiators. The major findings obtained from follow-up of these subcohorts were presented at the American Heart Association 53rd Scientific Session in November 1980 and published in Circulation, Volume 62, (supplement III), page 300: "Current OCA-use was associated with a 4.6 mm Hg increase in systolic blood pressure ($p<0.001$) and with a 1.9 mm Hg increase in diastolic blood pressure ($p<0.05$). This association was not materially altered by past OCA-use or differences in age, body weight, parity and cigarette smoking habits among OCA-users compared with non-users. These blood pressure increases were reversible following discontinuation of the contraceptive drugs, but were not compatible with the hypothesis that OCA-induced hypertension results from certain women experiencing marked changes in blood pressure after starting contraceptive drugs. These data suggest that the small shift in the distribution of blood pressure at all levels among OCA-users cannot fully explain the several-fold increased risk of myocardial infarction associated with OCA-use.

Significance to Biomedical Research and the Program of the Institute: Many publications reported a several-fold increase of hypertension associated with OCA-use. Presumably this contributed to the fears and the marked decline in use of these drugs. The results of this contract, supported by two others (-HD-75-30 and -31) of NICHD's recent program contribute to provide a more balanced view of the risks associated with OCA-use.

Proposed Course: The project has been completed, but not yet fully reported. The Project Officer cannot fully accept the interpretations of the investigators from Harvard Medical School, published in 1980: Circulation, Volume 26, supplement III, page 300. A hypothesis was proposed for an alternative explanation: oral contraceptives may not cause hypertension among very young women <22 years of age, but may cause hypertension among older women, e.g. those >30 years of age who experience myocardial infarctions. Testing of this hypothesis was attempted in Contract -HD-5-2830 (Evans County Health Department, Claxton, Georgia. The results of this project supported this hypothesis, but could not be conclusive, since the median age of the population was 21, the mode 18. For further testing of the hypothesis workshops and analyses have been proposed in NICHD's Contract Plan for FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Case-Control Study of Myocardial Infarction in Young Women With Special Reference to the Role of Oral Contraceptives
Contract Number: NO1-HD-6-2849
Contractor: Boston University, Boston, Massachusetts
Money Allocated: 0

Objectives: To evaluate the relationship between oral contraceptive (OC) use and the occurrence of non-fatal myocardial infarction (MI) women. Special emphasis is placed on evaluation of possible interactions between OC use and other MI risk factors such as cigarette smoking, hypertension, blood lipids, etc.

Major Findings: Five hundred and fifty-six women with MI have been compared to 2,036 age-matched hospitalized controls. The overall risk of MI for current OC users (within the month preceding hospitalization), relative to the risk for non-users, is estimated to be 3.5 (95% confidence limits 2.2-5.5). Among non-OC users, the risk of MI for heavy cigarette smokers, relative to the risk for non-smokers, is estimated to be 7 (95% confidence limits 5-12). For women who both smoke cigarettes heavily and are current OC users, relative to women who neither smoke nor are current OC users, the risk estimate is 39 (95% confidence limits 22-70). For past OC users 40-49 years of age, the risk of MI is related to duration of OC use. For past OC users with a total duration of use of less than 5 years, the risk of MI is not increased. However, for past OC users with a total duration of use of five to nine years, the risk of MI, relative to the risk among non-users, is 1.6 (95% confidence limits 1.1-2.5). For past OC users with a total duration of use of ten or more years, the risk of MI, relative to the risk among non-users, is 2.5 (95% confidence limits 1.5-4.1). This trend is statistically significant ($p<0.01$).

Significance to Biomedical Research and Program of the Institute: The data from this study confirm previous reports that current OC use increases the risk of MI, and that current OC use interacts with the effects of cigarette smoking in a multiplicative fashion. In addition, the very large size of this study has permitted the first rigorous assessment of the risk of MI among long-term past OC users. The finding that long-term past OC use does increase the risk of MI is new, and is of major importance. This finding indicates that the risk of MI that is attributable to OC use is greater than previously believed, and is consistent with the concept that some OC formulations may accelerate the process of atherogenesis.

Proposed Course: This project is complete.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Oral Contraceptives on Folic Acid Metabolism
Contract Number: NO1-HD-74-2862
Contractor: University of Alabama School of Medicine
Money Allocated: $98,797 (1978); $79,115 (1979); $19,195 (1980)

Objectives: The single objective of the final clinical phase of this contract was to exploit the knowledge generated by its preceding basic research on the interaction or oral contraceptives with folic acid metabolism in the cervical epithelium. This drug-nutrient interaction appeared causally associated the occurrence of intraepithelial cervical neoplasia among oral contraceptive users. For this exploitation a clinical trial was conducted to quantitate the efficacy of folate therapy for reversing cervical dysplasia and preventing its progression to cervical cancer.

Major Findings: These are described in a manuscript, entitled IMPROVEMENT IN CERVICAL DYSPLASIA ASSOCIATED WITH FOLIC ACID THERAPY IN USERS OF ORAL CONTRACEPTIVES, accepted for publication in the American Journal of Clinical Nutrition: 47 women with cervical intraepithelial neoplasia, diagnosed by cervical smears, received oral supplements of folic acid, 10 mg, or a placebo (ascorbic acid, 10 mg) daily for 3 months under double-blind conditions. All had used a combination-type oral contraceptive agent (OCA) for at least 6 months and continued it while returning monthly for follow-up examinations. All smears and a biopsy obtained at the end of the trial period were classified without knowledge of treatment status using an arbitrary scoring system (1 normal, 2 mild, 3 moderate, 4 severe, 5 carcinoma in situ). Mean biopsy scores from folate supplemented subjects were significantly better than in folate-unsupplemented subjects (2.28 versus 2.92, respectively; $p<.05$). Final versus initial cytology scores were also significantly better in supplemented subjects (1.95 versus 2.32 respectively; $p<.05$), unchanged in patients receiving the placebo (2.27 versus 2.30 respectively). Prior to treatment the mean red cell folate concentration was lower among OCA users than non-users (189 vs 269 ng/ml, respectively; $p<.01$) and even lower among users with dysplasia (161 vs 269 ng/ml, respectively; $p<.001$). Morphologic features of megaloblastosis were associated with dysplasia and also improved in folate supplemented subjects. These studies indicate that either a reversible, localized derangement in folate metabolism may sometimes be misdiagnosed as cervical dysplasia, or else such a derangement is an integral component of the dysplastic process that may be arrested or in some cases reversed by oral folic acid supplementation.

Significance to Biomedical Research and Program of the Institute: The findings of this contract were not considered of significance to the research program of the Institute.

Proposed Course: Research on the interaction of oral contraceptives with essential nutrients has been terminated.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Relative Risk of Diabetes in Users of Steroid Contraceptives
Contract Number: N01-HD-8-2818
Contractor: Emory University
Money Allocated: $96,300

Objectives: The purpose of this study is to determine whether steroid contraceptive use, which is known to result in changes in glucose tolerance, is associated with an increased risk of overt clinical diabetes mellitus. The contract supports a classical case-control study comparing current and past use of contraceptive steroids in 150 women with newly diagnosed diabetes mellitus and 450 control women matched for age, race and socio-economic status. Data on known or suspected risk factors for diabetes are also being obtained.

Major Findings: Data from this study do not demonstrate any association between the frequency or duration of use of contraceptive steroids and subsequent development of diabetes mellitus. In the diabetic cases, 36.0% had ever used combination type oral contraceptives as compared with 51.6% of the controls. Duration of use was essentially the same for the two groups, 21.6 months for the cases and 22.8 for the controls. Depo Provera was used in 12.7% of the cases for an average duration of 40.1 months, and in 16.9% of the controls, for an average of 20.7 months. Exposure to any type of contraceptive steroid occurred in 44% of the cases and 62.2 % of the controls, for durations of 33.1 and 26.7 months. The total duration of steroid use in this population is quite low, and it remains possible that longer term use of contraceptive steroids may result in deterioration of pancreatic function and development of clinical diabetes.

Significance to Biomedical Research and the Program of the Institute: Steroid contraceptives are now used by millions of American women. Effects on glucose tolerance have been reported since the early 1960s, and it has been postulated that steroid-induced changes in carbohydrate metabolism may, with continued exposure, lead to frank clinical diabetes. No evidence on this point has been obtained from general prospective studies of OC users and no retrospective studies of the risk of diabetes have previously been reported. This study provides negative data which are encouraging. Reservations as to their broader applicability relate to the fact that the population was entirely black and no comparison with white subjects is available, and also to the relatively short periods of exposure to steroid contraceptives in this population.

Proposed Course: This study has been completed and a manuscript reporting the findings is in preparation.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Long Term Effects on Mothers and Offspring of Diethylstilbestrol Treatment During Pregnancy
Contract Number: NO1-HD-8-2829
Contractor: University of Chicago
Money Allocated: $208,280 (FY 80)

Objectives: The purpose of this project has been to determine the effects of prenatal exposure to diethylstilbestrol (DES) on the health of male and female offspring, especially on reproduction and the risk of cancer, and also to determine whether there is a risk of cancer of the breast and other organs associated with the use of DES by the mothers.

Major Findings: Male offspring exposed in utero to DES revealed a greater proportion with minor abnormalities of the external genitalia including epidymidal cysts and also low sperm counts and decreased sperm mobility. Among female offspring no case of clear-cell adenocarcinoma of the vagina or cervix has been identified. A high proportion reveal vaginal and cervical adenosis and other related findings typical for DES in utero exposure. Extended follow-up suggests the possibility of increased primary infertility among DES-exposed daughters and a higher rate of unfavorable outcome among those becoming pregnant. Among the mothers exposed to DES while pregnant a greater risk of cancer of the breast and of the ovary and cervix was reported after the first review of the cohort which was of borderline significance.

Significance to Biomedical Research and the Program of the Institute: This is a unique study based upon a controlled clinical trial to evaluate the effectiveness of DES in improving pregnancy outcome which was conducted at the Lying-In Hospital in Chicago during the early 1950s. The determination of adverse effects of prenatal hormonal exposure on the reproductive system of female and male offspring is of critical importance to the program of this Institute.

Proposed Course: This project was extended without further cost to the Government and will terminate in June 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Research on the Presence of Abnormal Proteins in the Serum of Users of Oral Contraceptives
Contract Number: N01-HD-0-2823
Contractor: Emory University
Money Allocated: $214,706 (FY 1980 Funds)

Objectives: The purpose of this project is to confirm and extend work of Beaumont et al reporting on the presence of abnormally precipitating globulins (GAP) in the serum of some women using oral contraceptives. Beaumont reported the protein fraction to be elevated in a significant number of healthy users and ex-users of oral contraceptives and markedly elevated in most women with serious thrombotic complications of pill use. The contractor will try to reproduce the laboratory procedures of Beaumont and to measure levels of GAP in serum of healthy women who are current users, ex-users, new users, and never users of oral contraceptives and in serum from patients with thrombotic episodes associated and not associated with pill use.

Major Findings: The investigators supported by this contract and N01-HD-0-2824 have worked collaboratively in efforts to establish laboratory procedures comparable to those used by Beaumont in Paris. Extensive modifications of published procedures were arrived at by letter, telephone, and a visit to Paris, and assays in the three laboratories now appear to be reasonably comparable. The contractors are ready to start testing sera from their patient populations.

Significance to Biomedical Research and the Program of the Institute: This research may help to elucidate the pathogenetic mechanism involved in thrombosis related to the use of oral contraceptives and may furnish a method useful in identifying women who are at special risk of thrombosis while using oral contraception.

Proposed Course: It is anticipated that assays on serum from 100 never-users, 50 users, 50 ex-users, 50 new users studied before and after initiation of therapy, and about 50 thrombosis patients will be completed during FY 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Research on the Presence of Abnormal Proteins in the Serum of Oral Contraceptive Users
Contract Number: N01-HD-0-2824
Contractor: Wayne State University
Money Allocated: $93,481 (FY 1980 Funds)

Objectives: The purpose of this project is to confirm and extend work of Beaumont et al reporting on the presence of abnormally precipitating globulins (GAP) in the serum of some women using oral contraceptives. Beaumont reported the protein fraction to be elevated in a significant number of healthy users and ex-users of oral contraceptives and markedly elevated in most women with serious thrombotic complications of pill use. The contractor will try to reproduce the laboratory procedures of Beaumont and to measure levels of GAP in serum of current OC users, ex-users and never-users, and in serum from patients with thrombotic episodes associated and not associated with pill use. This contractor will include in the user group women taking high and low-dose oral contraceptive preparations.

Major Findings: The investigators supported by this contract and N01-HD-0-2823 have worked collaboratively in efforts to establish laboratory procedures comparable to those used by Beaumont in Paris. Extensive modifications of published procedures were arrived at by letter, telephone, and a visit to Paris, and assays in the three laboratories now appear to be comparable. The contractors are ready to start testing sera from their patient populations.

Significance to Biomedical Research and the Program of the Institute: This research may help to elucidate the pathogenetic mechanism involved in thrombosis related to the use of oral contraceptives and may furnish a method useful in identifying women who are at special risk of thrombosis while using oral contraception.

Proposed Course: It is anticipated that assays on serum from 100 never-users, 50 users of low dose and 50 users of higher dose contraceptive pills, 50 ex-users, and up to 50 thrombosis patients will be completed during FY 1982.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Longitudinal Studies of Lipoprotein Changes in Users of Various Oral Contraceptive Preparations
Contract Number: NO1-HD-1-2803
Contractor: George Washington University
Money Allocated: $375,868

Objectives: To evaluate the effects of three currently marketed oral contraceptive formulations on lipoprotein patterns, glucose tolerance, and insulin secretion in women.

Major Findings: This project, which began June 1, 1981 is a prospective clinical investigation of the effects of three currently marketed oral contraceptive (OC) formulations on lipoprotein patterns, glucose tolerance, and insulin secretion in women. Three hundred consenting women 18-30 years of age who wish to initiate OC use will be randomly assigned to one of 3 OC formulations, each containing 50 mcg of ethinyl estradiol and either 1 mg of ethynodiol diacetate, 1 mg of norethindrone acetate, or 0.5 mg of norgestrel. These formulations have been selected so as to test the effects of different progestogens at the same dose of estrogen. A control group of 100 women not using oral contraceptives will also be studied.

One month prior to the initiation of OC use, fasting blood specimens will be obtained and analyzed for glucose, hemoglobin A_{1c}, and a variety of lipid components, including high-density lipoprotein cholesterol. These analyses will be repeated just prior to the initiation of OC use, along with additional lipid analyses ("beta quantification"), an oral glucose tolerance test, and insulin measurements. Also, a medical history and physical examination will be performed, and a 24-hour dietary recall and exercise history obtained.

After OC use is initiated (and at parallel times for the controls), fasting blood glucose, hemoglobin A_{1c}, and lipid analyses will be carried out at 2, 6, and 10 months. At 4, 8, and 12 months, additional lipid analyses, an oral glucose tolerance test, and insulin measurements will be performed, and a 24-hour dietary recall and exercise history obtained. OCs will then be discontinued and follow-up studies similar to those described above (fasting blood glucose, hemoglobin A_{1c}, and so forth) will be carried out over a period of two months. The data from the project will then be analyzed to determine how the 3 OC formulations being studied influence lipoprotein patterns (especially high-density lipoprotein cholesterol), glucose tolerance and insulin secretion.

Significance to Biomedical Research and Program of the Institute: It is currently thought that some OC formulations may increase the risk of cardiovascular disease by accelerating the process of atherogenesis, and may do this by altering lipoprotein patterns (e.g., by decreasing high-density lipoprotein cholesterol), glucose tolerance, and insulin secretion. This issue is of considerable public health importance.

Proposed Course: This project has just begun, and is scheduled to require 3 years for completion.

Contract Title: Health Status of American Men

Contract Number	Contractor	Money Allocated
NO1-HD-6-2807	University of California at Los Angeles	$425,100
NO1-HD-7-2801	Mayo Clinic	59,605
NO1-HD-7-2802	University of Southern California	642,778
NO1-HD-7-2803	University of Minnesota	517,200

Objectives: The purpose of this collaborative study is to identify long-term health hazards which may be associated with vasectomy as a contraceptive procedure. The current health status and medical histories of previously vasectomized men are to be compared with those of non-vasectomized control groups. The contracts with the Mayo Clinic, USC and the University of Minnesota provide for data collection according to a common protocol, and the contract with UCLA provides for project coordination and data analysis.

Major Findings: In the first phase of this study, a detailed protocol and manual of procedures was developed and subjects for the study were identified. The study is now in the data collection phase and information has been obtained and processed on over 21,000 eligible subjects. Plans for analysis are well under way but analyses of possible differences between cases and controls have been deferred pending completion of data collection in order to avoid any possibility of introducing bias into the collection of the remaining data.

Significance to Biomedical Research and the Program of the Institute: Vasectomy is a popular method of contraception which has generally been considered to have no adverse effects. However, it is known that a substantial proportion of vasectomized individuals develop circulating antibodies to spermatozoa and that, in rabbits, guinea pigs, and monkeys, this immune response can result in disease in the testis and sometimes kidney. Furthermore, in monkeys there is now strong evidence that vasectomy leads to exacerbation of atherosclerosis, presumably through immunologic injury to arterial walls. This study will be able to identify differences in incidence of common and also quite rare diseases in vasectomized men as compared to controls, or to demonstrate that there is no increase in risk within certain defined limits of probability, for intervals up to 8-10 years after vasectomy.

Proposed Course: Data collection has been completed at the Mayo Clinic and will be completed in Los Angeles and Minneapolis in the spring of 1982. The remainder of calendar year 1982 will be devoted to analyses of the data and preparation of a summary report and a monograph describing the study.

Contract Title: Effect of Bilateral Vasectomy on the Progession and Regression of Atherosclerosis in Macaca fascicularis
Contract Number: N01-HD-8-2827
Contractor: Oregon Regional Primate Research Center and Bowman Gray School of Medicine
Money Allocated: $106,496

Objectives: The purpose of this contract is to pursue preliminary findings which indicate that vasectomy may significantly increase the severity of atherosclerosis in monkeys. The effects of vasectomy on atherogenesis are being studied in normocholesterolemic and hypercholesterolemic monkeys fed a diet comparable in cholesterol content to that of the average American man. The possible therapeutic role(s) of a more prudent diet and/or vas reanastomosis on regression of established atherosclerosis will also be evaluated.

Major Findings: Sixty cynomolgus monkeys which have been under study at Bowman Gray have now been necropsied and the extent of atherosclerosis in the thoracic and abdominal aortas has been quantitated. There appears to be a clear exacerbation of atherosclerosis by vasectomy and an increased effect of vasectomy in the animals with higher cholesterol levels.

Fifteen of the 75 monkeys at Oregon have been necropsied for similar evaluation. Half of the remaining 60 have had vasovasostomies and half remain vasectomized, and half of each group has been put on a diet very low in cholesterol. These four groups of 15 monkeys each will be used in regression studies.

Significance to Biomedical Research and the Program of the Institute: These contractors previously demonstrated that vasectomy markedly increased the extent of atherosclerosis in a small group of cynomolgus monkeys fed a high cholesterol diet and more recently they have demonstrated significantly increased atherosclerosis in rhesus monkeys maintained for 9-14 years after vasectomy on a diet free of cholesterol. If these findings are applicable to man, vasectomy, an increasingly popular method of permanent contraception, could lead to a significant increase in cerebrovascular and cardiovascular disease. This contract is designed to validate and extend the preliminary monkey data and to determine the possible therapeutic role of vas reanastomosis and/or restriction of dietary lipids.

Proposed Course: The extent of atherosclerosis will be quantitated in the coronary, carotid, cerebral, iliac and femoral arteries of the 60 monkeys sacrificed at Bowman Gray to evaluate further the effect of vasectomy and interactions between lipid levels and vasectomy. Efforts will be made to identify deposits of immune reactants in tissues, particularly atherosclerotic plaques, from these monkeys. The 60 monkeys in the regression studies will be maintained for 18 months and then sacrificed to evaluate possible reductions in established levels of atherosclerosis.

Contract Title: Epidemiologic/Clinical Studies of Vasectomy and Atherosclerosis
Contract Number: N01-HD-0-2806
Contractor: Medical College of Wisconsin
Money Allocated: $24,000

Objectives: The purpose of this study is to determine whether there is a relationship between vasectomy and the extent of coronary artery atherosclerosis in man. Cardiovascular status in the 11,000 members of the Milwaukee Cardiovascular Data Registry has previously been evaluated by coronary angiography. The men are now being queried as to vasectomy history and the combined data will then be analyzed to determine the relationship between vasectomy (and duration of exposure to the effects of vasectomy) and the extent of coronary artery occlusion in various age groups. Data on a number of established cardiovascular risk factors are also available for analysis.

Major Findings: Data collection has been completed and analyses are in progress but not yet completed. Information has been obtained on 658 vasectomized men, including 168 vasectomized ten or more years prior to angiography, and 9354 who had not been vasectomized at the time of angiography.

Significance to Biomedical Research and the Program of the Institute: Data from two NICHD contract studies have now demonstrated that vasectomy results in marked exacerbation of atherosclerosis in two species of monkeys. The possibility has thus been raised that vasectomy, an increasingly popular method of permanent contraception, may lead to a significant increase in risk of human cardiovascular disease. This contract should contribute important information on the effect of vasectomy on the development of atherosclerotic lesions in human coronary arteries at an early stage, before they may become evident in increased rates of clinical disease.

Proposed Course: Analyses of data from this study are expected to be completed this year.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Epidemiological Study of Vasectomy and Coronary Heart Disease
Contract Number: N01-HD-0-2809
Contractor: Battelle Memorial Institute
Money Allocated: $242,105

Objectives: The purpose of this project is to determine the relationship between vasectomy and the subsequent development of coronary heart disease. The study involves men enrolled in the University of Washington Exercise Testing Registry who have had angina pectoris, myocardial infarction or resuscitated cardiac arrest, and control subjects who have no indication of coronary heart disease. Vasectomy history will be obtained by questionnaire. Data on the prevalence of vasectomy and duration of exposure will be compared in the two groups. Data will also be analyzed to take into consideration other known and suspected risk factors for cardiovascular disease.

Major Findings: Data collection is under way and progressing well, but no findings can be expected at this time.

Significance to Biomedical Research and the Program of the Institute: Data from two NICHD contract studies have now demonstrated that vasectomy results in marked exacerbation of atherosclerosis in two species of monkeys. The possibility has thus been raised that vasectomy, an increasingly popular method of permanent contraception, may lead to a significant increase in risk of human atherosclerotic disease. This contract is expected to provide important information on the role of vasectomy as a possible risk factor for coronary heart disease in man.

Proposed Course: Another year will be required to complete the collection and analysis of data in this study.

Contract Title: Study of Myocardial Infarction in Relation to Vasectomy
Contract Number: N01-HD-0-2810
Contractor: Boston University Drug Epidemiology Unit
Money Allocated: $339,300

Objectives: The purpose of this contract is to determine the relative risk of myocardial infarction in vasectomized as compared to non-vasectomized men. A classical case-control study is being conducted in some 75 hospitals in New England and New York State. Cases are male patients under age 55 admitted for first myocardial infarction and controls are men admitted to the same hospitals for unrelated acute conditions. Interviews are used to obtain information on vasectomy history and other known or suspected risk factors for cardiovascular disease. It is anticipated that some 2000 cases and 1-2 controls per case will be enlisted over a three year period of data collection.

Major Findings: Data collection is underway and progressing well, but no findings can be expected at this time. It is noteworthy that 3% of the control subjects report having been vasectomized 10 or more years before hospitalization, indicating that the study will be able to detect a relative risk of 1.5 for myocardial infarction at 10 years after surgery.

Significance to Biomedical Research and the Program of the Institute: Data from two NICHD contract studies have now demonstrated that vasectomy results in marked exacerbation of atherosclerosis in two species of monkeys. The possibility has thus been raised that vasectomy, an increasingly popular method of permanent contraception, may lead to a significant increase in risk of human atherosclerotic disease. This contract is expected to provide important information on the role of vasectomy as a possible risk factor for myocardial infarction in man.

Proposed Course: This project is expected to require another 18 months for data collection, plus 6 months for final analyses of the data.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Survey of Women's Health and Birth Control Methods
Contract Number: NO1-5-2852
NO1-7-2811
NO1-6-2806
NO1-7-2807
NO1-6-2804
NO1-7-2808
NO1-7-2809
NO1-7-2812
NO1-6-2805
NO1-7-2810

Contractors: Biostatistics Center of George Washington University and nine clinical centers
Money Allocated: 0

Objectives: These contractors have collaborated to conduct a multi-center case-control study of the relationship between IUD use and the occurrence of seven serious gynecological and obstetrical disorders (pelvic inflammatory disease, septic and non-septic fetal loss, ectopic pregnancy, vaginal hemorrhage, uterine perferation, abruptio placenta, and placenta previa).

Major Findings: Using a common protocol and set of data forms, data has been gathered in 9 collaborating clinical centers concerning nearly 9,000 cases (all diagnostic categories) and 10,000 controls. These data have been analyzed and reports have been prepared. These reports: 1) confirm that IUD use increases the risk of pelvic inflammatory disease, but also show that oral contraceptive use and use of barrier methods (diaphragm, condom) decrease risk; 2) confirm that women with an IUD in place at the beginning of the second trimester of pregnancy are at increased risk of septic fetal loss, and show that this increased risk is not present for women with an IUD in place at conception but removed in the first trimester of pregnancy; 3) provide evidence that IUD use is not responsible for the tripling of ectopic pregnancies in the United States during the years 1965-1977; 4) provide evidence the IUD use does not in general increase the risk of vaginal hemorrhage serious enough to lead to hospitalization; 5) show that the risk of uterine perforation following IUD insertion is greatest for post-partum insertion in women who are lactating; 6) show that IUD use does not increase the risk of abruptio placenta or placenta previa.

Significance to Biomedical Research and Program of the Institute: The findings from this study will improve the quantity and quality of information that is available to women and their physicians when assessing the benefit/risk characteristics of the IUD as a method of contraception.

Proposed Course: This project is complete.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Induced Abortion on Subsequent Reproductive Function and Pregnancy Outcome in Hawaii
Contract Number: NO1-HD-6-2801
Contractor: University of Hawaii at Manoa
Money Allocated: 0

Objectives: To evaluate the effect of induced abortion on subsequent reproductive function and pregnancy outcome by linkage of state abortion records with live birth and fetal death statistics. Specifically, to study the effect of induced abortion upon rates of subsequent spontaneous abortion, premature birth, and other birth-related disorders.

Major Findings: Pregnancy outcomes that occurred between January 1971 and December 1978 are being compared for 16,500 women who collectively underwent 17,702 recorded induced abortions between July 1970 and June 1974 and two control groups: 1) all live births and fetal deaths that occurred in the State of Hawaii between January 1971 and December 1978, minus those that occurred to women in the "recorded induced abortion" group; 2) all live births and fetal deaths that occurred between January 1971 and December 1978 to women without a prior recorded induced abortion who are matched to the women with such a history for maternal age, racial background, residence (urban vs. rural), and birth order.

Previous reports from this study have suggested that women with a history of induced abortion have a somewhat (about 23%) higher risk of subsequent first trimester spontaneous abortion than women with no history of induced abortion. However, recent analyses indicate that this finding is an artifact that can be explained on the basis of a higher probability of repeat abortion among women with a history of induced abortion than among women with no history of induced abortion. In addition to this finding, recent analyses have also shown that when induced abortion is accompanied by infection or retained placenta, the risk of subsequent ectopic pregnancy is increased about 5-fold (relative to when induced abortion is not accompanied by infection or retained placenta).

Significance to Biomedical Research and Program of the Institute: This broadly based study is expected to provide comprehensive information concerning the effect of induced abortion on subsequent reproductive function and pregnancy outcome, an issue of considerable public health importance.

Proposed Course: Final analyses of the study data are now underway, and a final report will be submitted upon termination of the project on September 21, 1981.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Prospective Study of the Outcome of Pregnancy Following Induced Abortion
Contract Number: NO1-HD-6-2803
Contractor: State University of New York
Money Allocated: 0

Objectives: To evaluate the effect of the induced abortion on subsequent pregnancy outcome by comparing pregnancy outcomes of women with a history of one or more induced abortions (and no other pregnancies) to pregnancy outcomes of : 1) women who have previously had one spontaneous abortion (and no other pregnancies), 2) women who have previously had one live birth (and no other pregnancies), and 3) women who have never previously been pregnant.

Major Findings: 2,409 pregnant women have been followed from registration in prenatal care until delivery. 520 of these women had had one or more induced abortions prior to the current pregnancy, 180 had had one spontaneous fetal loss, 800 had had one live birth, and 909 had had no previous pregnancies.

The women with prior induced abortions have been compared to the control groups for outcomes and complications of pregnancy. The results of these comparisons indicate that, in general, the risk of adverse pregnancy outcomes (low birth weight, gestation less than 37 weeks, spontaneous mid-trimester fetal loss, reduced Apgar score, pre-eclampsia premature rupture of membranes, or late bleeding) is no greater among women with a history of one or more induced abortions (and no other pregnancies) than among women with no previous pregnancies. Thus, the findings from this study suggest that termination of one or more pregnancies by induced abortion prior to actually giving birth has the effect of delaying the risks of adverse pregnancy outcome that are normally associated with first pregnancy.

Significance to Biomedical Research and Program of the Institute: The effect of induced abortion on subsequent reproductive function and pregnancy outcome is an issue of considerable public health importance. This study has provided reassurance that induced abortion does not lead to a major increase in the risk of subsequent adverse pregnancy outcomes. However, this study is not large enough to preclude a modest adverse effect of induced abortion on subsequent pregnancy outcome. Thus, the results of this study will need to be evaluated along with the results of other, larger studies that are being carried out in this program area.

Proposed Course: This project is complete.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Contraceptive Evaluation Branch
Contract and Collaborative Research

Contract Title: Birth Control and the Risk of Infertility
Contract Number: NO1-HD-0-2821
Contractor: University of Washington
Money Allocated: 0

Objectives: To evaluate the relationship between birth control practices and the occurrence of subsequent, undesired infertility by means of a population-based case-control study.

Major Findings: In this study, cases are all women 20-39 years of age with non-congenital, female-factor infertility that are identified from medical evaluations for apparent infertility in King County, Washington during a three-year period. It is anticipated that approximately 1500 such cases will be studied. For each case, one matched (for age, census tract, marital status, and pregnancy order) control will be studied. This will be a woman giving birth during the calendar year following the year that the associated case began trying to become pregnant. Cases and controls will be interviewed to obtain demographic, personal, medical, and reproductive history, and a detailed history of birth control practices. Data analysis will focus on estimating the relative and attributable risk of infertility for users of the intrauterine device, for women who have had induced abortions, and so forth.

This project began June 30, 1980. The first six months was devoted to preparatory work. The study protocol was refined, an interviewer's manual was prepared, and forms were developed for recording interviews and for abstracting medical records. Approval for investigating human subjects was sought and received at the institutions to be involved in the study, and procedures for identifying and contacting potential cases were established.

The data collection phase of the study began in January 1981. Since that time, interviews have been completed on 207 infertility patients. An additional 192 are presently being contacted, interviewed or having their completed interviews edited. Only 15 patient refusals have been encountered. Using data tapes containing the birth records of King County, Washington, a computer program has been devised to select controls based upon the matching criteria described above.

Significance to Biomedical Research and Program of the Institute: Considerable concern has been expressed over the possibility that the use of intrauterine devices may increase the risk of subsequent infertility. This project is designed to investigate this issue, and to provide an estimate of the risk of infertility that is attributable to the use of intrauterine devices or other birth control practices.

Proposed Course: The data collection phase of this study is scheduled for two years. During this time, cases and controls will be identified and interviewed, and the medical records of cases will be abstracted. The data obtained will be computerized, and prepared for eventual analysis. When the data collection phase of the study is complete, analysis will commence. The analysis phase of the study is scheduled for six months.

Contract Title: The Relationship Between Birth Control Practices and Subsequent Undesired Infertility
Contract Number: NO1-HD-0-2822
Contractor: Boston Hospital for Women
Money Allocated: 0

Objectives: To evaluate the relationship between birth control practices and the occurrence of subsequent, undesired infertility by means of a clinically-based, case-control study.

Major Findings: In this study, cases are married women over 18 years of age who are identified as having female factor infertility at seven clinical centers located across the United States. Controls are of two categories: 1) women from infertile couples whose infertility is due to male factors, and 2) recently delivered women. Cases and controls are interviewed to obtain information concerning birth control practices and other relevant data. Analysis will focus on estimating the risk of female factor infertility in women who have used intrauterine devices, undergone induced abortion, etc. relative to the risk for women who have not used these methods of birth control.

The study began June 30, 1980. During the first nine months, the study protocol was refined, a manual of operations developed, and a questionnaire designed. Subcontracts were negotiated with the clinical centers, and with the statistical subcontractor. A meeting of the clinical collaborators was held. Research assistants were hired for each clinical center, and they met to receive instruction in the design and conduct of the study. The questionnaire underwent pilot testing at each center. Data collection began April 1, 1981, and between April 1, 1981 and June 15, 1981 198 cases and male factor infertility controls, and 283 recently delivered controls were studied. It is anticipated that the rate of case and control ascertainment will increase as the study progresses.

Significance to Biomedical Research and Program of the Institute: Substantial concern has been expressed that the use of intrauterine devices may increase the risk of subsequent infertility. This project will provide quantitative assessment of this issue.

Proposed Course: It is anticipated that data collection for this project will require two years of work. During this period, it is expected that approximately 1700 cases of female-factor infertility, 1700 male-factor infertility controls, and 3400 recently delivered controls will be studied. Data analysis will begin during the latter portion of the data collection period, and will proceed for approximately 6 months after data collection has been terminated. The project will then be complete.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

The Center's grant and contract program in the behavioral-social sciences focuses on the size, rate of growth, and composition of our nation's most important asset, its population. The topics addressed by specific research projects derive from fundamental questions concerning the growth and distribution of our population, and their relation to individual and societal welfare. For example: What accounts for changes in the number of children couples decide to have? How successful are they in controlling their reproduction? How do families and households form and reform? What are the consequences of many or few children for individuals, families and the nation? What are the effects of age at childbirth and birth spacing on mothers and their children? How does internal migration affect the welfare of the people moving and the communities from which they come and to which they go? How would a rise or decline in the rate of population growth rates from a combination of natural increase and net immigration affect our economy and our environment in the future? Answers to these and many other questions are needed in order to deal with population changes now and in the years ahead. The results of research are intended ultimately to help individuals and couples understand the personal and societal consequences of their behavior and to help the government evaluate the impact of a variety of policies and programs on both the quantity and quality of our human resources.

The staff of the Social and Behavioral Sciences Branch uses grants to support research on these broad objectives, and uses contracts to support research on more specific research goals identified by the Institute. The Branch's strategy has been to concentrate on topics of current societal importance as well as questions of scientific concern. Consequently, the program has emphasized not only such topics as social and economic factors affecting the level and trend in national birth rates, but also the effectiveness of contraceptive practice, changes in family size and their consequences, the determinants and consequences of adolescent fertility, trends and implications of delayed childbearing, the implications of immigration for the U.S., and an analysis of changing family and household structure. One result of this strategy is that the Branch's research program changes in response to changing social conditions as well as in response to developing scientific knowledge. At present, its program focuses on the topics listed below.

Family and Household Structure

Changes in fertility, mortality and migration have combined to alter the American population in recent years, raising new problems and scientific questions. An important way in which these combined forces are expressed to society is through changes in the number and structure of households. It is the household that defines the living context of the population, and it is through the household that demographic events are typically measured. The family is a mediating influence that interacts with the changing forces of fertility, mortality and migration to produce changes in households. Accordingly, much attention has been given to family/household demography.

Research has been conducted on the measurement of demographic events associated with family and household change. In measuring family demography it is essential that we develop a thorough understanding of the trends of family formation (mar-

riage) and family dissolution (death and divorce). One study involving a comparison among the United States, Sweden, England and Belgium over the time period 1910-1975 has concluded that there has been a substantial movement toward earlier and more universal marriage, although there has been some reversal of that trend during the last 10-15 years. The most striking finding is the rise in the proportion of marriages ending in divorce which has accelerated since 1965. For example, 12.5 percent of American women married in 1915 will see their marriages end in divorce; this probability grows to 31.6 percent for those women married in 1945, falls to 25.1 percent for those married in 1955, and accelerates sharply thereafter to an estimated 41.6 percent of American women who married in 1975. Another study has concluded that once divorce occurs among blacks, the probability of remarriage is not influenced by either the number of children or the age of the youngest child at divorce. However, among white divorced women, women childless at the time of divorce have a higher probabilty of remarriage than divorced women with children.

Work has begun on estimating the extent to which children are involved in changing marital patterns. A study of women and children in remarriages from 1960 to 1975 indicates that one-third of the children in recent remarriages were under the age of five and one-sixth were teenagers aged 14-17. Most of the children involved in remarriage had siblings and half had two or more. The stepfathers of most of these children had been married before and about half of these stepfathers had children from previous marriages. For many of the children studied, remarriage followed very soon after divorce, but for almost a fifth, their mothers waited five or more years before remarrying. The study reports that children's experience of a second marital disruption has doubled in recent years. Also, about a third of the children are combined into households with half siblings and this proportion rises to two-thirds among children under age five at remarriage.

Family trends have altered the household structure in this country. One study indicates that the major change in household patterns seems to center around two age groups and processes: young adults who are leaving home and entering into marriage and/or the labor force, and elderly people who are changing their residential pattern. Many young adults are abandoning the traditional pattern of living with relatives (other than their parents) or as a lodger as a transition step to the establishment of their own households. Among 20-24-year-olds, this type of living arrangement declined from 18 percent for those born in the early 1900s to approximately three percent for young adults today. Young people are now more likely to set up their own households. Another study has discovered that the pattern by which children break from their parental households and form their own varies markedly among major ethnic and religious groups. Two dominant patterns were observed, one with children remaining home after completing school and providing financial support to the family and another consisting of children breaking away from their parental households at an early age and being subsidized by parents to continue their education.

Work has begun on identifying the causes of the recent changes in family and household demography. One study has attempted to develop an economic paradigm to express the observation that other institutions have taken on many responsibilities once borne by families. Thus, the role of family as an insurer against losses of earning power and health has declined with the growth of social security, health insurance, and so forth. As a result, kinship is less important in modern than in traditional societies. In marriages, as well, the importance to the extended family of a marriage match has declined, and the importance of affection and love to partners themselves has become more important. As these characteristics may be

more difficult to ascertain before marriage than the traditional characteristics of family reputation and position, the secular rise in divorce rates may in part reflect this shift. The demographic transition or shift from high birth and death rates to low birth and death rates can be viewed in terms of changes in family functioning. In traditional societies that have not undergone a demographic transition, the family is used as a vehicle to produce child labor and insure the security of parents in old age. As public mechanisms develop to provide labor markets and old age security, the role of the family changes and motivation to bear children lessens.

A study in Taiwan reports that despite widespread use of contraceptives, the decline in the birth rate is probably being retarded by the fact that traditional familial forms and relationships are still very important there. As an indication of the relation between traditional familial elements and reproductive patterns, the study finds that the length of co-residence of a couple with the husband's parents, the importance attached to having a male heir, and the expectation of future co-residence with their own married sons are all related to lower age at marriage, a larger number of births, a higher preferred number of children, and a lower percentage initiating contraception to space their children. In advanced stages of demographic transition and high levels of economic development, another class of models is hypothesized to explain fertility and family life which view the primary, parental motivation for family life and childbearing as the receipt of direct psychic benefits from the number of children born and concerns about the lifetime welfare of children as a type of philanthropic motivation.

Another project has been assessing whether household crowding (i.e., high density of people in the household) and social isolation(i.e., living alone) are associated with pathological behavior. Individuals raised in cities were, as adults, less reactive to household overcrowding than persons raised in the country or in towns. When the composition of the household is taken into account, it appears that the interpersonal relationships in a household, not just the amount of space, influence whether crowding has negative effects. For example, couples with children reacted less to crowding if other adults were present, but non-parents in households where children were present reacted more to crowding than adults living with other unattached adults. Persons living in multiple-dwelling units or apartments reacted more to crowding than those living in detached houses. Among persons living alone, it was found that no adverse mental health effects could be attributed to their solitary living arrangements in this study of the general population. Apparently, reductions in demands made by others and intrusions on privacy offset other effects of living alone. This will require some reassessment of previous research on the role of social integration. For example, among the elderly, previous research had shown higher mortality rates for those living alone.

Other studies have indicated that high levels of out-of-wedlock childbearing among adolescents have produced households of complex structure and that structure can be an important element in determining the well-being of the teenage mother and her child. Many teenage mothers live in households containing other adult members, most notably the grandparents. The existence of this type of multigenerational household seems to be in response to economic hardship because State levels of Aid for Dependent Children payments appear to be strong determinants of whether a teenage mother would live with her parents or establish an independent household. The physical status of children born to adolescent mothers seems to be more sensitive to the availability of economic resources and to family structure than any other characteristics of children. Given the findings on effects of crowding, it

will be important to assess the effects of these complex families on the grandparents, not on just the teenage mother and baby.

Factors Affecting the Control of Fertility

Research is being carried out in the U.S. today to determine how people manage their reproductive lives, but it is also important to look at other societies and other eras in our own society to understand fully the processes underlying the control of fertility. Present knowledge shows that fertility is determined by a variety of factors including the biological ability to bear children, the use or non-use of methods to control fertility, the exposure to sexual activity, the entry into marriage, and the motivation to have children. Thus, studies are under way on these determinants of fertility in the U.S. and other societies from both an historical and contemporary perspective.

A study pertaining to women born from 1901-1910 in the U.S. was undertaken in an effort to understand better the low fertility of the 1920s and 1930s. These women had the lowest total fertility rates ever recorded in the United States before efficient contraception became widely available. In general, lower fertility was found among women who married at later ages and who experienced shorter durations of marriage. The lowest levels of fertility were observed for women who delayed marriage until after the Depression, though it is likely that this reflects the effects both of higher education and lower fecundity of those marrying at later ages. That fertility was deliberately controlled is evidenced by the fact that three-quarters of the women surveyed used contraception at some time. Surprisingly, the fertility of women hardest hit by the Depression was at least as high as and often higher than the fertility of women less severely affected.

Analyses of the contraceptive behavior of a national U.S. sample indicate that between 1970 and 1975 there has been a sizeable decline in the rate of intended conceptions. Also, in this period, there was a large increase in the delay of intended conceptions, an unprecedented decrease in failure rates by couples attempting to delay or to terminate fertility, and widespread use of sterilization to end reproduction. A study of the factors involved in the effective, correct, and continuous use of the pill, IUD, diaphragm, and other contraceptive methods by unmarried women aged 20-29 is now in progress.

Analysis of changing attitudes toward abortion between 1970 and 1975 show some changes, but not marked ones. Those found to be negative toward abortion in 1970 changed relatively little, with few major shifts to positive attitudes. Those who viewed abortion positively in 1970 tended to remain positive. Those who were in the more neutral area and who changed were more likely to move to negative or inconsistent positions than to become more positive.

As the focus moves from the techniques of fertility control to the motivation for fertility control, there are several levels of analysis. The motivation to bear children may be influenced by societal-level factors, by the husband-wife relationship, or by intrapersonal factors. On the societal level, an analysis of 1900 census tapes has shed light on the transition from high to low fertility in the U.S. By the late 1800s, large segments of the rural population were engaged in manufacturing activity, which brought about a decline in fertility in rural areas. Further, although most people still lived in rural areas, the urban populations had typically achieved very low levels of fertility which had pronounced effects on overall U.S. fertility. It appears that variations in fertility were directly

related to the changing roles of children, shifting from important contributors of labor to the household to prolonged school attendance. Mandatory school attendance and increasing mechanization of agriculture helped alter the economic need for children and suggests that the economic contributions and costs of children affected fertility decisions. Overall, this study suggests that fertility declines need not be a direct consequence of only urban-industrial changes. Rather, changes within agricultural sectors can also precipitate fertility declines. The implications of this conclusion have important extensions in societies which today remain largely agricultural, and are concerned with reducing their current rates of fertility.

Other research continues to explicate the role of economic factors in the determination of fertility. One investigator observed that a history of female labor force participation may have little effect on fertility during the early marital years when most women have one or two children, but that it contributes to the lowering of the total number of children born over a longer period of time. A positive relationship between women's intelligence and labor force participation was found, and there was evidence to suggest that the more intelligent women may have lower (total) fertility because they delay the initiation of childbearing. The occupational resources with which a woman entered marriage, including her educational attainment, earning potential, and past employment tended to increase her labor force participation. On the other hand, the husband's occupational resources tended to lower the likelihood that the wife would be employed.

The rapidly accumulating evidence concerning the importance of economic factors in the control of fertility seems to be forming a unified model of fertility. Fertility levels are directly related to the amount of economic resources available to the family (e.g., income), and inversely related to the cost of raising a child (e.g., cost of direct support of children and opportunity costs). In recent years, opportunity costs may be the dominant force in the dynamic competition between these economic forces. As the value of a woman's time rises in the market place, it becomes more costly to remain home and raise children (opportunity costs) and it is predictable that women will increase their labor force participation and compress their childbearing into short time intervals. A number of studies also suggest that increased labor force competition has resulted from the historically large "baby boom" cohorts now entering the labor force. This competition may depress the labor market and actually lower the "opportunity cost" of childbearing, thereby stimulating fertility.

Attitudinal studies are useful as measures of the pressures placed on individuals to behave one way or another. A study has shown that between 1970 and 1975 there was a change in the attitudes of white married American women about the ideal time for them to have their first child. While the amount of change is related to religious affiliation, education, and attitudes toward women's rights, almost every group of women gave a higher age for first birth in 1975 than in 1970.

In another study, it was found that the higher the status of the wife's job, the more likely it is that she will want fewer children than her husband. It was also found that husbands with more education than their wives are more likely to want fewer additional children than their wives, especially at higher family incomes. The influence of female employment on fertility reflects societal-level factors such as the need for and acceptance of women workers but also individual-level characteristics such as anticipated rewards of employment and child care and husband-wife division of labor within the home.

More recent changes in fertility patterns are highlighted, and future patterns anticipated, in an exciting longitudinal survey of mothers and their "Baby Boom" offspring. Results of the study offer a new perspective on the changes in the orientation of people toward family size in the U.S. While mothers in the sample had had an average of 3.9 children in 1980, they reported that if they could begin again they would have an average of only 3.3. Shifts in the attitudes of mothers toward childlessness were even more marked: in 1962, 84 percent said that they believed that all married couples who can ought to have children, while in 1980 only 43 percent held this opinion. In contrast, the sons and daughters of these women said they would like to have, on average, 2.9 children, an average of one-half child more than their mothers said they would like their children to have, but one child less than the average number the mothers themselves had borne. In addition, a comparable proportion of children--39 percent--said they thought all married couples ought to have children. These facts combine to suggest that the generation of children about to begin childbearing themselves will not be experiencing substantial pressure from the older generation to have more children than they want. Also, the evidence of a substantial reduction in the normative sanctions against childlessness could have enormous ramifications for future fertility behavior.

Another study has found that certain psychological variables of alienation, meaninglessness and isolation, impede rational control of sexual and marital activities as well as contraceptive behavior. The more isolated were more likely to have engaged in premarital intercourse, to have cohabited, and to have preferred delaying of marriage. High scores on meaninglessness were associated with greater perceived disadvantages of birth control and lower levels of knowledge about sex and reproduction. People with such scores had premarital intercourse as well as intercourse in early marriage without using contraception effectively and consistently; nearly half of the first pregnancies were either unintended or unwanted.

Studies of decision-making addressed the question of sex selection and preference for family size. Results suggest that sex preselection would not be likely to affect fertility rates substantially in the U.S., and that the majority of U.S. women do not desire to control the sex of offspring. Preselection might affect the sex ratio and birth order, with more males likely to be firstborns and more daughters likely to be second children. Family size preferences dominate sex preferences for many couples. Also, many couples stop having children before reaching their ideal number because of fear of having another child of the "wrong" sex. That is, sex preferences can actually reduce fertility below an "ideal" level.

While most childbearing takes place within marriage, rising divorce rates draw attention to the interplay of marriage, divorce and childbearing. A study of women who married before 1960 shows that there is a lower probability of divorce for those who have at least one child than for those who do not have any children. This pattern is attenuated among women married after 1960 and is particularly reduced among those married after 1965. An analysis of the probability of divorce by length of time from the birth of a child gives no support for the suggestion commonly advanced that women with preschool children are less likely to terminate their marriages than those whose children are above the age of five. This study also reports that while women are now taking longer to enter a second marriage after divorce, there is no increase in fertility during the intervening single state. Only teenagers fail to fit this pattern. After 1970, teenage marital fertility continued to fall, but their post-divorce fertility rose. This study also reports on childbearing in second marriages and indicates that women who first

married in their teens and then entered into a second marriage will have higher fertility than their contemporaries who first married before age 20 but remained in that marriage. However, for women who first married after the age of 20 there is no such clear difference between women who divorced and remarried as compared with those who stayed married.

Consequences of Family Size

Study of the consequences of family size continues to form a major portion of the social and behavioral sciences program. An important reason for this is that the consequences of having one or more children is an important factor determining fertility. Clearly, understanding the determinants of fertility cannot be complete without taking into account the impact of consequences of fertility. A major ongoing program initiative is concerned with the consequences of low fertility (the effects of childlessness and of one-child families) on the adults and children involved, since there has been practically no research in the past on the consequences of low fertility, although this phenomenon is increasing. In addition, there is reason to believe that many people have a child or have more than one child at least partly because they believe that they or the child will suffer adverse effects for not doing so.

Four studies of only children using large data sets show no adverse effects of being an only child. Only children were found to be more like firstborns in larger families, and were shown to be slightly superior on cognitive abilities and achievement to children with siblings. Preliminary findings from one study show that "only" girls exhibit greater sex-role flexibility than other children (including "only" boys). On the whole, however, "onlies" simply have not been shown to differ from children with siblings on a wide range of factors including physical development, use of medical care, behavioral and psychological factors, marital status, number of children they had, divorce rates, occupational choice, or levels of income. A study which compared "onlies" with children with siblings in one-parent households also showed no negative results of being an "only." In fact, "onlies" from these one-parent households showed fewer interpersonal, emotional, and behavioral problems and were more mature, independent, and comfortable with adults than comparable children with siblings. Since the consequences of family size may vary with age of the child, two continuing projects are doing in-depth studies of adolescent only children and comparing them to first and lastborns in larger families. These studies are concerned with the consequences of being an "only" on intelligence, interpersonal orientation, attitudes, time use, reciprocal interaction skills, and ego identity formation.

It is hypothesized also that family size may have different effects for parents and children. Several studies are under way which compare the consequences of having 0, 1, and 2 or more children on a variety of factors relating to social and psychological well-being. Three of these studies have preliminary findings which show that mothers of only children have greater continuity of employment than mothers of large families, while childless wives have the greatest continuity of employment. The same relationship is shown for level of occupational aspirations and attainment. One of these studies shows that living standards decline most sharply as family size increases above three children. Another study finds that women who delay childbearing until age 30 are better off economically than either younger childbearers or childless women. Consumption patterns of childless and small families differ somewhat from those of larger families, but the primary difference as family size changes appears to be in workload of family members and in use of time.

Four studies are under way on the economic consequences of family size. One is concerned with the difference family size makes on level of living, and the other three are attempting to estimate actual expenditures on children in families of various sizes, incomes, ages, residences, and occupations. These studies will also predict, in constant dollars, the cost of raising a child born in 1980.

Several ongoing studies are concentrating on the consequences of both involuntary and voluntary childlessness. These studies are looking at the effects on men, women, and married couples at different points in the life cycle and are assessing the relationship of childlessness to educational and occupational attainment, social and economic well-being, measures of personal and marital satisfaction, and indicators of quality of life. One recently completed study shows that there is a continuum and no clear demarcation between the voluntarily and involuntarily childless on several factors. For instance, while the involuntarily childless have a greater desire for children, less desire for activities competitive with having children, more willingness to undergo treatment for fecundity impairments, and a more favorable attitude toward adoption, there is overlap with the voluntarily childless and some sharing of views. This in-depth study identifies several factors that affect strength of desire for children and also notes that there is a cohort difference in factors contributing to attitudes toward childlessness. The couples who entered their years of potential reproduction during the late 1960s and 1970s perceive childlessness as a more acceptable option and deal with it more openly and directly than do those from earlier cohorts. Another study shows that shifts in attitudes regarding childlessness are marked, with 84 percent of couples in 1962 believing that almost all couples who can should have children, whereas only 43 percent held this opinion in 1980.

Adolescent Pregnancy and Childbearing

Concern about early pregnancy and childbearing revolves around the effects on the young woman, her child, the father, and other family members involved, as well as society as a whole. While birth rates of older adolescents have declined in recent years, birth rates have increased and then decreased among younger adolescents. There has been an increase in the likelihood of out-of-wedlock births, and adolescents still account for one-third of the legal abortions performed in this country each year. There is substantial evidence of high levels of unintended pregnancy and childbearing among adolescents, and research is generating an improved understanding of the effects of this behavior. In previous years, the effects of childbearing on children's early and school-age development as well as the adolescent's educational, occupational, fertility and marital experiences have been reported. Effects on the fathers and other family members have also been addressed.

Early childbearing also has impact on society, for when it prevents individuals from achieving their educational and occupational goals, society loses their contributions to the economy and the tax base. More directly, if early childbearing leads to greater use of public services, there is a direct impact on public expenditures. These public sector costs include Aid to Families with Dependent Children (AFDC), Medicaid, food stamps, foster care, and so forth. AFDC mothers were more likely to have been teen mothers than were American women in general. Among AFDC mothers under age 30, 64 percent had been teenage mothers, whereas only 24 percent of all American women aged 20 to 30 in 1975 had given birth before age 20. Initial estimates of the public sector costs related to early childbearing indicate that in 1975 a total of $8.55 billion was expended on AFDC households in which the mother was a teenager at the time she bore her first child. This total includes $5.00

billion expended on AFDC, $1.45 billion on Food Stamps, $.93 billion on Medicaid to the children of AFDC mothers, and $1.17 billion for Medicaid for AFDC recipients who were teenage mothers, including the cost of prenatal care and delivery for teenage mothers still under the age of 20. This total does not necessarily represent the amount that could be saved if all these mothers had postponed their first birth, since some would have required public assistance regardless of their age at first birth. A more complete picture of the public sector expenditures that could be saved if the incidence of teenage childbearing were reduced should emerge next year in subsequent analyses.

Research on the determinants of teenage pregnancy and childbearing includes studies of individual, couple, familial and societal level factors affecting adolescent behavior. In 1979, data for metropolitan areas show that about half of women aged 15-19 have experienced premarital intercourse. There was no lowering of the age at first intercourse as noted earlier in the decade, but the proportional increases have been greatest at ages 15 and 16. The probability of intercourse increases with age, exceeding 50 percent only for 18- and 19-year-olds. While age and race are strong predictors of sexual activity, religion also has an effect. Analysis of national data from 1971 shows that teenagers who report believing religion is important to them or who attend religious services regularly are less likely to be sexually experienced when other factors are taken into account.

Information about sexual activity is important because it tells how many teens are at risk of pregnancy or in need of services and also because aspects of sexual behavior influence contraceptive practice. Both the consistency and effectiveness of contraceptive use increase with the frequency of sexual activity and the number of sexual partners. In the late 1970s, several changes occurred in contraceptive practices among teenage women. There was a substantial decline in the proportion using the pill as their first method, but only a slight decline in the proportion using the pill as their present method. In 1979, 41 percent of women aged 15-19 living in metropolitan areas who had ever used a contraceptive had most recently used the pill. Of those same women, 23 percent reported using the condom and 19 percent withdrawal. To the extent that pill use declined, the slack was taken up by withdrawal and to a lesser extent by diaphragm and rhythm. From 1976 to 1979, more teens were contraceptively protected all the time but also more reported never using contraception. The risk of pregnancy remained the same over this time period, even within categories of contraceptive use and non-use. This finding will be examined in the coming year.

In another study, sexually active teens report embarrassment in obtaining contraceptives as a barrier to using them. The most important predictors of contraceptive embarrassment were parental attitude toward premarital intercourse and guilt regarding sexual behavior, as well as perceptions of the difficulties in obtaining contraceptives. The ambivalence of teens about parental influences on their willingness to obtain medically prescribed contraception is demonstrated by their frequent mention of the importance of parental approval while concurrently stressing their interest in establishing an independent relationship with the physician. When teens consider seeking reproductive health care, confidentiality becomes paramount and their evaluations of a doctor's trustworthiness on this matter can determine whether they visit the doctor for contraceptives. In other studies, socioeconomic status (SES) was related to the extent of contraceptive use--the higher the SES, the more likely contraception was used at first intercourse, last intercourse, and was always used. Another study confirms previous reports of adolescents' ignorance about contraceptive methods and the fertile period of the

menstrual cycle. Furthermore, teens often think they understand a contraceptive method when, in fact, they do not.

Other research supports earlier findings in demonstrating that many young women do not view abortion as a substitute method of birth control and that those who report they would choose an abortion, if pregnant, were more likely to have used a highly effective means of contraception at last intercourse. Preliminary results from several studies indicate the need to understand how teens think about personal dilemmas and what role models they have for the solution of problems they face in their personal lives. Findings suggest that when parents or others demonstrate effective, goal-directed behavior they provide the opportunity for adolescent females to learn these skills and to apply them to the proper use of contraceptives. In one study, the family experiences of those who never achieved proper contraceptive use provide instead an impression that life's problems are too difficult, complex, and unpredictable to master.

Some of the complexity of the problem of fertility control among teenagers can be seen in studies which include teenage couples. One study showed that the partners frequently reported discrepant information about birth control. For example, 70 percent of the couples disagreed about which birth control methods they had used in their relationship. Much of this disagreement, however, is attributable to differing reports concerning withdrawal and rhythm. The absence of communication between partners also extends to the possible consequences of their sexual activity. When asked what they would do if the woman became pregnant, more than half of the men and women gave different responses. In some cases, the different responses were the result of the male deferring to his girlfriend's decision. In many cases, however, both partners had strong preferences, those preferences were in conflict, and there was no indication that the matter had ever been discussed. To date, the clearest conclusions to be drawn from this work are that teen couples have poor sex education and that there is inadequate discussion between partners.

In another study of decision-making regarding the resolution of pregnancy, it was found that two-thirds of the pregnant girls studied reported having contingency plans before they became pregnant. Partners were significant in most decisions. Those having abortions characterized the impact of the partner as a direct influence, while the respondents who kept the child more often described their partners' influence as unsuccessful. A study of teenage mothers who became pregnant again with those who did not indicated that the important predictors of repeat pregnancy include low socioeconomic status, educational attainment, and I.Q.; problems with contraception as indicated by frequent changes in method; history of prior pregnancy often terminated by abortion; and missed clinic appointments. The relationship between the number of contraceptive changes and the risk of repeated pregnancy is of particular interest and may provide a useful indicator for intensified preventive efforts.

National data have been collected on sexual and contraceptive behavior and attitudes of males aged 17-21--the age group that includes the partners of most 15-19-year-old women. Preliminary analyses show males are more likely to be sexually active than females of the same age. Other research on males confirms their greater sexual activity and their greater expectation of benefits from such behavior than the expectations reported by girls. Interviews with parents show the mother's age at first intercourse to be predictive of the child's, an echo of earlier research which showed the mother's age at first birth to be predictive of the daughter's. As with research on teenage couples, research on parents and their children shows

discrepant reports of communication regarding sex. Parents seem to underestimate seriously the likelihood that their child is sexually active. The role of the family appears strong in terms of transmitting general values which affect sexual behavior (e.g., religiosity and educational aspirations), but their influence on specific sexual and contraceptive behaviors is less clear.

Policies and programs relating to family planning and welfare are presumed to influence adolescent behavior. Contrary to expectations, AFDC benefits--when they were statistically significant--consistently decreased the incidence of teenage childbearing and AFDC policy variables did not exert any influence. While in one analysis the availability of family planning services was not significantly related to teenage fertility, another project presented a more intensive analysis which showed that areas with higher proportions of adolescents enrolled in family planning programs were also areas with higher adolescent birth rates. This is a common finding in many countries, due to the location of clinics in high fertility areas and to differences in socioeconomic characteristics and levels of sexual activity. This study also showed that areas with greater increases in the proportion of adolescents enrolled in family planning clinics between 1970 and 1975 generally had greater decreases in adolescent birth rates. The family planning program averted an estimated 119,000 births in 1975/1976 among women aged 15-19, 82,000 to whites and 37,000 to nonwhites.

Migration and Population Distribution

While birth and death rates are the major determinants of national population size and age composition, the movement of people is the important third side of the demographic triangle. The size or rate of growth of the U.S. population may be of little interest or importance to a community that is experiencing a flood of immigrants. Similarly, the growth of the U.S. population will likely be increasingly influenced by the movement of people from other countries. The new and powerful forces governing population movement in the U.S. have combined to pose new population problems and scientific questions. While the Social and Behavioral Sciences Branch does not deal with many legal or practical problems engendered by migration, attention is paid to basic scientific questions regarding how a population changes through migration.

Last year, an initiative to determine the implications of international migration for the United States was begun. Projects were supported to ascertain the level and volume of international migration in the United States with special reference to Latin America, to estimate the impact of international migration on the U.S. economy and social structure, and to study the adjustment process that immigrants undergo after moving to this country. The latter projects are attempting to distinguish the factors that will determine whether a migrant will make a successful adjustment to the U.S., or will only stay a short time and then return to his homeland.

Being an immigrant may be viewed as a vulnerable condition and is not infrequently associated with health risks and other problems. The adjustment of migrants is an important research question for it tells us how migrants lose--or do not lose--their risk status. One study attempts to understand the process by which Asians adapt to an American environment. It has been observed that from the early 1960s to the mid-1970s, Asian immigrants (exclusive of Indochinese refugees) rose from eight to 35 percent of all legal immigrants to the U.S. The Asian immigrants are distinctly "professional" in occupational composition relative to other types of

immigrant streams to the United States. This aspect has been reduced in recent years by the family reunification policies of the United States which have subordinated occupational considerations to the goal of allowing families to reunite in the United States. Asian-Americans, especially native-born, are more likely to have higher educational attainment but lower earnings attainment than whites, and large numbers of Chinese and Philippine immigrants are in the low-wage service and trade sectors.

The study of internal migration within the United States has taken on new dimensions to accommodate the recent changes in the destinations and types of internal migration and to focus on the types of socioeconomic issues that will emerge in the latter part of this century. One study has identified three aspects of population change, sensitive to the migration process, that will shape the future demands for health services and the provision of health care in the United States. They are: (1) shifts in the age distribution which will give greater prominence to the health-care needs of the elderly; (2) changing settlement patterns which will alter the geography of demand for health care, shifting some of it away from large population centers to places where specialty medicine is less readily accessible; and (3) increasing concentration in large central cities of the disadvantaged and of deportable aliens, whose needs tend to strain health care delivery systems in areas where they are a sizeable presence.

Work is also under way to relate the processes of household change to migration. A considerable amount of population movement is attributable to young adults breaking away from their parental households. One study reports that approximately 13 percent of the young men 14-24 years old migrated annually between the years 1966 and 1975. Rates of mobility were considerably greater for whites than for blacks, and whites seemed to be more successful than blacks in using migration as a tool for occupational mobility. Migration seems to be strongly related to job change and occupational mobility, and migrants seem to benefit economically by moving. Perhaps an even more interesting question relates to why, in the face of the evidence that migration improves occupational mobility, do not more disadvantaged people move? One study reports that there is little evidence that black suburbanization, as a parallel process to white suburbanization, has become a continuing process. Instead, it is the non-migrating (as opposed to the in-migrating patterns characteristic of the late 1950s and 1960s) black population which will have an increasing influence on city economies. This study suggests that the concentration of poor, immobile black populations in central cities will become a considerable, if not dominant, problem of urban economies in the future.

Several studies have shown that housing and neighborhood characteristics are more important than employment opportunities in determining trends in residential location. Many households have members with jobs in the suburbs, and this growth has encouraged both the growth of the suburban housing stock and suburban employment, with the responsiveness of the housing stock being greater than that of employment. Also, there is a tendency for suburban employment to follow the growth of suburban populations. Race does not appear to have been as significant a determinant of location during the 1960s as it was in the 1950s. Also, one study reports that household characteristics have an important role in residential mobility. The gain or loss of family members, or simply children's growth, lead to mobility.

Workshops

A contractor/grantee workshop on Family and Household Structure was held on 5-6 March 1981. Eleven investigators working in this area of research described their progress, problems and findings. The workshop provided staff with a sense of the current state-of-the-art, progress on research underway, and assistance in forming our next programming efforts in this area of research.

The second contractors' workshop on Economic Determinants of Fertility was held on 27 May 1981 to bring together four contractors working on "expenditures on children" contracts to discuss problems they were encountering, results, and future plans.

Two contractor/grantee workshops on Adolescent Childbearing were held during this fiscal year. The workshop held on 5 December 1980 brought together four contractors working on the societal costs of adolescent childbearing to present summaries of their progress, problems encountered, results, and future plans. The workshop held on 29-30 June 1981 included twenty-three investigators working on adolescent sexual, contraceptive, and fertility behavior. The investigators discussed the findings from their projects and shared ideas with the other investigators and the staff of the Center for Population Research. In addition, there was a discussion of future research and policy implications of this work, and a discussion of the research funded by the Office of Adolescent Pregnancy Programs, DHHS.

Targeted Research

Requests for Contract Proposals (RFPs)

Research contract proposals were solicited for research on Changing Patterns of Household Structure (NICHD-SBS-81-3). This research will fill gaps in research funded under a previous solicitation which documents the current status of changes in and projections of family and household structure. Proposals were requested to study how changes in the family, e.g., marriage, divorce, and childbearing, relate to changes in the household. The RFP specifically requested research on the Hispanic population, and also comparisons of the patterns of family and household structure between the U.S. and other developed countries.

An RFP entitled The Effects of Family Size: A Critical Review of Research Since 1973 (NICHD-SBS-81-4) requested proposals for a critical review of the research done since 1973 dealing with the consequences of family size. This includes the actual, perceived and anticipated consequences of family size for children, parents and the relationship between children and parents. Family size includes zero or more children and one or more adults, therefore including childless couples and one-parent families as well as the usual nuclear family.

An RFP for Research on the Effects of Fertility on Changing Roles of Women and Men (NICHD-SBS-81-5) requested proposals for research on the consequences of childbearing and childspacing for men and women in the U.S. which take into account age of parents, age of children, family size, family income and other factors, such as region of the country and urban/rural residence. The focus of this RFP was on research dealing with the effects of childbearing and childrearing on activities changed, added and foregone by both men and women.

Research contracts were also solicited for Research on Delayed Childbearing (NICHD-SBS-81-7). Delayed childbearing has considerable social, economic and

health significance. The goal of this RFP was to enhance the understanding of delayed childbearing in the U.S. by supporting research on the trends, in terms of numbers of births and birth rates; the social and demographic characteristics of couples who delay childbearing; and the correlates, determinants and consequences of delayed childbearing.

Institutional Programs in Multidisciplinary Population Research

The Social and Behavioral Sciences Branch support three kinds of Institutional Programs in Population Research: Program Projects, Population Research Centers, and Specialized Population Research Centers at major institutions in the United States. Support for these institutional programs enables coordinated, highly skilled groups of behavioral-social scientists in a variety of disciplines to organize and conduct multidisciplinary population research programs. These programs are designed to attack complex population problems that cannot be adequately studied by an individual investigator working alone.

A. Population Research Centers (A Population Research Center grant provides for centralized services and facilities required for enhancement of the quality and productivity of existing population research projects.)

Population Research Centers supported by SBSB, together with their major research areas, are located as follows: (1) University of Wisconsin at Madison--demography and human ecology; (2) University of Texas at Austin--demography of minority groups and population change and distribution; (3) The Johns Hopkins University--demography, teenage fertility, and reproductive biology; (4) Princeton University--demography, population economics, and statistics; (5) University of Washington--fertility, mathematical demography, and human ecology; (6) University of Michigan--fertility, population distribution and differentiation, and economic demography; (7) University of Pennsylvania--fertility, population movement, and economic demography; (8) University of North Carolina--fertility, family planning, migration, and population statistics.

B. Specialized Population Research Centers (Specialized Population Research Center grants provide for the support of a comprehensive population research program that is specifically responsive to research areas specified by the CPR.)

The SBSB is funding two Specialized Population Research Centers. The Center at the Rand Corporation, Santa Monica, California, has two major foci: (1) the demography of families and households and (2) regional and local population changes. The Center at the National Opinion Research Center, Chicago, Illinois, specializes in the economic demography of the family.

Population Research Manpower Development

A. Institutional Fellowships

The NICHD awards grants for the support of institutional behavioral-social population research training programs, behavioral-social predoctoral and postdoctoral trainees, research career development of individuals with outstanding potential for independent behavioral-social population research, and

senior postdoctoral fellowships. During fiscal year 1981, the NICHD awarded population research training grants to 12 behavioral-social programs at the following institutions: Princeton University; University of Wisconsin at Madison; University of Michigan, Economic Demography; University of Michigan, Population and Human Ecology; Brown University; University of Texas at Austin; Florida State University; University of Pennsylvania; Yale University; University of Chicago; University of North Carolina, Population Studies; University of North Carolina, Population Statistics.

B. Individual Postdoctoral Fellowships

Five behavioral-social postdoctoral fellowships were awarded during fiscal 1981.

C. Research Career Development Awards

One Research Career Development Award was made in fiscal 1981.

D. Senior Postdoctoral Fellowships

One Senior Postdoctoral Fellowship was awarded during fiscal 1981.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Support of Bibliographic Preparation of Population Index
Contract Number : NO1-HD-12138
Contractor : Princeton University
Money Allocated : $98,288 (1971); $72,984 (1973); $90,879 (1974); $99,333 (1975); $94,235 (1976); $93,089 (1976 TQ); $99,821 (1977); $110,193 (1978); $116,123 (1979); $122,412 (1980)

Objectives: The purposes are to support the bibliographic preparation by Office of Population Research staff of Population Index as a quarterly journal containing bibliographic information and special articles in the population field. Printing and distribution costs are paid by other sources of funding.

Major Findings: A 1977 site visit to the Office of Population Research (OPR) reaffirmed the value of the Index, now in its 47th year, as a comprehensive guide to articles, books, and other significant materials from the world literature, including in-house publications of relevant research organizations that deal directly with population. Publication will continue in essentially its present form. The front, author, geographic, and statistical sections are computerized. Following publication of an introductory paper (Oct. 1979), "Section T--Machine Readable Data Files" was added to the Bibliography beginning with the Jan. 1980 issue. Following up 1935-1968 cumulative publication of items, computerization of citations for the years 1975-78 are now ready (with the support of the Agency for International Development) for incorporation into a new POPLINE retrieval service under the National Library of Medicine, and citations for the period 1978-1980 will be available in the summer of 1981.

The 1980 Population Index included a special annotated guide to over 800 regularly recurring series of national and international official vital statistics (including natality, mortality, abortion, and marriage) and migration statistics. Articles published by the Index during the past year include a description of demographic data available from the Geneological Society of Utah, and a methodological study on the completeness of reporting of adult deaths in populations that are approximately stable.

Significance to Biomedical Research and Program of the Institute: Used throughout the world, Population Index is indispensable to demographic research and teaching, family planning, and to approaches to economic and social development. Its circulation of 4,770 includes all members of the Population Association of America and of the International Union for the Scientific Study of Population. References to health research, planning, and delivery systems make it essential to the health mission of NIH in the population sciences.

Proposed Course: This contract, initially funded in 1971, was renewed in 1978 for three years to 1981.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	A Study of Couples' Motivations for Parenthood, Decision-Making, and Fertility Regulation
Contract Number	:	NO1-HD-52807
Contractor	:	University of California, Los Angeles
Money Allocated	:	$125,209 (1975); $62,925 (1977); $142,463 (1978); $133,915 (1979)

Objectives: This project examined the relationships among motivations for parenthood, division of power and responsibility in the marital dyad, and fertility behavior. Six hundred couples, with wives aged 15-34, were interviewed twice during a three-year period. Half the couples were recently married for the first time, and the other half recently had a first child. In each subgroup, one member of each couple--half the husbands and half the wives--was designated as a primary respondent from whom most of the information was collected.

Major Findings: Individuals reported more agreement regarding fertility decisions and preferences between spouses than actually existed. It appears that preferences for children are reassessed after the birth of a child rather than emerging from a gradual crystalization of individual preferences into a joint decision during the early years of marriage.

Among couples without children, both husbands and wives who decided not to have a child in the next two years evidenced concern about its negative effects on employment. For the entire sample, spouses' wishes regarding a/another child also were an important factor differentiating persons who decided to have or not to have another child in the next two years. When spouses disagreed on whether to have a child in the next two years, the wife appeared somewhat more influential in determining actual fertility outcome almost two years later, especially in cases where she was the one who did not want the child.

Demographic factors generally influenced desires, intentions, and decisions indirectly through their effects on attitudes and motivations. A significant exception was that age had a direct positive effect on short-term fertility desires. Among the married (no child), husbands' attitudes and motivations strongly influenced wives' attitudes and motivations, while among the babied (one child), the opposite pattern occurred.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the antecedents, determinants, and correlates of fertility.

Proposed Course: This study was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Longitudinal Study of Intended and Actual Fertility: The 1975 National Fertility Study
Contract Number : NO1-HD-52819
Contractor : Princeton University
Money Allocated : $589,950 (1975); $228,945 (1976 TQ); $230,614 (1977); $215,990 (1979); $214,398 (1980)

Objectives: Toward the end of 1975, some 3000 white women who were interviewed in the 1970 National Fertility Study, specifically all those married at less than 25 years of age, and not before 1951, whose first marriages are still intact, were reinterviewed, along with some 1000 white women of comparable marital status and age at marriage who married during the years 1971-75, to collect complete pregnancy histories and a record of use of the various modes of fertility regulation (contraception, abortion, and sterilization) vis-a-vis each pregnancy. Data collected consisted of both retrospective and prospective fertility intentions, attitudes toward reproduction and modes of fertility control, as well as information of explanatory value concerning such topics as experience in the labor force, attitudes toward sex roles and population problems, etc.

Major Findings: An analysis of marriage cohorts reveals that the era covered by the survey has been characterized by a large decline in the number of intended conceptions, a decline in rates of failure to delay or to terminate pregnancy, an increase in discontinuation rates for both the pill and the IUD, and the widespread resort to sterilization as the way to end reproductive life. Concomitantly, there was an increase in the age that women thought was the ideal age at which to have a first birth.

Women who work between marriage and the birth of their first child were likely to delay the second birth also, but others who wanted to work eventually stayed out of the labor force for the time being and had their second child sooner. After having two or more children, taking a job was likely to be associated with the decision to give up further births orginally intended.

The conclusions of the 1975 study regarding sex pre-selection were similar to those derived from the 1970 study. It was found that control of the sex of offspring is not the desire of the majority of U.S. women, but that if preferences prevailed, there would be a significant increase in sons as firstborn and daughters as second children. The overall sex ratio would be little changed from that occurring naturally except at very low fertility levels with universal use of such technology. Fertility is influenced by gender preference, but that influence is minimal in a low fertility population.

Significance to Biomedical Research and Program of the Institute: The study will contribute materially to the Center's program of understanding changes in fertility behavior of American women by allowing genuine causal analysis of the dramatic changes in fertility during this period.

Proposed Course: This study was initiated in FY 75, and the current series of analytical studies was completed during FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	A Study of Low Fertility Cohorts in the United States
Contract Number	:	NO1-HD-32799; NO1-HD-62818
Contractor	:	Georgetown University
Money Allocated	:	$99,911 (1973); $16,140 (1974); $22,913 (1975); $335,591 (1976); $424,410 (1977); $167,227 (1978); $70,516 (1979); $74,314 (1980)

Objectives: This study sought a deeper understanding of the low fertility of the 1920s and 1930s in the United States, by studying cohorts of women born during 1901-1910. The survey questionnaire included items on background characteristics, pregnancy and contraceptive history, attitudes toward family size, as well as measures of the influence of the Depression on the respondents' lives.

Major Findings: Lower fertility was observed for higher age at first marriage and shorter duration of marriage. Lowest fertility was observed among women who delayed marriage until after the Depression, though this may in part reflect both the higher education and the lower fecundity of those marrying at later ages.

Sixty-one percent of the women had their first birth in their twenties; almost half had terminated their childbearing by age 30. Over 70 percent used contraception at some time, and another 16 percent reported using douche for cleanliness only. Condom, douche, and withdrawal were most commonly used; reliance on male methods highlights the important role of husbands in the control of fertility in these cohorts.

Characteristics associated with a relatively low probability of first birth and with relatively long birth interval included high levels of education for husband and wife, non-farm or urban residence during the first ten years after marriage, marriage after the start of the Depression, non-Catholicism, and husband's white collar occupation. Women who worked during the first interval also had relatively long first birth intervals.

The fertility of women hit hardest by the Depression was at least as high as and often higher than the fertility of women less severely affected. The data show a positive association between individual hardship and contraceptive practice, and a negative relation between individual hardship and wantedness of births.

Significance to Biomedical Research and Program of the Institute: As part of the Institute's interest in understanding the determinants of fertility, this project fills a major research gap. The women studied had the lowest total fertility rates ever recorded in the United States, a phenomenon that occurred before the most efficient contraceptives became available. These women's motivations and the means they used to achieve record-low fertility are important to further understanding of the practice of birth control and the motivations for parenthood.

Proposed Course: This project expired in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Alienation and Fertility Control in the Early Years of Marriage
Contract Number : NO1-HD-62833
Contractor : Bowling Green State University
Money Allocated : $142,361 (1976); $125,117 (1977); $111,309 (1978); $27,606 (1980)

Objectives: This research tested the relation between alienation and fertility with respect to a broad range of dependent variables, including family size preferences, early contraceptive and pregnancy decision-making, contraceptive use, premarital and unplanned pregnancies, and abortions. Interest centered on the early years of childbearing. Data were collected by means of self-administered questionnaires from 610 once-married couples, classified according to wife's age at marriage and marital duration. Methodologically, in addition to development of new measures of alienation, reversal of the alienation concepts resulted in positive measures of social integration. This work continued and expanded on previous work by the principal investigators.

Major Findings: Results showed that the alienation variables of meaninglessness and isolation were related to decision-making at several points in the family formation process. The more highly isolated were more likely to have engaged in premarital intercourse, to have cohabited, and to have preferred the delay of marriage to a later age. Those characterized by high levels of meaninglessness were likely to have begun dating at an early age, to have engaged frequently in premarital intercourse, to have terminated their education at the high school level or less, and to have had a short delay in the time interval between the decision to marry and marriage itself. High scores on meaninglessness were associated with greater perceived disadvantages of birth control and lower levels of knowledge about sex and reproduction.

The meaninglessness variable, in effect, operates as a self-fulfilling prophecy. People who perceive their broader social environment as being chaotic and unpredictable are likely to refrain from conscious and deliberate planning. Hence, they tend to develop a life style characterized by social drift, responding to events as they happen rather than deliberately causing them to happen through their own efforts. Having sexual intercourse without using effective methods of contraception frequently occurred in couples' premarital couple relationships. In early marriage relationships, also, fertility control was often haphazard and ineffective. Nearly half of the first pregnancies were either unintended or unwanted, suggesting that certain social, psychological obstacles are operating as impediments to rational control and mastery.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the antecedents and determinants of fertility.

Proposed Course: This study expired in FY 81.

Contract Title : Fertility Values and Family Growth
Contract Number: NO1-HD-62834
Contractor : Temple University
Money Allocated: $180,753 (1976); $30,049 (1978)

Objectives: This project examines the developmental effects of actual fertility on fertility values within religious and SES subgroups. A previous study indicated that the experience of bearing children was related to people's perceptions of a range of values related to fertility and family size desires. However, because longitudinal data were not available, it could not be determined whether people with three children had different values from those with no children because of the presence of children. The study proposed here will establish more clearly: (a) the effects of parity increase on the relationships of fertility values to desired family size; (b) the fertility value relationships that are associated with Catholic/Protestant affiliation, social class and those that are independent of these two factors; and (c) the parental experiences associated with an increase in parity.

Major Findings: Preliminary analyses of cross-sectional data produced a number of tentative conclusions which will be further tested when longitudinal data is available. Generally, the perceived costs of children are more related to family size desires than are the perceived benefits of children. Anticipation of the rewards of children were most important for the initiation of family building and recognition of costs of children were most important for the decision to terminate family growth only for upper status Protestants. The importance of the arrival of the first child in changing perceptions about childbearing is evident from two findings: (1) anticipated rewards of childbearing were found to be related to desires for larger families mainly for childless couples; (2) the proportion of respondents reporting unexpected problems when their (last) child arrived was significantly greater among women with only one child than among those with two children. However, the arrival and experience of each child in the family altered the relationships between fertility values and desired family size. The satisfaction of child care and development was an important fertility value only among Protestants. Concerns about family interactions in the form of giving attention among family members was an important fertility value, although its prominence varied among social subgroups. Financial matters were a special fertility concern of the Protestants, particularly the lower status ones. Protestants differed from Catholics in their developmental pattern of expectations of and reactions to the consequences of children, but fertility values generally were little related to fertility desires among the Catholics.

Significance to Biomedical Research and Program of the Institute: This project will enhance our understanding of the process of family formation and improve our understanding of the consequences of family size by elaborating how fertility influences future decisions about having children.

Proposed Course: The first stage of this project was completed in FY 80.

Contract Title : The Consequences of Family Structure and Maternal State for Child and Mother's Development
Contract Number : NO1-HD-82807
Contractor : University of Southern California
Money Allocated : $174,357 (1978); $98,849 (1979)

Objectives: This is a study of the effect of family size and childspacing upon the health and social development of children, using a Danish population of 9000 children born in 1959-61 in Copenhagen. Data will be analyzed to determine effects of order of birth and sex, age of mother, wantedness, spacing, perinatal factors, parental health and social and family structure variables. Additional data will be collected on development of children, school performance, delinquency, and psychological and social functioning.

Major Findings: Level of maternal education and mother's general contentment with her situation, as well as family stability, were most predictive of a child's later academic success. Mother's education and family stability had the greatest impact on psychosocial adjustment. The relevance of mother's age seemed to indicate that the increased degree of emotional problems of children whose mothers had started childbearing at an early age may be related to both the higher degree of instability that frequently characterizes such homes and to other factors related to the behavioral characteristics of the younger, less experienced mothers. Physical status is more sensitive to the availability of economic resources than to any other class of variable. Consistent relationships were found between family size and measures of physical growth, academic performance and psychosocial functioning.

Birth order showed virtually no relationship with the measures of growth, academic performance and psychosocial functioning. For the low socioeconomic status group, the relationship between family structure and academic functioning was similar to that generally found, i.e., older children and children from smaller families were found more competent. In the middle-to-high SES group, no effects were found on academic outcomes. On the psychosocial outcomes, firstborns, especially those in large families, showed more emotional responsivity. Spacing less than two years to a younger sibling appeared to be detrimental to academic performance. Spacing of less than two years or more than four years to a younger sibling was less conducive to optimal psychosocial functioning of the child.

Significance to Biomedical Research and Program of the Institute: This study explores the consequences of family size, birth order, and childspacing for a variety of health, psychological, and social outcomes.

Proposed Course: This study was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : American Attitudes Toward Abortion
Contract Number : NO1-HD-82832
Contractor : University of California at Los Angeles
Money Allocated : $92,668 (1978); $13,725 (1980)

Objectives: This study analyzed existing data on abortion attitudes during the past two decades as expressed in U.S. national samples. The emphasis was on demographic and social-psychological correlates of attitudes toward various aspects of the abortion issue. The two phases are interrelated. The first emphasized descriptive, sociodemographic and locational aspects of abortion attitudes, including changes over time. The second major component studied sociological and personality predictors of abortion attitudes. The project utilized Gallup survey data going back to 1962, data from the National Opinion Research Center (NORC) surveys, and information from the 1965 and 1970 National Fertility Surveys (NFS). Disaggregation of abortion attitudes as the dependent variable--timing of abortion, belief about when life or personhood begins, government aid for abortion, etc.--appeared to be increasingly significant as people become more aware of the complexity of the abortion problem.

Major Findings: Examination of changing attitudes over a five-year period revealed that the apparent stability of 1970-1975 cross-sectional data on abortion attitudes conceals a great deal of movement among categories, although typically the shifts are not great. That is, those characterized as being negative in 1970 moved to adjacent categories; there were few major shifts to positive categories. Those positive in 1970 demonstrated a high retention rate. Those in the middle category at the earlier point in time who shifted were more likely to move to negative or inconsistent positions than to the positive pole.

Consistent with prior analyses, it was found that religious affiliation, combined with an indicator of the frequency of attendance of church or of communion is the most important predictor of abortion attitudes. Frequently communing Catholics were the most negative, followed by frequently attending fundamentalist Protestants.

Following the religious variables in importance is a cluster of influences related to familialism--ideal family size and attitudes toward women's roles, plus changes in these attitudes. During the time period 1970-1975, few major changes in overall attitudes were taking place. But, such as did occur, took place primarily among women who did not already have certain structured religious or familialistic positions.

Significance to Biomedical Research and Program of the Institute: This research is central to the Program of the Center and is highly relevant to an understanding of the determinants of choice among various methods of fertility control.

Proposed Course: The project expired in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Psychological Factors Associated with Fertility Regulation: A Comparison of Pregnant and Non-Pregnant Adolescents
Contract Number : NO1-HD-82833
Contractor : The Philadelphia Health Management Corp., Philadelphia, PA
Money Allocated : $85,585 (1978); $115,055 (1979); $61,499 (1980)

Objectives: The primary purpose of this study is to examine the psychological/thought processes operating when a female adolescent does or does not become pregnant. A secondary purpose is to conduct an exploratory study of male adolescents' knowledge and attitudes toward contraception and attitudes toward teenage fatherhood.

The subjects for this study are predominantly black female and male urban adolescents of low socioeconomic status (SES). The first sample consists of 90 adolescents in the third trimester of pregnancy who have volunteered for one of two alternative programs run by the Philadelphia School District; these adolescents will be interviewed once, as they enter the program. The second sample is a comparison group of 194 non-pregnant adolescents, matched (2 to 1) with the first group on age, residential location (as an indicator of SES), and race. These adolescents will be interviewed twice. At the initial interview they will not be pregnant; they will be interviewed a year later, when it is expected that approximately 25 percent will have become pregnant. The third sample consists of 90 male black adolescents who have volunteered for a school district group discussion series on sexuality.

Major Findings: Second interviews with adolescent girls focused on familial communication, particularly between the mother and the adolescent. Efforts were made to determine whether girls who are contraceptive users or who are virgins exhibit greater interpersonal skills than do those who do not use contraception or who are non-virgins. No difference in problem-solving skills was found among the adolescent subgroups for the different messages transmitted by the mother regarding pregnancy. Some distinctions were evident between the subgroups for the different messages concerning sexual activity and contraceptive use. Those who were given advice about sex and birth control were more likely to use contraception. Non-contraceptors and virgins were more likely to have heard criticism of contraception. In the performance of means-goals tasks, virgins and contraceptors stated more means of achieving goals. Interpersonal skills seem to be taught by mothers, independent of IQ and problem-solving abilities. The transfer of skills seems most dependent on the mothers' own skills and with their efforts to communicate with their daughters. The daughters of non-communicative mothers show fewer interpersonal skills. What emerges as most important in the communication of sex-related topics is the communication in and of itself; the specific content of the conversation is less critical.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the correlates of adolescent fertility behavior.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Adolescents' Use of Information about Conception and Contraception
Contract Number : NO1-HD-82836
Contractor : Planned Parenthood Association/Chicago
Money Allocated : $91,224 (1978); $61,419 (1979); $15,000 (1980)

Objectives: Couples of different ethnicities from five Chicago area teen family planning clinics were chosen by quotas within each clinic to obtain five females seeking contraception, five who are pregnant, and five with a negative pregnancy test or "pregnancy scare." At the end of one year, 50 additional female adolescents will be interviewed as controls. Piaget's theoretical work suggests that mere assessment of knowledge levels among adolescents concerning contraception and other sexual matters is insufficient to understand the role of that knowledge in behavior , i.e., that adolescents assimilate knowledge selectively and then use it differently depending on their own situations and cognitive processes.

Major Findings: Six patterns were found in the histories of contraceptive use of female teen clinic attendees: (1) proper or almost proper use from first intercourse onwards; (2) proper use after an initial period of adjustment to the fact that they will be sexually active; (3) proper use after a sometimes lengthy period of repeated sexual intercourse; (4) less direct paths to proper use; (5) some, but no consistently proper, method use; (6) no use of contraceptive methods.

Almost 90 percent of the young women follow one of the first four paths leading to consistent and proper use. All daughters of highly educated parents and most daughters of those with only elementary education do so. Those in the fifth and sixth patterns have lower ages at first intercourse and more different partners than young women who, sooner or later, achieve proper use; they have also often experienced abandonment, abuse, and alcoholism.

Findings suggest that when parents or others model efficacy and goal-directed behavior they provide the opportunity for adolescent females to learn these skills and to apply them to the proper use of contraceptives. The family experiences of those who never achieve proper use provide instead an impression that life's problems are too difficult, complex, and unpredictable to master.

Significance to Biomedical Research and Program of the Institute: This research is central to the Institute's program and is highly relevant to departmental initiatives and to Congressional interest in the effectiveness of present modes of presenting such information to adolescents.

Proposed Course: This project is scheduled to be completed in FY 81.

Contract Title : Adolescents' Perspectives on the Health Care System: A Determinant of Fertility
Contract Number : NO1-HD-82837
Contractor : University of North Carolina
Money Allocated : $105,786 (1978); $108,120 (1979); $58,225 (1980)

Objectives: The purpose of this research is to identify the nature and sources of adolescent women's perspectives on the established medical care system, and the impact of these perspectives on their use of the system for fertility-related services. The specific objectives are: to identify the reasons why adolescent women may be reluctant to use the established medical care system for fertility-related services, and to assess the usefulness of various techniques for collecting data about adolescents.

Major Findings: Major findings related to adolescents' perspectives of the medical care system and use of the system for contraceptive purposes can be grouped according to parent-related issues, such as fear of discovery and reluctance to reject parental values overtly, and thoughts and feelings about doctor-teen interactions. The ambivalence of teens about parental influences on their medical care is demonstrated by their frequent mention of the importance of parental approval and support while concurrently stressing their interest in establishing an independent relationship with the physician. These concerns are heightened when teens seek reproductive system care; confidentiality becomes paramount and their evaluations of a doctor's trustworthiness on this matter can determine whether they visit the doctor for contraceptives.

Teens want doctors to discuss health matters directly with them, to give thorough explanations of procedures and options, and to show a personal interest in them. Most teens agree that doctors should maintain a professional attitude, but show humor and flexibility. These aspects of the physician-patient relationship increase in importance when applied to reproductive health care. Here, the need to enhance teens' comfort and ease their embarrassment is critical. Physicians offering to provide information is preferable to their questioning the teen. A non-judgemental, matter-of-fact presentation which stresses confidentiality is most valued.

Significance to Biomedical Research and Program of the Institute: Other studies have pointed to the high proportion of adolescent contraceptors who use clinic services, but little research has addressed adolescents' avoidance of the established medical care system. This system may be the only one large enough to meet adolescents' demands for family planning and related services.

Proposed course: The project was begun in FY 78 and is scheduled for completion in FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on the Determinants of Repeated Adolescent Pregnancy and Childbearing
Contract Number : N01-HD-82838
Contractor : Johns Hopkins University
Money Allocated : $52,752 (1978)

Objectives: This project used the extant data base of a Johns Hopkins longitudinal research and service program established in 1973 for adolescent mothers to identify those young women who had a subsequent (unwanted) pregnancy and to compare them with matched controls who did not experience a subsequent pregnancy. The study, which included about 90 cases and an equivalent number of controls matched by race and age, analyzed data collected during the first pregnancy and at the four-week postpartum visit. The data available about mother, father, and pregnancy included: demographic, educational, and family background variables; contraceptive use; amount of prenatal education; and contraceptive prescribed postpartum. These objectives were to provide: a broad and complete picture of the problem among these urban adolescents; gross indicators of risk of repeated pregnancy; and information about the outcome of subsequent pregnancy.

Major Findings: During the past two years the frequency of repeated pregnancy has been only between five and six percent per year. Two problem areas were identified and amended by instituting the supply of counseling and a temporary method of contraception at the time of hospital discharge, and a follow-up visit at 21 months, reducing by half the span of six months between 18 and 24 months during which the frequency of pregnancy tended to increase.

Comparison of teenagers within the follow-up who became pregnant again with those who did not indicated that the important predictors of repeat pregnancy include: marriage, low socioeconomic status, school dropout, low I.Q. (about one-fifth of the repeaters functioned in the mildly retarded range), problems with contraception as indicated by frequent changes in method, history of prior pregnancy often terminated by abortion, and missed appointments. The relationship between the number of contraceptive changes and the risk of repeated pregnancy is of particular interest and should provide a useful indicator for intensified preventative efforts. The white teenagers in this population appear to be at greater risk than the black.

Significance to Biomedical Research and Program of the Institute: This research, requested under RFP 78-8 relating to "the determinants of adolescent pregnancy and childbearing," is central to the program of the Institute and is highly relevant to Congressional interests. The project's considerable value lies in the study of young people who create hardships for themselves and society--adolescents with repeat pregnancies. The research may generate predictive data about individual characteristics as well as identify the structural sources of repeat pregnancies.

Proposed Course: This project expired in FY 80.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sociosexual Development in Black Adolescents within the Family Context: A Longitudinal Study
Contract Number: NO1-HD-82840
Contractor : Howard University Hospital
Money Allocated: $99,499 (1978); $107,253 (1979); $112,630 (1980)

Objectives: The purpose is to explore the effects of psychological adjustment, sexual knowledge and attitudes, family interaction, moral and ethical reasoning, socioeconomic status and environmental impact on sexual behavior. The project involves a five-year longitudinal study of 99 black early adolescents and their parents. Periodic medical examinations, unstructured interviews using a variety of stimulus materials, and videotaping of family interaction will be used. Participants will be a volunteer non-representative sample of healthy 10-year-olds from low and middle income families, evenly split male and female. Test materials (pictures, incomplete stories and simulated taped conversations) will be developed. Some family interaction observations will be made.

Major Findings: Eighty-six percent of the girls and eighty-nine percent of the boys reported that their main source of reproductive information was another family member, usually but not always the mother. Older age of respondent, middle income status, and single-parent family structure were each positively correlated with higher levels of reproductive information, as indicated by content, sophistication of language used, and accuracy of the process described. Biological maturation showed significant but opposite relationships with level of information for both sexes. For girls, there was a positive relationship between development and level of information, whereas the association was inverse for boys. This inconsistency may be due in part to the greater number of girls as compared to boys who had their onset of pubertal maturation at this point in time.

Achievement scores as measured by the Wide Range Achievement Test (WRAT) had differing levels of significance when related to the participant's level of information about the reproductive process. For girls, there was an inverse relationship, with higher WRAT socres correlating with less reproductive information. For boys, there was a positive but insignificant relationship. Higher achieving girls appear to have less information in this realm, though higher achieving males have similar levels of information in the basic areas of arithmetic, spelling and reading as they do about the reproductive process.

Significance to Biomedical Research and Program of the Institute: This study addresses the social and physiological changes around adolescence relating to a sexual development. Concern about adolescent sexual and reproductive behavior is shared by biomedical and social scientists. This proposal addresses a critical juncture of the two fields on an issue of considerable importance.

Proposed Course: This project began in FY 78 and is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : An Analysis of the Determinants of Adolescent Pregnancy and Childbearing
Contract Number: NO1-HD-82841
Contractor : Mathematica Policy Research, Inc.
Money Allocated: $83,695 (1978); $49,120 (1979)

Objectives: Data from the 1971 Kantner and Zelnik national probability sample of females aged 15-19 were used to analyze four components of adolescent fertility behavior: sexual activity, contraceptive practice, pregnancy, and decisions about the outcome of pregnancy. These components were examined in relation to pertinent socioeconomic variables: labor market experience, family structure and background, education, and sexual and contraceptive knowledge. The research was undertaken to develop a model of adolescent fertility behavior that would incorporate these socioeconomic variables.

Major Findings: The probability of intercourse increases with age, particularly at ages 18 and 19. Teenagers who believe religion is important or who attend church frequently appear to be less sexually active than otherwise similar teenagers. Black teenage women are significantly more likely to be sexually experienced at each age, yet are much less likely to possess correct knowledge of the timing of conception, thereby increasing the risk of an unplanned pregnancy.

The most important predictors of contraceptive use are the sexual activity variables. Both the consistency and effectiveness of contraceptive use depend positively on the frequency of sexual activity and the number of sexual partners. This suggests that sexually experienced teenagers reduce the risk of pregnancy associated with increased sexual activity by a higher probability of contraceptive use and by using more effective contraceptive methods. Family structure, family income, number of siblings, and parental education have virtually no influence on any of the contraceptive use variables.

The family background variables do appear to affect the probability of a pregnancy among white adolescents. The likelihood of pregnancy is lower among whites from high income households and whose mothers are highly educated. Blacks appear significantly more likely to become pregnant than whites, suggesting there are racial differences in either the ability or the motivation to prevent pregnancy. For both races, the likelihood of pregnancy is negatively related to educational expectations. Educational expectations is also an important predictor of pregnancy outcome, as teenagers with high educational aspirations are less likely to have a baby if pregnant. Black teenagers are more likely to have a live birth than are whites, and are less likely to legitimate the birth by marriage than are whites.

Significance to Biomedical Research and Program of the Institute: Adolescent pregnancy is a problem of high priority for the Institute. This research on fertility behavior pertains directly to the antecedents and prevention of adolescent pregnancy.

Proposed Course: This project was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Policy Determinants of Teenage Childbearing
Contract Number : NO1-HD-82843
Contractor : The Urban Institute
Money Allocated : $64,253 (1978); $30,657 (1979)

Objectives: This research assessed the relations of various state and federal programs to adolescent childbearing. The programs presumed to have a direct effect were family planning services, abortion availability, age of consent laws concerning contraception and abortion, Medicaid coverage of abortion, welfare benefit levels and acceptance rates, welfare coverage of unborn children and unemployed fathers, and benefit levels in food stamp and Medicaid programs. Indirect policy influences included local unemployment rates, female wages, and educational opportunity. State information on these programs and policies was added to the 1976 public use tape of the Survey of Income and Education. This survey provided data on fertility, demographic, and family characteristics of an estimated 30,000 females aged 14-19. Two sets of analyses were performed: (1) characteristics of individual females, plus state-level policy and control variables, were regressed on whether a female aged 14-19 had a child under one year of age; and (2) state-level policy variables plus state-level control variables were regressed on adolescent fertility rates in each state and a subset of cities.

Major Findings: The research results did not substantiate the hypothesized influence of state and federal programs on adolescent childbearing. In fact, measures of benefits from Aid to Families with Dependent Children (AFDC), when statistically significant, were consistently negative, and AFDC policy variables were consistently unimportant. Similarly, most measures of abortion availability were not significantly related to the dependent variable; however, the several significant measures suggested that greater availability was associated with lower fertility. Most family planning measures were not significant, and the significant measures varied in direction. Measures of educational opportunity did show a fairly consistent relation between higher female educational achievement and lower adolescent fertility. In this analysis the availability of sex education did not affect fertility, but a more refined measure of availability is needed. Only age and race were found to be strong and consistent predictors of the incidence of teenage childbearing. Greater educational opportunities and better employment opportunities for women were related to somewhat lower fertility, while higher income levels in the state or SMSA seem related to higher fertility.

Significance to Biomedical Research and Program of the Institute: This research addresses a topic of considerable importance and interest in both biomedical and social science areas--adolescent fertility.

Proposed Course: This project was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Exploration of the Effects of Organized Family Planning Programs in the United States on Adolescent Fertility
Contract Number: NO1-HD-82844
Contractor : The Alan Guttmacher Institute
Money Allocated: $74,546 (1978); $56,601 (1979)

Objectives: This study assessed the impact of family planning clinic programs in the United States on the fertility of black and white female adolescents (aged 15-19). Several measures of social, demographic and family planning service characteristics were compiled for Statistical Analysis Units (counties or clusters of counties) and then subjected to multivariate analysis to determine: (1) the association between adolescent birth rates and the availablility and utilization of family planning clinic services; and (2) the association between changes in the availability and utilization of clinic services and changes in adolescent fertility rates from 1970-1976. The multivariate analysis controlled other determinants of adolescent birth rates and of the impact of abortion and private physician services as alternatives to clinic services. Data sources included: (1) county-level data from special tabulations of the 1970 Census to provide 1970 fertility rates for women aged 15-19 by race, as well as family planning program data for 1969 and socioeconomic and demographic control variables for 1970; and (2) newly available data for 1975 and 1976.

Major Findings: Preliminary multiple regression analysis showed that on a cross-sectional basis (1970 and 1975), areas with higher proportions of adolescents enrolled in family planning programs were also areas with higher adolescent birth rates. This is a common finding in many countries, due to the location of clinics in high fertility areas and to differences in socioeconomic characteristics and levels of sexual activity.

As expected, early findings showed that areas with greater increases in the proportion of adolescents enrolled in family planning clinics between 1970 and 1975 generally had greater decreases in adolescent birth rates. The family planning program averted an estimated 119,000 births in 1975-1976 among women aged 15-19, 82,000 to whites and 37,000 to nonwhites. This equalled .101 births per adolescent patient or one birth averted for every 10 program patients.

Significance to Biomedical Research and Program of the Institute: This research is central to the program of the Institute. Though essentially an evaluation of family planning clinic services to adolescents, and thus highly focused on one determinant of adolescent fertility, the study addresses an important policy issue: whether differential availability of and enrollment in organized family planning programs measurably affects adolescent fertility. The study design should provide indirect evidence of this effect by presenting areal or ecological relations between the variables.

Proposed Course: This project was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Determinants of Fertility Behavior Among U.S. Females Aged 15-19, 1971 and 1976
Contract Number : NO1-HD-82848
Contractor : The Johns Hopkins University
Money Allocated : $109,576 (1978); $116,951 (1979)

Objectives: The researchers analyzed adolescent sexual, contraceptive, and reproductive behavior vis-a-vis clusters of independent variables: family background; socioeconomic status (SES); relative influence of parents and peers; religion; residence; plans for marriage, childbearing, education, and career; perception of norms about illegitimacy and abortion; employment; social life; and dating behavior. Data were from two national surveys of women aged 15-19, conducted in 1971 and 1976 under a grant from NICHD. The research methods included cross-tabulations, with race controlled and multivariate analysis.

Major Findings: Statistical analyses were employed to determine variables influencing various aspects of use of contraception (use at first and at last intercourse; regularity of use and of nonuse; ever-use of a medical method; use of a medical method at first and last intercourse; and premarital pregnancy (prevalence; mean age at first pregnancy; outcome of first pregnancy; marriage during first premarital pregnancy; and illegitimacy.

Analysis showed no difference of contraceptive use between whites and blacks. Socioeconomic status was related to the extent of contraceptive use--the higher the SES, the more likely contraception was used at first intercourse, last intercourse, and was always used. Current age, age at first intercourse, previous use, and pregnancy experience were all predictors of use. Race, current age, and religion were all predictors of use of a medical method of contraception; however, method used at first intercourse and experience of pregnancy are the two most important predictors of current use of a medical method. With respect to pregnancy, variables involving age and pregnancy intentions are the most important predictors. Unexpectedly, religious commitment is associated with lower mean age at first conception. Higher SES is important in the legitimation of a birth. Race is not significant in terms of whether or not a pregnancy occurs, but becomes an important factor once a pregnancy has occured in terms of outcome and whether legitimation occurs, with blacks more likely to have a live birth, and whites more likely to marry.

Significance to Biomedical Research and Program of the Institute: This research on adolescent fertility behavior addresses a topic of high priority to the Institute, using a major data source for the United States.

Proposed Course: This two-year project was completed in FY 81.

Contract Title	:	Social and Cognitive Development of Only and First-Born Children
Contract Number	:	NO1-HD-82849
Contractor	:	Educational Testing Service, Princeton, New Jersey
Money Allocated	:	$92,576 (1978); $95,844 (1979); $49,690 (1980)

Objectives: The research used data from a longitudinal study of children, their mothers, and mother-child interaction to make longitudinal and cross-sectional analyses contrasting the social behavior and cognitive development of only and firstborn children at ages 3, 12, 24, and 36 months. Planned and unplanned children also were compared. The decision whether or not to have a second child was examined from the perspective of the mother's locus of control, femininity, concept of ideal family size, and quality of interaction with the first child.

Major Findings: The data analyses on children and their parents indicated that differences in only and firstborn groups were related to three possible sources of variation: 1) the birth of a second child; 2) child characteristics; and 3) parent characteristics. The birth of a sibling was noted to change the congnitive performance of firstborn children and was related to a drop in skill performance. This suggests that firstborn children are different from onlies as a result of experiencing the entrance into the family of a new infant who diverts parental attention. Differences in the temperament characteristics of firstborn and only children may be reflected in the tendency of only infants to cry more and smile less than firstborn infants, which may affect parents' decisions of whether or how soon to have another child. Parents of onlies and firstborns may be different themselves and consequently engender differences in their children. Mothers of only children were older at the birth of their first child, expressed the desire for less children, and indicated a less positive attitude toward pregnancy and delivery as compared to mothers of firstborns. In terms of parental behavior, mothers of onlies were more involved with their infants.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the determinants and consequences of fertility behavior. More specifically, the findings represent a needed contribution to objective information about only children.

Proposed Course: This project was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Remaining Childless or Having Only One Child: An Analysis of Selected Outcomes for Husbands and Wives
Contract Number : NO1-HD-82850
Contractor : The Urban Institute
Money Allocated : $88,267 (1978); $89,107 (1979)

Objectives: This study analyzed the consequences of family size, specifically, zero and one-child families in relation to families with more children. The issues addressed were: economic well-being; time availability; schooling, training and labor force experience; geographic mobility; marital stability; and health. Besides the particular number of children, a concern of this research was the timing of births within the marital cycle. The analysis was based on the Michigan Panel Study of Income Dynamics, which studied heads of families, spouses, and their families from 1968-1976.

Major Findings: Although in the short run a birth reduces family income and lowers family living standards, the number of children has no long-term effects on a family's economic well-being. Impacting more strongly on the long-term well-being of a family is the timing of a birth. Women who delay childbearing until age 30 are better off economically than either younger childbearers or childless women. The addition of children changes family time use. For example, women drop out of the labor force after a first birth; however, they quickly return. Indeed, women with older children are employed more hours than childless women, although childless women do tend to be employed more years over their lifetimes.

The workload of the family increases differentially by birth. It increases the most with the first birth, and the least with the second; much of the increase is due to child care. Husbands and other family members assume more of the family workload as family size increases, as children grow and as mothers return to the work force. Finally, a first birth is associated with a dramatically reduced divorce probability.

Significance to Biomedical Research and Program of the Institute: Because increasing numbers of couples are electing not to have children or to have only one child, the consequences of such behavior are of growing interest and concern. This study assessed both the social and health consequences of very low fertility.

Proposed Course: This project was completed in FY 81.

Contract Title : Studies on the One-Child Family
Contract Number : NO1-HD-82851
Contractor : University of California, Berkeley
Money Allocated : $91,452 (1978); $69,592 (1979)

Objectives: This project compared only children and children with siblings on various physical and behavioral measures. Data, consisting of records of children enrolled in the Kaiser Health Plan, were collected during the mother's pregnancy and when the child was 5, 10, and 15-17 years old. The records included information on the mother's reaction to the pregnancy; the child's physical growth, cognitive abilities, and behavioral characteristics; and prospective data on intentions to have future children and contraceptive use. Consequences of being an only child (compared to being oldest and youngest in two- and three-child families) were analyzed, and the determinants of the one-child family were described.

Major Findings: One-child families apparently were rarely the result of deliberate choice. Only 5 percent of the mothers who had a 5-year-old only child reported having planned for this outcome. Late marriage or marital disruption after the birth of the first child had occurred in about half of the single-child families. One-child families, compared to those with two or three children, showed no significant differences at the time of the oldest child's birth in mother's or father's education, father's occupation, family income, mother's work history, or mother's occupation. The mother of an only child was no more likely than other mothers to have furthered her education, but she was more likely to have taken work outside the home, to have experienced marital disruption and/or remarriage, and to have relied on a child-care service or nursery school.

The research showed no adverse effects of being an only child. There were no important differences at ages 5 years, 10 years and 15-17 years between only children and the eldest of two or three children in physical development, utilization of medical care, or behavioral ratings; and there was some evidence that only children had marginally superior cognitive abilities.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the determinants and consequences of fertility behavior. More specifically, the findings represent a needed contribution to objective information about only children. At adolescence, some differences were noticeable in use of leisure time: onlies spent more time on reading, less time with clubs or groups. The onlies gave less importance to expectations of getting married, having children, and spending time with family.

Proposed Course: This project was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Personal Meaning of Voluntary and Involuntary Childlessness
Contract Number : NO-HD-82853
Contractor : American Institutes for Research in the Behavioral Sciences
Money Allocated : $47,981 (1978); $6,822 (1980)

Objectives: This study examined the antecedents to and personal meanings of childlessness within a marriage. In-depth psychiatric interviews were conducted with individuals from 74 intact marriages. The couples were of four types: voluntarily childless, involuntarily childless, natural parents, and adoptive parents. A composite picture of the main pathways through which married individuals and couples arrive at a childfree state was drawn from analysis of in-depth interviews, and a picture presented of the personal meanings of this state near the end of the reproductive period and during the post-reproductive period. In addition, a descriptive account of the life course of childfree individuals and couples was developed.

Major Findings: Results indicate that there is no clear demarcation between voluntary and involuntary childlessness and that many couples fall in a continuum between the two polar types. Whether a couple remains childless and where they fall on the voluntary-involuntary continuum depends upon the operation of many developmental processes and other factors that exert their influence throughout the individual's life course from infancy and childhood, through adolescence, to unmarried and, eventually, married adulthood. These causal factors may be grouped into six clusters: those that affect the individual's desire for children; those that affect the individual's desire for activities competitive with having children; those that affect the individual's opportunity for childbearing; those that affect the individual's fecundity; those that affect the acceptability and availability of evaluation and treatment for fecundity impairments; and those that affect the acceptability and availability of adoption to the individual. Marital couple interaction also has a major impact on ultimate childbearing status. Although there are numerous couple processes that contribute, three factors are particularly important: the sum total of both members' desire for children; the degree of difference between the two members' desire for children; and whether it is the wife or husband who has a greater desire for children. There were substantial differences between cohorts in the factors contributing to childlessness. The couples who entered their years of potential reproduction during the late 1960s and 1970s perceived childlessness as a more viable option and dealt with it more openly and directly.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants, and consequences of fertility.

Proposed Course: This study was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Fertility Research Resource
Contract Number : 1-YO1-HD-91041
Contractor : National Technical Information Service
Money Allocated : $110,868

Objectives: This project is for the generation of public use tapes with uniform documentation and their distribution through the National Technical Information Service (NTIS). The tapes involved are major United States surveys related to fertility, specifically: the National Surveys of Family Growth (NSFG), 1973 and 1976; the National Fertility Surveys (NFS) for 1965 and 1970; the Growth of American Families Surveys (GAF) for 1955 and 1960; and the June 1977 Current Population Survey (CPS) fertility and child care supplement. The Family Growth Surveys and the National Fertility Surveys will be structured into a hierarchical format in addition to the present rectangular format. Documentation will be standardized for the seven files and will include a machine readable CENTS-AID data base dictionary and a machine readable SPSS and OSIRIS codebook for the NSFG and NFS and GAF files. Comprehensive technical documentation also will be provided. An appendix will be created for the present CENTS-AID users' manual, illustrating procedures for using hierarchical files and will be based on NSFG and NFS examples. The NTIS also will provide a plan of distribution for the tapes once they are available.

Major Findings: All files, except the 1977 CPS, are being restructured into a hierarchical format. The manner in which the data were collected suggests three potential units of analysis: (1) respondents and their general attitudes about family size, contraception, etc.; (2) pregnancy intervals, including use of contraceptives and outcomes of pregnancies; and (3) live births. All files also are being restructured into a rectangular format at the pregnancy interval level. SPSS control cards are being prepared to serve as machine readable documentation for the rectangular files. A detailed users' directory is being written to describe the 1973 and 1976 NSFG data files, provide a thorough background of the two surveys, and index variables common to them. All files will have machine readable codebooks, prepared so that the CENTS-AID tabulation package can be used for analysis. The tapes are expected to be available in the Fall of 1981.

Significance to Biomedical Research and Program of the Institute: This project will provide an important new resource for persons wishing to study changes in fertility and fertility-related behavior that have occurred during the past 25 years.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Computer File of Studies on the Psychosocial Antecedents of Adolescent Pregnancy and Childbearing 1970-1980
Contract Number : NO1-HD-92800
Contractor : Western Washington University
Money Allocated : $122,509 (1979); $114,461 (1980)

Objectives: The purpose of this study is to construct a computer file of major studies of the psychological and social antecedents of adolescent pregnancy and childbirth. Nine studies conducted during the 1970s have been identified; however, others might be added. A major goal of the project is to produce an easily used data file available for secondary analyses. This will entail obtaining the data from principal investigators of the studies, establishing the quality of the data and the comparability of information from different studies, completing the file construction, testing the file, and writing a user's codebook and manual. Studies included in the file will be those that have measured psychological or social variables, have collected data on large samples of individuals aged 13-19, of either or both sexes, and have met accepted standards for scientific reporting of research methods and sampling.

Major Findings: There has been found to be a substantial degree of equivalence from one study to another in the measured variables. Up to 25 percent of the variables are equivalent across all studies, and an even greater proportion are common to two or more studies.

Work continues on preparation of questionnaire and interview items for inclusion in the computer file, and on compilation of the user's manual. The manual will contain condensed reviews of salient findings for many substantive areas in the file, such as sexual knowledge and education, contraceptive use, and attitudes toward sex and contraception. The methods and problems of secondary analyses also will be summarized in the manual. The file is expected to be available in the Fall of 1981.

Significance to Biomedical Research and Program of the Institute: This project will make available to the scientific community a large data set containing information on an important biomedical and social topic--adolescent pregnancy and childbearing. Early childbearing has been shown to affect both the health and the social well-being of the mother and child through factors such as low birthweight, complications of pregnancy, mother's education, and socioeconomic status. The collection of data is both costly and time consuming, and frequently the data are analyzed only by the original investigator. Further analysis of previously collected data can aid in answering many questions in the area of adolescent pregnancy and childbearing.

Proposed Course: This project began in FY 79 and is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Intellectual, Physical, Psychological and Social Consequences of Being Reared an Only Child
Contract Number : NO1-HD-92802
Contractor : Emory University
Money Allocated : $69,938 (1979); $71,756 (1980)

Objectives: This study undertook cross-sectional and longitudinal analyses of Cycles II (1964-65) and III (1966-70) of the National Health Examination Survey data on the only child compared with children in multiple-child families, to identify differences in characteristics between only children and others and to ascertain whether the differences were accounted for by mediating and control variables, such as parents' education and childrearing practices.

Major Findings: The major findings show no negative consequences of being reared an only child. If there are any differences between only and sibling children, they are slightly positive in favor of onlies. The so-called birth order effect of I.Q. disappears when level of parents' education is statistically controlled. The differences between siblings analyzed from the sibling sample also turned out to be insignificant and thus confirmed the non-existance of the birth order effect. Racial and social class differences in I.Q. scores and in academic achievement were not related to birth order but were highly positively related to the education level of the parents. When level of parental education is taken into account in respect to the I.Q. of black children and white children, two interesting results occurred: 1) the usual differences in I.Q. of black children and white children disappeared when the parents of the children had only five years or less of education, and 2) when the parents had thirteen or more years of education, the usual racial differences showed up, that is, white children scoring significantly higher. This may be a function of the differences in the quality of education received by black and by white parents in the 1930s and 1940s.

Mean I.Q. differences were stabilized by age six and remained parallel through age seventeen, suggesting the strong need to determine if these differences in I.Q. exist from the earliest ages of the children or develop subsequently before age six.

Significance to Biomedical Research and Program of the Institute: Considerable public and private interest centers on the main question addressed in this research: whether or not only children are different from or at a disadvantage to children with siblings. The research found no reasons why couples who wish to have just one child should not do so. The research makes an important contribution to the study of the consequences of fertility behavior.

Proposed Course: This project, initiated in FY 79, was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Childlessness and Status Attainment
Contract Number : NO1-HD-92804
Contractor : University of Texas
Money Allocated : $40,974 (1979)

Objectives: The aims of this study divide into three groups: (1) the detailed demographic description of childlessness from 1910 to the present by cohort; (2) the development and implementation of a measurement strategy for separating the phenomenon of childlessness into voluntary and involuntary categories, and an attempt to see how these two categories have changed over time; (3) the assessment of the impact of voluntary childlessness on wives' status attainment variables such as family earnings and occupational prestige, and the comparison of the status attainment effects of voluntary versus involuntary childlessness.

Major Findings: Three phases of voluntary-involuntary childlessness were identified as occurring in the U.S. since the 1920s. In the first phase (1920-1940), there was a moderate percentage of couples having no children by choice. Phase two is characterized by an overall reduction in childlessness with little if any of the remaining childlessness due to voluntary factors. Phase three began in the 1960s and is characterized by increases in overall childlessness, as well as increases in voluntary childlessness.

The investigator used two models, cognitive and behavioral, to separate voluntarily and involuntarily childless women; similar models were used for women with one child to classify them as voluntarily or involuntarily single-childed. The cognitive approach, focusing on knowledge of one's fecundity, produces a set of results different from that determined when one relies on the practice or non-practice of contraception as an indicator (the behavioral model). According to the cognitive approach, in 1955 and 1960 there were more women involuntarily childless than voluntarily childless. In 1965, 1970, and 1973 there were more women in the voluntarily childless category with the exception of women thirty and over in 1970 and 1973. On the other hand, the behavioral approach always yields greater numbers in the involuntary category for all ages, except in 1973 where the voluntarily childless women were more numerous among all childless women and among women under thirty. For women under thirty, there is a pattern of increasing levels of childlessness for each year sampled since 1955.

Significance to Biomedical Research Program of the Institute: This research provides demographic descriptive data on childlessness and addresses the consequences of childlessness for individuals and families, relevant to departmental and Congressional concerns.

Proposed Course: This project was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of the Consequences of Deciding to Remain Childfree, Compared with Deciding to Have Children and Deciding to Postpone Children
Contract Number : NO1-HD-92805
Contractor : University of North Carolina
Money Allocated : $71,425 (1979)

Objectives: This project is analyzing consequences of remaining voluntarily childless. The voluntarily childless are being compared with postponers, undecided, and parents to test the hypothesis that voluntarily childless wives will be more likely to: (a) emphasize the costs of children, negative motivations for parents having children and positive motives for remaining childless; (b) be characterized by dyadic withdrawal; (c) have an egalitarian division of labor in the home; (d) have consistently high achievement for the wives; (e) have high marital satisfaction; (f) have higher combined income, less debt, and higher satisfaction with their standard of living. The study is also investigating whether voluntary childlessness is a qualitatively unique phenomenon or a quantitative extension of a model that explains low parity processes. Analyses are being performed on extant data from a random sample of 186 voluntarily childless and 598 other married women.

Major Findings: Analyses show that the three childless groups have significantly higher marital satisfaction than the parents. The predictors of marital satisfaction, which included variables related to satisfaction with husband's economic provision, the woman's education, and dimensions of the couples' division of labor, were similar for the mothers and the postponers. Most differences between the postponers and the parents were due to differences on individual variables in the model. The processes leading to marital satisfaction for the voluntarily childless were different from those of the parents and the postponers. Both spouse involvement variables and satisfaction with husband's economic provison significantly influence marital satisfaction among the undecideds. The women in the three childless groups did a significantly smaller proportion of the total housework than the mothers. However, a substantial similarity was found between the sets of variables predicting division of labor for the parents and the postponers. The predictors of division of labor among the voluntarily childless were essentially the same as those for the undecideds.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the antecedents, determinants and consequences of fertility.

Proposed Course: This study expired in FY 80.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Long Term Consequences for Couples of Childlessness and One-Child Families
Contract Number : NO1-HD-92807
Contractor : The Pennsylvania State University, University Park
Money Allocated : $59,812 (1979); $31,562 (1980)

Objectives: This research utilized a 24-year longitudinal study of married women in Pennsylvania to evaluate the consequences for parents of being childless or of having only one child and also to examine several antecedent factors. Some 915 women from rural backgrounds, married only once and still living with their husbands, were interviewed as high school sophomores in 1946 (at about age 16), in 1957, and again in 1971. The data set, which included 45 childless women and 75 currently married women with one child, permitted an examination of the consequences for parents of childlessness and the one-child family. Antecedent factors, such as personality, family of orientation and birth order, and occupational aspirations, were available from the early data set. Consequences, including occupation and income aspirations and achievement, life satisfaction, aspiration for social mobility, and social participation, were available in the last data set.

Major Findings: The effects of a number of adolescent social and personality factors as well as adult family and socioeconomic status variables were statistically controlled in order to make clear the effect of fertility status on each of 14 consequence indicators; the indicators were related to life satisfactions, mobility aspirations and occupational attainments. While relatively few of the differences were statistically significant or large in absolute value, the findings were that involuntarily childless women had somewhat lower levels of life satisfactions while unintentionally one-child women had higher levels of life satisfactions and occupational attainment on a few key indicators than did their peers with two or more children. Implications of the findings include (1) the desirability of detecting infertility early in marriage so that advice and counsel can help women find satisfying alternative roles to homemaking, and (2) the dissemination of information to prospective parents concerning the advantages of the one-child family for satisfying work and homemaking roles for married women.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the biomedical and behavioral programs of the Institute in its focus on the consequences and correlates of fertility behavior.

Proposed Course: This project was completed in FY 81.

Contract Title : Sexual and Contraceptive Attitudes in Behavior of Young, Single Females
Contract Number : NO1-HD-92809
Contractor : The University of Guelph
Money Allocated : $26,354 (1979); $14,119 (1980)

Objectives: This project involved analysis of two data sets on adolescent sexual activity and contraception. Both data sets were collected in Ontario, Canada; one was from a film evaluation study of 514 females ages 17-22, and the other from a birth control clinic study of 486 females aged 13-20. The women were classified three ways: as virgins unlikely to have premarital intercourse; as virgins likely to have premarital intercourse; and as nonvirgins. The research focused on measurement and prediction of contraceptive use, and embarrassment about obtaining contraceptives.

Major Findings: Significant differences were found between virgin females who said they were and those who said they were not likely to engage in premarital intercourse. An examination of the characteristics of these two groups suggests that studying the transitional group of potential nonvirgins can help to better understand factors influencing the transition from virginity to nonvirginity. The most important predictor of virginity status was peer experience, followed by dating commitment and religiosity. The most important variable in predicting contraceptive use was the belief in the necessity of using birth control.

An eight-item contraceptive embarrassment scale was analyzed. The most important predictors of contraceptive embarrassment were parental attitude toward premarital intercourse and sexual guilt, as well as perceptions of the difficulties in obtaining contraceptives. The embarrassment scale had significant negative correlations with contraceptive use.

The relationship between attitudes toward abortion and contraceptive behavior was analyzed. The results indicated that the young women did not view abortion as a substitute means of birth control, and that those who would choose an abortion, if pregnant, were more likely to have used highly effective means of contraception at last intercourse.

There were significant differences in the contraceptive attitudes and practices of younger adolescents as compared with older adolescents. The younger adolescents were less likely to be using effective methods of contraception, and were more embarrassed about obtaining contraception.

Significance to Biomedical Research and Program of the Institute: Adolescent pregnancy is a research area of high priority for the Institute. This research on sexual and contraceptive behavior pertains directly to the antecedents and prevention of adolescent pregnancy.

Proposed Course: This project was funded in FY 79 and was completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on Childlessness and the One-Child Family
Contract Number : NO1-HD-92814
Contractor : Steiger, Fink, and Kosecoff, Inc.
Money Allocated : $39,719 (1979); $50,674 (1980)

Objectives: This research will assess the social-economic, psychological, and physical effects of parity (number of children born) on couples who either have completed their childbearing or are in the later years of childbearing. The social-economic variables to be examined are social adjustment, friends and social networks, career patterns, and income. The psychological variables are social and psychological well-being, depression, and perceived quality of life. The physical variables are current health status, resistance-susceptibility to illness, and concern about health. Couples will be divided into three groups, based on the wife's age (30-39, 40-49, and 50-59), so as to obtain information about changes in the effects of parity during the life cycle. The data to be used are from the RAND Corporation's Health Insurance Study (HIS), which included more than 8,000 individuals (2,750 families) in four geographic areas of the United States. Some additional data needed to ascertain the voluntary or involuntary nature of fertility will be collected by RAND. Analytical techniques will include discriminant function analysis and multiple regression.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interests in the consequences of fertility behavior. Childless couples and those with only children are still comparatively rare in the United States, and they tend to be negatively stereotyped as selfish, maladjusted, lonely, unhappy with marriage, etc. This research will use an excellent data source to provide objective information about childless and one-child families, including previously unavailable information about physical health.

Proposed Course: This project began in FY 79 and is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Disruptive Effects of Fertility: A Longitudinal Study of the Sequential Consequences of Childlessness and Childbearing on the Educational and Occupational Pursuits of College-Educated Women
Contract Number : NO1-HD-92816
Contractor : DePaul University
Money Allocated : $42,098 (1979)

Objectives: This research studied the effects of being an only child, of having one or more children, and of being childless on such variables as educational and occupational aspirations and attainment. The goal was to develop a parsimonious model of initial aspirations, fertility and occupational choices, and the relationships between these. Data were from the National Opinion Research Center's longitudinal survey of 1961 college graduates. The first wave was conducted in 1961 on a representative sample of 41,116 graduates; a subsample of 4,868 graduates completed follow-up waves in 1962, 1963, 1964, and 1968. The present research used data from a subsample of 1,685 female graduates.

Major Findings: The level of career aspirations of college graduates is predictive of labor force participation seven years after graduation, but not of parity level. Aspirations also do not predict plans seven years later to follow or not follow a career.

Childbearing generally has a disruptive effect on the career and later educational courses of female college graduates. Women bearing their first child while employed or while in graduate school typically drop out, and are not employed four years later. Further, the number of children born within 3.5 years after graduation has a negative effect on both full-time labor force participation and graduate school attendance, although it shows a positive effect on later plans to be employed.

Childless women are more likely to both be in the full-time labor force (especially those with early career aspirations) and to complete expected graduate degrees. Those women intentionally childless are more likely to have consecutive full-time employment and graduate school attendance. For childless women planning to have children, the positive effect of not having children on work seems due both to the freedom to pursue careers, and to those factors fostering the planfulness necessary to maintain a childless state.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility behavior.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Reciprocal Interaction Skills and Ego Identity Formation in Adolescents
Contract Number: NO1-HD-92819
Contractor : The University of Texas at Austin
Money Allocated: $79,380 (1979); $72,122 (1980)

Objectives: The study proposes to analyze data on only children and on children from two- and three-child families. Three clusters of variables believed to mediate the consequences of being an only child will be examined. These are: parental attitudes concerning family size and achievement; sibling and peer interaction; and family interaction and climate. Because only children may have different experiences than children with siblings relative to these three factors, it is possible that they will exhibit distinctive patterns of ego identity and reciprocal interaction skills. The investigators will also study the relationship of process and status to each other and their joint effects.

Major Findings: The present analyses concern the frequencies with which families encounter situational stresses in addition to the normative developmental challenge of launching their adolescents. These data are expected to contribute to an understanding of how patterns of family individuation, adaptability, and cohesion are related to stressful experiences families encounter.

Respondents identified stressful events which happened to any members of their families within the past year and indicated whether aspects of life related to communication, interaction and decision-making have increased, stayed the same, or decreased for them in the past year. For all family members, beginning or terminating job responsibilities occurred most frequently, with adolescents exceeding their parents in such changes.

Findings of parents' perceptions of changes in family life over the past year indicate that not all families experience the transition time of adolescents' last year in high school in the same way. Nonetheless, a cohesive pattern seems to emerge. Although most parents feel that they spend less time with their adolescents, they also feel emotionally closer, more able to express themselves candidly, and no need to increase the level of enforcement of family rules with their children. Almost two-thirds of the parents reported that their adolescents' influence on decision-making increased over the past year.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interests in the consequences of fertility.

Proposed Course: This study is scheduled to be completed in FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Sex Role Development and the Single Child Family
Contract Number : NO1-HD-92820
Contractor : Institute for Research on Social Problems
Money Allocated : $93,285 (1979); $96,169 (1980)

Objectives: The major objective of the proposed research is to study sex-role development, together with its parental antecedents, in only children. Attention will be given to the consequences of being an only child for sex-role development and identity. Also, the effects of having an only child on the parents' family planning decisions and attitudes toward sex roles will be considered. The research will explore the strength of the parents' need for child gender diversity, the relation of this to family size decisions, and the parents' sex-role expectations in relation to family planning decisions.

The investigator will first compare the parents of only children with the parents of two children in such characteristics as sex-role attitudes, degree of sex-typing, perceptions of their child's sex-role orientation, and factors related to family size decisions. Secondly, the study will compare the sex-role identity of the only children with both the first- and last-born children at the pre-school (ages 4-6) and fourth grade (ages 8-10) levels.

Major Findings: Preliminary findings show that by age 8-10, grade school children have already developed negative attitudes and stereotypes about only children. Age and gender differences in the cognitive components of sex-role acquisition are evident, but are not uniformly related to the presence or absence of siblings. It is the case, however, that only children, particularly females, are more knowledgeable about stereotypes. "Only" girls also exhibit greater sex-role flexibility than do boys in single-child families or children of either sex from two-child families. This may be less due to parental encouragement of gender diversity than to encouragement of more highly valued masculine traits as they show themselves in girls. It is also possible that the generally greater stringency associated with the sex-typing of boys may simply permit less behavioral flexibility, irrespective of family constellation. The degree of flexibility shown by children from two-child homes does not seem to be associated with whether the child has a same- or opposite-sex sibling or whether they are first- or second-borns.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interest in the social, psychological, and economic consequences of childlessness or of having one child.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Study of Consequences of Remaining Childless or Having Only One Child
Contract Number: NO1-HD-92821
Contractor : Research Triangle Institute, Research Triangle Park, N.C.
Money Allocated: $199,074 (1979); $87,006 (1980)

Objectives: The investigators (1) analyzed the 1973 National Survey of Family Growth and the 1971 and 1975 Current Population Surveys and (2) held discussions with 60 couples to assess the long-term social, economic, and psychological consequences of childlessness and of having one or two children. The research focuses on important economic, demographic, and social-psychological aspects of the quality of life: (1) marital dissolution and remarriage; (2) female labor-force participation and occupational attainment; (3) family income and standard of living; and (4) the degree of congruence between women's educational and occupational attainments, and between spouses' occupational attainments. The analyses of large-scale studies of the "objective" aspects of quality of life will be combined with in-depth studies of couples' experiences in these areas and in marital and parental roles, friendships, and use of financial resources and leisure time.

Major Findings: Interviews with couples who were childless or who had one or two children revealed that the childless are similar to those with children in the following ways: the women have a similar range of occupations and of leisure activities; most couples have friends and acquaintances from work, neighborhood, and recreational activities, and do not appear to be socially isolated; and many have close relationships with their families.

Some aspects of childless life reveal differences among the couples. At least some of the childless women, when they come to a decision to remain childless or find they are unable to have children, appear to make a greater commitment to career development. Those who do not have children of their own are not isolated from children; many childless couples, especially the involuntarily childless, have developed particularly close, continuing relationships to one or more other children.

The effects of childlessness and of children on the probabilities of marital dissolution and of remarriage after divorce were also examined, using life table analyses of the 1975 Current Population Survey data. It was revealed that the probabilities of divorce for childless women were not statistically significantly different from those for women with no children, nor were there significant differences regarding the probabilities of remarriage.

Significance to Biomedical Research and Program of the Institute: This research is important to the biomedical and behavioral programs of the Institute and is policy-relevant to departmental and Congressional concerns.

Proposed Course: This project is scheduled to be completed in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Societal Consequences of Adolescent Childbearing
Contract Number : NO1-HD-92822
Contractor : The Urban Institute
Money Allocated : $117,398 (1979); $199,579 (1980); $208,385 (1981)

Objectives: The proposed research will provide information at the national level on public expenditures to teenage parents through various programs: AFDC, Medicaid, Food Stamps, etc. Three types of estimates will be calculated. First, there will be calculation of actual expenditures for a given year (1975) on births to teenagers and non-teenagers. Next, the 1975 data will be projected forward to 1990 using a number of different assumptions (seven in all). These assumptions relate to different probabilities of birth among teenagers and others, different assumptions about illegitimate pregnancy, and school dropout rates among teenagers if they get pregnant. The third estimate will cumulate over the period 1975 to 1990 the cost to the government per mother for teenage versus non-teenage mothers who bore their first child in 1975. This group of teenage mothers will be treated as a cohort and the cost of their cumulative fertility to the government will be examined.

Major Findings: Initial estimates of the dollar public sector costs related to early childbearing were prepared for this project. It is estimated that in 1975, a total of $8.55 billion was expended on AFDC households in which the mother was a teenager at the time she bore her first child. This total includes $5.00 billion expended on AFDC (Aid to Families with Dependent Children), of which 2.80 billion was directed to teenage mothers currently 30 years of age or less, $1.45 billion on Food Stamps, $.93 billion on Medicaid to the children of AFDC mothers, and $1.17 billion for Medicaid for AFDC recipients who were teenage mothers, including the cost of prenatal care and delivery for teenage mothers still under the age of 20. This total does not necessarily represent the amount that could be saved if all these mothers had postponed their first birth, since some would have required public assistance regardless of their age at first birth. A clearer picture of the public sector expenditures that could be saved if the incidence of teenage childbearing were reduced should emerge from the DYNASIM analyses. Behavioral analyses conducted to enable re-programming of modules for DYNASIM (a microsimulation computer model) comfirm earlier work indicating that a birth to a teenager acts as a strong impetus to early marriage. Childbearing, and even more so marriage, predicts school dropout. In addition, marriage strongly inhibits school re-entry for those who have dropped out, while both marriage and motherhood predict failure to complete the grade for young women enrolled in school.

Significance to Biomedical Research and Program of the Institute: The project offers an ideal opportunity to assess the public sector costs of adolescent childbearing, a topic of considerable interest to the Institute with both social and biomedical implications.

Proposed Course: This project is scheduled for completion in FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Estimating and Forecasting Money Expenditures on Children
Contract Number : NO1-HD-92823
Contractor : MATHTECH, Inc.
Money Allocated : $94,802 (1979)

Objectives: This project will analyze the cost of children based on the economic theory of indirect utility as embodied in a transcendental log functional form. The investigators are using the 1972-1973 consumer expenditure survey as the basis for estimating the cost of children, and combining these cost estimates with a separate analysis of life cycle income to predict cost of children from the year 1980 through 2000.

Building on consumer demand theory, demand functions are to be estimated in which consumption is a function of prices and income. The resulting demand functions are substituted back into the utility function, producing an indirect utility function. The transcendental logarithmic indirect utility function is adopted as the functional form. This combined with Roy's identity permits derivation of the implied consumer demand functions for each commodity. Lifetime income profiles for head and spouse are being developed and used in the total expenditures equation to forecast expenditure category. The marginal cost of an additional child is then to be computed using several standard of living measures and a "relative expenditures" approach.

Major Findings: Preliminary analysis of the standard of living indicators shows that either total food expenditure or simply expenditures on food consumed at home yields more well-behaved indicators than any other consumption category. Either total consumption unit income after taxes or just total current consumption expenditures is the most promising denominator for the ultimate indicator. Employment status of the wife has an important effect on expenditure patterns, and will be incorporated into continuing analyses.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility.

Proposed Course: This project is scheduled for completion in FY 82.

Contract Title : Estimating the Cost of Children in the United States
Contract Number : NO1-HD-92824
Contractor : Carolina Population Center, University of North Carolina
Money Allocated : $119,553 (1979); $81,213 (1980)

Objectives: Three different methodological approaches will be used to estimate the direct, out-of-pocket costs of rearing children to an age of financial independence. Each method will utilize the 1972-1973 Consumer Expenditure Survey data for preparing basic estimates, supplemented in some instances with other data, such as the 1976 Health Interview Survey. The first method will estimate child costs in terms of additional family income required to maintain a given standard of living as children are born and mature ("a standard of living" approach). The second method constructs unit consumer scales and estimates child costs in terms of unit proportions for each category of expenditure (an extension of the "unit consumer scale" approach). The third method makes use of recent developments in demand system analysis that incorporate demographic variables directly into household demand equations (a "utility-based" approach). A major aspect of the proposal will be comparative assessment of the strengths and weaknesses of the different approaches. In addition, 1972-1973 data will be compared to 1962 data for examination of trends and change. Each method can be used to provide cost data by year of child's age and by a variety of family characteristics, including family size, family income, age of head, race, occupation and education of family head, region and type of residential area. Projections of costs of rearing a child born in 1980 to age 22 will be calculated using three different assumptions of future inflation trends. Estimates will be disaggregated by age of child, family size, age of parents, family income, race, occupation, education, region, city size, and life cycle changes in family income.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility.

Proposed Course: This project is scheduled for completion in FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Research on the Economic Determinants of Fertility
Contract Number : NO1-HD-92825
Contractor : Data Resources, Inc.
Money Allocated : $84,371 (1979); $67,117 (1980)

Objectives: Cross-sectional estimates of the costs of maintaining a child through its dependent years have been calculated using data derived from the 1972-1973 Consumer Expenditure Survey Summary and Detailed Interview tapes. Estimates of child costs have been made by selected family characteristics including family size, family income, age of parents, residential area, occupation and education of family head. Separate estimates of expenditures have been made by broad categories of consumer goods and services such as food, housing, medical care, and education. The allocation of expenditures by category among family members have been made using a modification of a method developed by Edward Lazear of the University of Chicago and Robert Michael. The cross-sectional estimates have been used as the basis for forecasting year-by-year expenditures for a child born in 1980 through its period of dependency. The forecast method utilizes an age-income model developed by Data Resources, Inc. which is linked to the agency's macroeconomic model of the U.S. economy. Separate forecasts will be developed for families of different characteristics, including three levels of income. Forecasts of summated costs will be expressed in discounted and undiscounted form under three different assumptions of inflation trends.

Major Findings: Preliminary results of past family expenditures on children show that higher income families spend more on children than do lower income families, but the percentage increases in spending on children are smaller than the percentage increases in income. Source of income also matters; families spend more of each incremental dollar on children when it is earned by a child. Expenditures display a U-shaped pattern across children's ages--high for young children, lower for children in the middles ages (roughly 5 to 12), and higher again for older children. Finally, non-white families appear to spend more on their children, holding family income constant, than do white families. Future work will be directed toward generating forecasts of expenditures on children born in 1980.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the consequences of fertility.

Proposed Course: This project is scheduled for completion in FY 81.

Contract Title : Societal Consequences of Adolescent Childbearing
Contract Number : N01-HD-92826
Contractor : Analogs
Money Allocated : $109,705 (1979); $54,771 (1980)

Objectives: The research will result in the development of computer simulation methodology for estimating public sector costs consequent to adolescent childbearing as a function of current or projected birth rates by age of mother, various rules for determining which costs should be associated with adolescent childbearing, and various life cycle periods. This methodology will be used to estimate public sector costs and the model will assess the sensitivity of these costs to various rules for making associations of cost factors with events following adolescent births.

In addition to total costs associated with a current year's birth cohort the estimation methodology will be able to accept projected fertility rates and will also be able to develop confidence limits on estimates as well as ranges for various life cycle periods and assumptions regarding "associated costs" such as those consequent, subsequent births to past teenage mothers now on welfare. The methodology will be validated by comparing predictions of future costs based on past birth cohorts with current AFDC caseloads and associated public sector costs.

Significance to Biomedical Research and Program of the Institute: The project offers an ideal opportunity to assess the public sector costs of adolescent childbearing, a topic of considerable interest to the Institute with both social and biomedical implications.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : The Consequences of Being and Having an Only Child on Intelligence, Interpersonal Orientation, Attitudes and Time Use
Contract Number : NO1-HD-92830
Contractor : The University of Texas at Austin
Money Allocated : $134,575 (1979); $76,941 (1980)

Objectives: This research comprises a series of three interrelated studies to assess the consequences of being and having an only child on intelligence, interpersonal orientation, attitudes, and time use, over a two-year period. The research is designed to examine the consequences of growing up without siblings particularly (but not solely) on the development of interpersonal skills and IQ. The research studies are grounded on the confluence model of intellectual development proposed by Zajonc and Markus, which accounts for the relationship between family size and intelligence by explaining the IQ discontinuity of only children as caused by the fact that only children do not have younger siblings to tutor.

Major Findings: Preliminary findings from Study 3, which is an experimental investigation of the sibling tutoring factor of the Zajonc and Markus confluence model, suggest the effects of the sibling tutoring factor on intellectual development are not clear-cut and seem to be considerably more complex than the confluence model would suggest.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the antecedents, determinants, and consequences of fertility.

Proposed Course: This study is scheduled for completion in FY 82.

Contract Title : Contraceptive Decision Making in Adolescent Couples
Contract Number : NO1-HD-92835
Contractor : American Institutes for Research in the Behavioral Sciences
Money Allocated : $54,510 (1979); $43,228 (1980)

Objectives: This study investigates couple communication and decision-making regarding contraception among young unmarried sexual partners. Factors which influence these interactions in teenagers and the nature of the effects of such communication on fertility decision-making and actual contraceptive practice have been examined. The goal was to provide a sufficient level of understanding of the processes underlying communication and decision-making in teenage couples, as they relate to contraception, to permit the development of a theory and model for these phenomena. Data for the study were collected by means of in-depth interviews with 83 couples, with the requirement that the female partner be between 15 and 18. Included were information on demographic factors, situational variables related to contraceptive knowledge and use, social interactions, and psychological variables.

Major Findings: A preliminary review of the data reveals that respondents' ignorance about contraceptive methods and female fertility cycles is quite extensive. Furthermore, they are often unaware of their ignorance, thinking they understand a contraceptive method when, in fact, they do not.

When individual partners' interviews were examined as a couple, it was seen that the partners reported discrepant information about the frequency of discussions about birth control. The majority (70 percent) of the couples disagreed about which birth control methods they had used in that relationship. Much of this disagreement, however, is attributable to differing reports concerning withdrawal and rhythm methods.

The absence of communication also extends to the possible consequences of their sexual activity. When asked what they would do if the female partner became pregnant, more than half the couples gave different responses. In some cases the conflicting responses were the result of the male deferring to his girlfriend's decision. In many cases, however, both partners had strong preferences, those preferences were in conflict, and there was no indication that the matter had ever been discussed. To date, the clearest conclusions to be drawn from this work are that teen couples have poor sex education and inadequate discussion between partners.

Significance to Biomedical Research and Program of the Institute: This project pertains directly to the Institute's interest in contraceptive behavior.

Proposed Course: This project is scheduled for completion in FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Marriage and Fertility Histories, July 1980
Contract Number : YO1-HD-01044
Contractor : Bureau of the Census
Money Allocated : $275,000 (1980); $75,000 (1981)

Objectives: This project is for the collection of data on marital history and living arrangements and support of children in the June 1980 CPS supplement. The June CPS includes fertility history and expectation data in addition to the usual sociodemographic data such as education, occupation, income, race, history, etc. In many respects this will replicate undertakings in 1971 and 1975 where marriage and fertility history data were collected. Marriage history data has been collected from all subjects 15-70, fertility histories from women 15-70 and fertility expectations from women 18-39. Men who have been married more than once were asked if they have children from a previous marriage(s) living elsewhere, how many, and whether the father provided financial support for such children. Following data collection, the Census Bureau will provide NICHD with a public use data tape.

Major Findings: During the past year, the data file was edited for internal use and public distribution. Production was completed within the Bureau on tabulations on birth expectations and fertility based on the June 1980 CPS.

The tapes were released for public use in July 1981. An advance report based on the June 1980 CPS is in preparation including data on birth expectations and period fertility rates for women in different socioeconomic groups. This latter statistic is a new feature to be included in the Bureau's annual reports. This rich, high quality survey will provide a fruitful source of data for many researchers investigating contemporary American marriage and fertility patterns.

Significance to Biomedical Research and Program of the Institute: With marital disruption rates at an all-time high and fertility at a similar low, research on the timing, correlates and interrelationship of marriage and fertility behavior is of very high priority and directly relevant to the Institute's interests in the determinants and consequences of fertility.

Proposed Course: This project will be completed in FY 81.

Contract Title	:	Collection of Data in Cycle III of the National Survey of Family Growth Concerning Unwanted Childbearing and Contraceptive Practice in the Later Reproductive Years
Contract Number	:	1-Y01-HD-01045-00
Contractor	:	National Center for Health Statistics
Money Allocated	:	$204,130 (1980)

Objectives: This project involves data collection and tape preparation for that portion of National Survey of Family Growth Cycle III (NSFG III) relating to contraceptive practices and unwanted childbearing among women 30-44. NCHS will deliver a complete documented data tape for the 1981 survey, i.e., all data for all women interviewed ranging in age from 15-44. The NSFG III is the latest in a succession of national fertility surveys that began in 1955. These surveys are the source of data about contraceptive practices, wantedness of births, sterilization, and correlates of these behaviors for the U.S. as a whole. The questionnaire deals specifically with knowledge of birth control and reproductive processes; detailed pregnancy history; history of use of contraceptives and wantedness and planning status of births; sterility and subfecundity; use of family planning and infertility services; and demographic characteristics, e.g., socioeconomic indicators, marital history, child care, race, and ethnicity. The structure of this survey is such that meaningful comparisons can be made with earlier time periods, and the oversampling of black women ensures comparisons between blacks and whites.

Significance to Biomedical Research and Program of the Institute: This project will provide valuable data about the contraceptive practices and fertility behavior of women in the U.S. These data will be useful to a wide range of biomedical and behavioral scientists.

Proposed Course: This two-year interagency agreement was begun in FY 80.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Contraceptive Decisions: Spousal Relationship, Method Commitment, and Expectancy-Values
Contract Number : NO1-HD-02802
Contractor : Center for Consumer Research, University of Florida
Money Allocated : $72,639 (1980)

Objectives: This research will integrate three areas of investigation related to contraceptive choices and practice: (1) theoretical and empirical efforts in the area of expectancy-value formulation; (2) joint consumer decision-making by marital partners; and (3) theory and research on the nature of the marital relationship (decision-making style). The purpose is to analyze the impact of joint decision-making on contraceptive use (continuous, episodic, or non-use), for different contraceptive methods. Three interviews within 12 months will be conducted with 360 sexually active white couples who are not planning a pregnancy. In half the couples the women will be aged 18-27; in the other half they will be aged 36-45. Both partners of each couple will be interviewed individually and jointly, to ascertain each one's expectations and values, perceptions about the other partner's expectations and values, and joint decision-making. The analysis will focus on the explanatory power of the expectancy-value conceptual framework, combined with joint decision-making, partners' perceptions, influence of marital decision-making style, and type of contraceptive commitment as these influence contraceptive choices and ultimate fertility intentions.

Significance to Biomedical Research and Program of the Institute: This research pertains directly to the Institute's interest in the antecedents and determinants of fertility.

Proposed Course: The research began in FY 80 and will continue into FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Interdisciplinary Assessment of Fertility Management in a High Fertility Community
Contract Number : NO1-HD-02803
Contractor : Department of Community Medicine, University of Kentucky
Money Allocated : $129,301 (1980); $154,456 (1981); $104,468 (1982)

Objectives: This interdisciplinary three-year project aims at describing fertility regulating practices, attitudes and perceptions, as well as the factors associated with these variables, in a high fertility Eastern Kentucky Appalachian county of about 13,000 people. Three strategies will be employed. First, a "community inventory" will be undertaken to determine general access to fertility regulation information, supplies, and services, and to assess the views of community leaders and the general community atmosphere regarding family planning; this will involve structured and unstructured interviews and a traditional ethnographic approach. The second strategy involves survey interviews with 400 married females, and 100 of their husbands, covering knowledge and attitudes regarding family planning, fertility history, family conjugal roles, value and personality factors, and sociodemographic variables. The third strategy entails in-depth interviews, based on factors which emerged using the other two strategies, with 100 women and their husbands. Analyses of the data will be both descriptive and exploratory.

Significance to Biomedical Research and Program of the Institute: This research should provide insights into the dynamics of fertility regulation that are unobtainable by single disciplinary approaches. The results are likely to increase understanding of the complex of variables that affect birth planning at the community, family, and individual levels.

Proposed Course: This project is scheduled for completion in FY 83.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Consequences of Childlessness for Older Men
Contract Number : NO1-HD-02804
Contractor : The University of California at Los Angeles
Money Allocated : $56,387 (1980)

Objectives: This is a study of the psychological, social, and emotional consequences of childlessness among married men 60 years of age and over. It is hypothesized that the relation between parity (childless or parenthood) and well-being is moderated by social-psychological variables such as social contact and congruence between fertility desires and outcomes. One hundred-fifty men over age 60 with children will be compared with 150 childless older men on a variety of variables including several measures of well-being, congruence between desired and actual family size, satisfaction with family size, expectations of parity outcomes and actual outcomes, social interaction, and sociodemographic variables.

Major Findings: Field work has been completed for this research. Owing to difficulties in locating childless men, interviews were obtained with 99 childless and 162 non-childless older men. Further, data are available from a previous study for the wives of 209 of the male subjects.

Preliminary analysis has shown no association between childlessness and dissatisfaction with life among older men. Among widowed women, there is an association between childlessness and negative psychological states. However, data are not available on whether this is true for widowed men.

Significance to Biomedical Research and Program of the Institute: This study is relevant to the Institute's interest in the consequences of fertility.

Proposed Course: This study is expected to continue into FY 82.

Contract Title : Research on the Societal Consequences of Adolescent Childbearing Public Assistance Costs, 1975 and 1977
Contract Number : NO1-HD-02837
Contractor : JWK International Organization
Money Allocated : $90,845 (1979); $33,506 (1980)

Objectives: This project uses the 1975 and 1977 AFDC Recipient Characteristics Surveys for an analysis of the costs of teenage childbearing among the population receiving public assistance. The direct dollar costs of being a prior teenage mother are being obtained for AFDC recipients aged 20 and 30 for AFDC payments, food stamps, other state and local assistance payments, and emergency payments. The indirect impact of teenage mother motherhood on these same costs will also be explored. For AFDC mothers currently in their teens, total direct costs will also be cumulated and indirect costs will be explored. In addition, reliance upon Social Services will also be tabulated. Finally, development of a causal model is proposed to examine the number of months mothers have received AFDC payments.

Major Findings: Of all AFDC assistance families, in about seven percent the mother was age 19 or younger, in both 1975 and 1977. In another 22 percent of AFDC families in 1975, and 24 percent in 1977, the mother started her childbearing while a teenager. AFDC mothers were more likely to have been teen mothers than were American women in general. Among AFDC mothers under age 30, 64 percent had been teenage mothers, whereas only 24 percent of all American women aged 20 to 30 in 1975 had given birth before age 20.

AFDC payments to current and prior teenage mothers under age 30 were estimated to total $2.5 billion in 1975 and $3.0 billion in 1977, or 65 percent of all payments to women under age 30. The analysis of the direct effect of mother's age at first birth on the components of an AFDC payment showed that age at first birth had a small but significant effect on the amount of payment in 1975. It exerted no effect on the amount of payment in 1977 once the effects of other relevant variables were controlled.

Significance to Biomedical Research and Program of the Institute: This project addresses an important consequence of adolescent fertility. It has high policy relevance for HHS programs.

Proposed Course: This project is scheduled for completion in FY 82.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Longitudinal Analysis of Family and Household Structure
Contract Number : N01-HD-02849
Contractor : The Urban Institute
Money Allocated : $787,000 (1981)

Objectives: This research consists of three studies of the structure and the behavioral patterns of families and households. The first study will describe the sequence and spacing of marital and childbearing events of women over their lifetimes. The second study will examine changes in marital status composition, applying techniques of multiregional population analysis to data on flows of individuals among marital status categories throughout their lifetimes. The final study will describe the frequency of family structures and living arrangements of children, and will estimate the probability of moving from one family type to another.

Major Findings: Three-parameter lognormal first-marriage hazard functions have been estimated for several birth cohorts. The estimated median age at first marriage fell from 22.7 years for females born 1900-04 to 21.6 years for the 1920-24 birth cohort to a low of 20.3 years for females born between 1935 and 1939. It has increased since then to an estimated 21.5 years for women born 1950-54. Preliminary marital status life tables have been estimated for U.S. white females based on their marital and mortality experience during 1970-75. Of 100,000 single white females starting out life together, 83,073 are expected to be alive at age 65 and distributed as follows: 4,130 single, 51,045 presently married, 8,175 divorced, and 19,724 widowed. Estimated life expectancy at birth equals 77.48 years of which 24.39 are expected to be spent single, 38.44 married, 4.79 divorced, and 9.86 widowed.

Significance to Biomedical Research and Program of the Institute: The proposed analyses will be useful not only for describing the micro-level processes that occur in the lives of individuals, but also for making projections of the future distribution of the population by family type.

Proposed Course: This contract is scheduled to run until FY 83.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title	:	Demographic Perspectives on the Consequences for Children of Changing Marital Patterns
Contract Number	:	N01-HD-02852
Contractor	:	University of Wisconsin at Madison
Money Allocated	:	$215,117 (1980)

Objectives: The proposed research will use data from the 1976 National Survey of Family Growth and from the 1975 and 1980 (June) Current Population Surveys to conduct five specific analyses: 1) life-table analysis of children's experience of marital disruption, estimating the cumulative experience of being in a single-parent family by time since disruption and cumulative proportions of children experiencing disruption by age; 2) a study of trends in children's experience with marital disruption; 3) a cross-tabulation of data to describe the household living arrangements of children who have experienced marital disruption, in terms of number and types of parents/stepparents and siblings; 4) an analysis of inter-marital fertility including analysis of contraceptive use and family planning status prior to the birth; 5) a life-table analysis of the occurrence and timing of second-marriage births.

Major Findings: Characteristics at the time of remarriage have been estimated for both women and children in remarriages from 1960 to 1975. Slightly less than a third of the children in recent remarriages were under the age of five; one-sixth were teenagers 14-17. Few of the children were without siblings at the time of remarriage and half had two or more siblings. The stepfathers of most of these children had been married before and perhaps half of the children had stepsiblings from these previous marriages. For many, the remarriage followed very soon after separation, overlapping the processes of adjustment to divorce and to the second family; at the same time, almost a fifth waited five or more years before their mothers remarried. The social and economic adjustments required of these children was emphasized by the fact that a high proportion had mothers who were high school dropouts (increasing poverty experience in the single-parent family) and by the fact that a quarter of the early 1960 remarriages had been disrupted by the date of the interview. Estimates by marriage cohort suggest a recent doubling in children's experience of a second disruption. Overall, about a third of the children share the second family with a half sibling, although the proportion is two-thirds among children under age five at remarriage.

Significance to Biomedical Research and Program of the Institute: A major strength of this research lies in its use of recently collected data to determine current patterns of marital disruption and the subsequent formation of new households.

Proposed Course: This project is expected to continue through FY 83.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Changing Patterns of Household Structure: A Cohort Analysis
Contract Number : N01-HD-02853
Contractor : University of North Carolina
Money Allocated : $21,810 (1981)

Objectives: The proposed research will graphically depict changing patterns of household structure for different cohorts by sex, urban-rural residence, and race. In addition to national results, information for 10-18 states, representing various regions, will be presented separately. Data from the 1940, 1950, 1960, and 1970 censuses will be used. Major results will show the percent of each cohort I) whose relationship to head of household is child by age, 2) classed as head of household by age, 3) classified as grandchild of head of household by age, 4) classified as parent of head of household by age, and 5) classified as other relative by age. In addition, the research will calculate the ratio of unmarried females ages 35-59 to persons 65 and over for whites and nonwhites in different regions for the period 1900 to 1980, to assess the extent of changes in the availability of such women for the care of elderly family members.

Major Findings: Preliminary results indicate that the major changes taking place in household patterns seem to center around two age groups and processes: young adults who are leaving home and entering into marriage and/or the labor force, and elderly people who are changing their residential patterns.

The peaks evident in cohort graphs at ages 20-24 in the proportion classed as "other relative" or "lodger" indicate this has been an important route of labor force entry and establishment of own household. However, the declines from 15 to 18 percent in early cohorts to three to five percent in recent cohorts in these categories at this age indicate that young people are now more likely to set up their own households.

An interesting finding that has emerged is the increase in the proportion of a cohort classed as "child of head" as the cohort ages from birth to 10-14. This is not attributable to underenumeration of children age 0-4; rather, there is a complementary decline in children classed as "grandchild of head" as the child ages, especially for the black cohorts.

Significance to Biomedical Research and Program of the Institute: This project is expected to extend appreciably the empirical knowledge on past trends and current patterns of marriage, divorce, and household formation.

Proposed Course: This project is scheduled to run through FY 81.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Social and Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Demographic Analysis of Family and Household Structure
Contract Number : N01-HD-12802
Contractor : The Johns Hopkins University
Money Allocated : $136,684 (1981)

Objectives: Data from the Public Use Tape from the June 1980 Fertility and Marriage History Supplement to the CPS will be used to deal with two major topics: 1) the extension of families over more than one household due to links formed by children from previous marriages; 2) current and projected rates of marriage, dissolution, and remarrriage. Separate analyses will be made for different subgroups, by age, sex, race, and education.

Using as units of analysis both "households of remarriage" and "divorced or separated persons", the investigators will show distributions of parents with own children living in other households, distributions of children with a parent in another household, households with children from a remarriage, and various combinations. New data on the proportion of men providing regular or occasional support to persons in other households will also be analyzed.

Studies on the second topic will involve life table analyses yielding cumulative probabilities of marital dissolution or of remarriage by duration of exposure and cohort. The initial analysis will concentrate on past and current patterns, but further analysis using Tukey's "median polish" technique will yield projections of future rates of marital dissolution and remarriage, for cohorts with incomplete marital histories. Finally, an attempt will be made to determine whether rates of dissolution and remarriage based on self-reports by males and by females are in substantial agreement.

Significance to Biomedical Research and Program of the Institute: This project is expected to extend appreciably the empirical knowledge on current patterns of marriage, divorce, and houshold formation.

Proposed Course: This project is scheduled to run through FY 83.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Center for Population Research
Reproductive Sciences Branch

Within the context of the mission of the Center for Population Research (CPR), NICHD, the Reproductive Sciences Branch (RSB) facilitates the acquisition and application of the knowledge required to enable men and women to: 1) control their fertility; and 2) free themselves from reproductive disease and disablement. The primary means by which such facilitation of the CPR mission is accomplished by the RSB is through the award of grants for the support of research and training in the reproductive sciences. During the period from October 1, 1980 to September 30, 1981, RSB provided funding for grants and other forms of support for activities subsumed by the following programs: 1) Fundamental Biomedical Research on Problems of Human and Relevant Animal Reproduction; 2) Institutional Programs in Multidisciplinary Reproductive Sciences Research; 3) Reproductive Sciences Research Manpower Development; and 4) Reproductive Sciences Research Facilitation and Augmentation.

1. Fundamental Biomedical Research on Problems of Human and Relevant Animal Reproduction

Mechanism of Steroid Hormone Action. Steroid hormone regulation of gene expression is directly implicated in many health-related problems involving reproductive tissues The recent development of an *in vitro* cell culture system for chick oviduct tissues has been reported by an RSB grantee. This technological advance is of significant interest to those researchers working on the mechanisms of steroid and peptide hormone action. It has been discovered that, in addition to estrogen, a somatomedin-like peptide hormone found in serum is essential for the induction of egg white (ovalbumin) gene mRNA in explanted oviduct cultures. It has been further shown that in addition to somatomedin, other peptides known to interact with somatomedin receptors are capable of substituting for the serum requirement. The effect of the peptide hormone has been demonstrated to occur at the transcriptional level within one hour after addition of the peptide to the medium. In contrast, the transferrin gene, which also is induced by estrogen in tubular gland cells, apparently does not require serum or peptide hormones for activation. It appears, therefore, that the peptide hormone may interact with a cell-surface receptor and trigger an intracellular signal capable of interacting with the ovalbumin gene. These results and the availability of the new model system offer an exciting promise for future progress in elucidating the mechanism of action of steroid and peptide hormones in reproductive tissues.

Purification of Steroid Receptors. Because of the unstable nature of the receptors for progesterone and androgen, much difficulty was encountered in clarifying the mechanisms of hormone action of these steroid hormones. Recently receptorologists have succeeded in stabilizing the steroid receptors by molybdate. This finding allows for the first time precise physical characterization of androgen as well as progesterone receptors. In the presence of molybdate, the androgen receptor is stable almost indefinitely at 4°C without steroid. This means that fractionation can be initiated on the unoccupied receptor. Preliminary studies indicate that this receptor can be concentrated from large volumes of cytosol by DEAE cellulose and heparin affinity chromatography. This provides about a 150- to 200-fold purification of the androgen receptor and concentration into a much smaller volume.

Genetic Regulation of Preimplantation Development. The genetic analysis of early embryonic development is an important technique for studying genetic mechanisms controlling the earliest processes associated with this vital phase of reproduction. The analysis of mutations expressed during early development are of importance not only for a general type of understanding of such processes, but also for understanding of the molecular basis for specific types of genetically determined developmental abnormalities leading to death or severe malformations. Using genetic analysis of the cell surface histocompatability antigen (H-2) system, an RSB grantee has investigated the synthesis and expression of a constituent of the histocompatability (H-2) antigen called Beta-2-microglobulin (Beta 2-M) in oocytes and preimplantation embryos. The results show that while Beta-2M is synthesized by both unfertilized eggs and all stages of preimplantation development, it is not expressed on the cell surface until the blastocyst stage of development. It first appears on trophectoderm cell surfaces and only later on the cells of the inner cell mass. In contrast, parallel studies of the complete H-2 antigen indicate H-2 is neither synthesized nor expressed on cell surfaces during similar developmental states. Thus, while capable of synthesizing Beta-2M, oocytes do not express it on their cells surfaces until the necessary "anchoring" molecules are also synthesized and in place. The recent recognition of a genetic variant of Beta 2-M by this grantee has made it possible to determine the time of expression of the gene in the embryonic genome by the appearance of the paternally determined gene product. The analyses of reciprocal-cross data has demonstrated that paternal gene activation occurs by the two-cell stage of early development. This is the earliest time at which expression of the embryonic rodent genome has been demonstrated. These results also identify the earliest appearance of a genetic-based molecular abnormality associated with aberrant embryonic development.

Localization of Androgen-Binding Protein. The androgen-binding protein (ABP) found in testicular and epididymal cytosols has been shown to be produced by Sertoli cells of the seminiferous tubules. The recent purification of homogeneous ABP from rat epididymis permitted the preparation of monospecific antibody to ABP and the immunocytochemical staining of the protein. Within the seminiferous tubules, a positive immunocytochemical reaction was noted in the apical portion of the epithelium, apparently in spermatids and/or Sertoli cells. ABP was also localized in granules in the apical cytoplasm of the principal epithelial cells of the proximal part of the caput epididymis and the epithelial cells of the ductili efferentes. The cells in the distal part of the caput as well as the corpus and cauda of the epididymis did not contain ABP. Numerous coated vesicles and multivesicular bodies were present in the supranuclear cytoplasm of the epithelium where ABP was taken up. The results indicate that ABP is taken up from the lumen as soon as it enters the ductili efferentes and proximal part of the caput epididymis.

Monoclonal Antibodies and Sperm Maturation. Significant progress continues to be made by an RSB grantee in identifying and characterizing specific surface components of sperm in order to determine their role in reproduction. Monoclonal antibodies produced by fused mutant myeloma cells and spleen cells derived from immunized donors (hybridomas) were used previously to demonstrate the appearance of four new proteins on the surface of mouse sperm during its maturation in the epididymis. Sperm maturation in the epididymis is required for the acquisition of fertilizing ability by sperm. The maturation process although critically important to the achievement of fertility, is poorly understood at the molecular level at present. Immunohistochemical studies have now shown that of the four antigens recognized by these monoclonal antibodies, one is only detected on sperm and not in male reproductive tract cells, two are present in the apical region of cells lining the caput and corpus epididymis, and one is found in a short length of epithelial cells in the distal caput

epididymis. These results support the hypothesis that sperm surface composition is molecularly altered during maturation either by modification of existing surface components produced by the epididymal epithelium, or by the attachment of new components produced by the epididymal epithelium. Further studies using this powerful new tool in reproductive biology have produced eleven new gametogenic monoclonal antibodies, several of which recognize antigens on specific regions of the sperm surface, including subregions of the sperm head. These studies have provided the first solid evidence that individual antigenic determinants are restricted to specific areas of the sperm surface and have demonstrated for the first time that sperm surface components produced by other cells in the reproductive tract (epididymal epithelium) become applied to and associated with specific topographically restricted areas of the sperm surface. These studies should provide information of considerable value in the design of studies on the regulation of male fertility and in the diagnosis and treatment of certain types of male infertility.

Chemically Defined Medium for Sperm Motility Research. Studies of sperm functions *in vitro* have been enhanced by the development and use of a new chemically defined medium. Recently, a synthetic polymer, polyvinyl alcohol, was discovered to have very useful properties for this purpose. Use of this polymer in place of serum protein permits separation of sperm motility from fertilizability. Both fertilization efficiency and sperm motility are usually reduced in diluted medium. Such reductions in fertilization efficiency and sperm motility are attributed to the "sperm dilution effect." Recently RSB grantees showed that addition of sperm motility factors, taurine or hypotaurine and catecholamine, to the chemically defined medium eliminated the "sperm dilution effect." It, therefore, appears that the "sperm dilution effect" may be, at least in part, due to a reduced availability of the sperm motility factors.

Mechanism of Gossypol Inhibition of Male Fertility. The drug gossypol is a non-steroidal compound that inhibits sperm production in animals and human beings. It is presently under study throughout the world as a potential male antifertility agent. Two studies by RSB grantees have further clarified the mechanism of the gossypol effect on fertility. For the first time, it has been demonstrated that gossypol interferes with testosterone production by the Leydig cells of the seminiferous tubules of the testis. Serum testosterone and leutinizing hormone (LH) levels, but not those of follicle stimulating hormone (FSH), were significantly reduced following the administration of gossypol to rats at concentrations which inhibited fertility without an apparent loss of libido. Sperm in the resulting ejaculates were reduced in number and rendered immotile. Ultrastructural examination of epididymal spermatozoa and late spermatids in the seminiferous tubules revealed degeneration in the tail region of sperm, particularly the mid-piece mitochondrial sheath. Most interestingly, Leydig cell production of testosterone was experimentally shown to be inversely proportional to the gossypol concentration. Short-term fertility restoration was accompanied by normal values for sperm structures, numbers and motility. Normal values for serum testosterone and LH levels occurred following a short-term recovery period.

The reversible and, thereby, temporary nature of the drug has often been cited in support of its potential for clinical application. Recent studies by another grantee have, however, raised doubts regarding the nature of the reversibility of this experimental antifertility compound. Using the polyene antibotic filipin to test the permeability of the blood-testis barrier in gossypol-treated guinea pigs, the grantee observed marked perturbations of this barrier. In particular, along with ultrastructural changes in Leydig and Sertoli cells, the common occurrence of vacuolization and distention of Sertoli-Sertoli junctions was demonstrated to cause

interrupted junctional strands which were noted to serve as a nidus for multiple "atypical rectangular gap junctions." In regions of intact junctions, basal structures were found to be accessible to the antibiotic filipin even though it was excluded from the intermediate compartment formed by the luminal and basal Sertoli-junctional strands. In membrane domains containing disrupted junctions, filipin perturbations were observed both within and around the intermediate compartment. The focal alterations in barrier permeability associated with the disruption of Sertoli-Sertoli cell junctional integrity have thus demonstrated the loss of compartmental organization along the seminiferous tubules of a type which may not be temporary in nature.

Regulation of Gonadotropin Secretion. Substantial progress has been made in the general area of hypothalamic control of gonadotropin secretion in the rhesus monkey. A model for neuroendocrine control of the menstrual cycle in this species has been developed that can probably be extrapolated, in a large degree, to the human female. New insights have been acquired concerning the significance of relatively small changes in frequency of hypophysiotropic stimulation on the level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in circulation and the ratio of one to the other. The central component of the control system which governs the menstrual cycle is an oscillator located in the arcuate nucleus of the hypothalamus. It generates a pulse of gonadotropin releasing hormone (GnRH) approximately once an hour, and electrophysiological studies indicate an apparent synchrony between multi-unit activity in the median eminence and circhoral LH discharges. The hypothalamic input into the menstrual cycle appears to be regular, with GnRH being discharged in a steady fashion over the whole menstrual cycle.

Studies of the effects of different frequencies of GnRH administration on the secretion of gonadotropins in monkeys show that increasing the frequency of administration to even two pulses per hour reduces the secretion of gonadotropins. When pulses of GnRH are reduced below 1 per hour, there is a shift in the FSH/LH ratio, and this appears to be due to the lower metabolic clearance rate of FSH, as compared with LH. Follicular development, as manifested by increments in plasma estradiol concentrations, is not initiated by this gonadotropin pattern which resembles that in neonatal females (high FSH, low LH, and no follicular development). Seemingly, small variations in FSH/LH ratio may lead to widely different ovarian responses.

The systems of pulsatile GnRH administration, which have been found to be requisite for the stimulation of gonadotropin secretion in the rhesus monkey, are being applied clinically, particularly in the treatment of infertility associated with hypothalamic, hypophysiotropic hypogonadism.

Brain Peptides and Gonadotropin Secretion. Recent advances in opiate research have provided a new dimension to our understanding of neuroendrocrine control of pituitary function. The highest concentration of beta-endorphin in the human brain is found in the arcuate-median eminence region of the hypothalamus. Since dopamine and GnRH neurons are also in this region, the interaction of their neuronal networks in regulation of gonadotropin secretion appears likely. Endogenous opioid peptides have an inhibiting role on LH release and augment prolactin secretion by the hypothalamic-pituitary system. Treatment with the opioid receptor antagonist, naloxone, can result in competitive inhibition of these effects. Pituitary microadenomas that secrete high levels of prolactin are often associated with reduced circulating LH levels. Patients with such tumors, when infused with naloxone, respond with an increment of LH release suggesting that an increased endogenous opioid activity may have a role in this clinical disorder.

Preliminary investigations in the monkey indicate that hypothalamic release of beta-endorphin is regulated by the ovarian hormones, estrogen and, more importantly, progesterone. Studies in women during the menstrual cycle suggest that endogenous opiates are involved in the regulation of LH during the late follicular (high estrogen) and mid-luteal (high estrogen-progesterone) phases but not in the early follicular phase of the cycle. Although estrogen and progesterone modulate LH secretion through direct action on the pituitary, their effects on hypothalamic GnRH are still not clear. These studies provide indirect evidence that endogenous opiates may play an important role in control of GnRH secretion during the menstrual cycle.

Calcium is Involved in GnRH Action. During the past year, RSB grantees have further elucidated the mechanism of action of GnRH. In order to investigate how this hormone interacts with the gonadotrophs of the pituitary gland, a method for visualization and localization of GnRH receptors on the cell surface was developed by using a rhodamine conjugated GnRH derivative. Using this method, it was made clear that the fluorescently (rhodamine)-labeled receptors were initially distributed on the cell surface and formed patches which subsequently internalized into endocytic vesicles. Internalization, however, does not appear to be required for LH release. When calcium ion was directly introduced into the pituitary cells in the absence of GnRH, LH was released. These observations have made it possible to postulate that GnRH action may be divided into three sequential steps: 1) interaction of GnRH with a specific plasma membrane receptor, 2) mobilization of ionic calcium, and 3) expulsion of the contents of the gonadotropin secretory granule into the extracellular space.

Norepinephrine Triggers GnRH Release. There is a build-up of new GnRH in the median eminence axon terminals and an increased release of norephinephrine which activates the discharge of this newly synthesized GnRH. The role of norephinephrine appears to be the triggering of GnRH release and not GnRH production because when LH surges are blocked with phenobarbital, GnRH concentration still increases in the median eminence. However, as the trigger for its release, that is, increased norepinephrine, is completely blocked by phenobarbital, an LH surge does not occur. Evidence appears to support the hypothesis that it is estrogen which sets into motion the increase in GnRH in the preoptico-suprachiasmatic-tuberoinfundibular system and which also regulates the catecholamine system.

Control of Prolactin Secretion. There is evidence in support of the existence within the hypothalamus of a specified prolactin releasing factor. A possible candidate has been vasoactive intestinal polypeptide (VIP) which was first isolated from porcine intestinal duodenum and subsequently identified in mammalian brain areas including the hypothalamus. It had been shown to evoke secretion of prolactin in rats and it is secreted into hypophyseal portal blood. Studies now indicate that VIP is also a potent stimulus for prolactin secretion in monkeys and that it exerts its effects, at least in part, by a direct action at the pituitary level. These investigations have raised the strong possibility that a new hypothalamic peptide, VIP, serves as the long sought after prolactin releasing factor in primates. The possible existence of such a releasing factor has relevance to our understanding of the galactorrhea-amenorrhea syndrome in women.

Gonadocrinins: Ovarian Fluid Peptides Which Stimulate the Secretion of Pituitary Gonadotropins. The gonad may contain and possibly secrete nonsteroidal substances that might be included in a multistep feedback system involving the hypothalamo-pituitary axis. Dialysates of crude acid extracts of ovarian follicular tissue and fluid, from rats pretreated with PMSG, stimulate the secretion of both LH and FSH but not prolactin, growth hormone or thyrotropin. This stimulating factor, named

gonadocrinin, is smaller than 3500 daltons and peptide in nature. Since cultured rat granulosa cells also secrete substances with gonadocrinin activity in vitro, it appears that granulosa cells are probably the source of gonadocrinin.

Endocrine Response to Exercise. A number of interesting new observations are being made regarding endocrine responses to exercise. Studies in normal women in the early follicular phase of the cycle using a bicycle ergometer indicate evidence of neuroendocrine stimulation of LH release with associated gonadal steroid secretion in anticipation of exertion. In contrast, prolactin, growth hormone and the ACTH-adrenal axis were activated in response to exercise. The increased secretion of hormones with potentially anti-reproductive action (prolactin, ACTH, beta-endorphin) during exercise may contribute to "hypothalamic amenorrhea" in women athletes. The athlete's amenorrhea syndrome is of increasing concern and a number of studies are being directed towards exploring the complex relationships of height/weight disparity or nutrition with amenorrhea. While height/weight disparity may be a critical factor affecting optimal reproductive function, preliminary studies indicate that alterations in hypothalamic control of gonadotropin release, independently of body composition, may also be operant in the development of "athletic amenorrhea."

Secretion of Progesterone by Corpus Luteum. RSB-supported studies have provided a more complete understanding of the endocrine factors which regulate the secretion of progesterone by the corpus luteum. Since synthesis and secretion of progesterone are initiated by the interaction of LH with specific receptors located on the plasma membrane of luteal cells, the mechanisms involved in regulating the concentration of receptors for LH were studied by examining the subcellular pathways involved in the loss and renewal of receptors for LH in luteal cells. There is evidence for the presence of two steroidogenic cell types in bovine corpora lutea, both of which secrete progesterone. A procedure was developed using an elutriator whereby two distinct populations of cells can be separated. Although the large luteal cells produce progesterone, their ability to respond to LH is on the order of 10-fold less than that of the small luteal cells. These observations in conjunction with results from autoradiographic analyses suggest that the difference in responsiveness between large and small luteal cells to LH is likely due to differences in the number of receptors for LH associated with the different cell types. The large cells have now been found to have approximately 3,000 LH receptors/cell, while the small cells have approximately 33,000 receptors/cell. Since the initial step in the complex sequence of events that results in progesterone synthesis and secretion is the binding of LH to its receptor, a better understanding of the life-cycle of LH receptors (i.e., pathways of synthesis and degradation), may reveal new mechanisms susceptible to contraceptive regulation.

This technique for separation of cell types also has provided an opportunity to determine how PGF_2alpha exerts its negative effect on LH stimulated production of cAMP and secretion of progesterone. The hypothesis that LH and PGF_2alpha may act primarily on these different cell types appears to be correct because the large cells have all the receptors for PGF_2alpha while the small ones have essentially none. As previously discussed, the converse is essentially true for LH. Thus, the two types of cells appear to interact to regulate the secretion of progesterone. These studies on the cell-to-cell interactions of two different steroidogenic populations of luteal cells enhance our basic knowledge of ovarian function.

Blood Lipoproteins in Ovarian Function. Recent studies have clarified the mechanisms by which ovarian cells acquire cholesterol from the circulating lipoprotein pool as a substrate for steroidogenesis. Lipoprotein-carried cholesterol was shown to be the

primary substrate for progestin synthesis and sterol ester storage by rat luteal tissue using both in vivo and in vitro systems. The availability of lipoprotein-carried cholesterol to the lutein cells was shown to regulate de novo cholesterol and progestin biosynthesis and cholesteryl ester storage in a reciprocal fashion. Two microsomal enzymes which catalyze rate-limiting reactions in cholesterol biosynthesis and cholesteryl ester storage are regulated in a reciprocal fashion by ovarian sterol balance. Both human and rat high density lipoproteins (HDL) were capable of stimulating progestin synthesis as well as cholesteryl ester storage during short term incubations. The stimulation of steroidogenesis by exogenous HDL was shown to be due to the sterol of HDL and not to the apoproteins of the lipoprotein. De novo sterol synthesis was found to play a negligible role in the supply of sterol for progestin synthesis. Ovarian cells have now been shown to have specific binding sites for high density lipoproteins and preliminary data indicate that LH plays a major role in determining the expression of lipoprotein binding sites. These studies increase our understanding of the control of steroidogenesis and should open up new avenues for investigation of the cause of defective corpus luteum function as well as providing new insight into pharmacologic approaches for the control of corpus luteum function.

Nutrition and Reproduction. The effects of nutrition on reproduction are not fully understood at present. Male reproductive tissues have, however, been found to contain large amounts of lipids, particularly polyunsaturated lipids. Such lipids are important to the integrity and function of membranes and, thereby, determine, in part, the ability of the tissue to perform its functions. The metabolic processes controlling the state of the lipids in the membrane-rich reproductive tissues are currently being actively studied. In particular, essential fatty acid deficiency is detrimental to male testicular tissue. Metabolic studies on testicular tissue from rats maintained on a fat-free diet post-weaning have been found to demonstrate biochemical signs of deficiency for certain fatty acids at even the earliest time periods studied. A higher initial content of fatty acids in Sertoli cells relative to germinal cells was shown to become nearly the same for both cell types in fat-deficient rats. As the rats become even more deficient, the concentration of phospholipids decreased further in the Sertoli cells while there appeared to be relatively no change in the germinal cells. Refeeding a fat-supplemented diet caused an increase in the phospholipid concentration in Sertoli cells, but not the germinal cells. It appears, therefore, that although both Sertoli cells and germinal cells are affected by fatty acid deficiency at about the same time, the Sertoli cells are affected to a greater degree and, thus, may be the primary site of the essential fatty acid deficiency effect on testicular function.

Oviductal Function and Ovulation. The prognosis for recovery of fertility following surgical repair of the Fallopian tubes is inexplicably low. Recent information from studies in a rabbit model indicate that alterations of oviductal structure and function influence ipsilateral ovulatory function. Removal of the fimbriae only on one side resulted in a significant decrease in the numbers of ovulations in the ipsilateral ovary. Although at present the explanations for this suppression of ovulation are speculative, i.e., disruptions of vascular, neural or hormonal linkages between the ovary and oviduct, these observations may have important implications for women who are unable to conceive after surgery in cases of hydrosalpinx corrected by salpingostomy. Such studies of oviductal function in relation to ovulatory function may lead to a better understanding of persistent infertility following restorative oviductal surgery.

A New Technique for Visualization of Cytoskeleton. The multifunctional granulosa cell in vivo undergoes dramatic shape and biochemical changes during its life

history. It is assumed that these shape and biochemical changes involve the cytoskeleton system. RSB grantees studied the cytoskeletal system in vitro by use of a sandwich technique that avoids detergent extraction and freezing. They demonstrated that the cultured granulosa cell is composed of branching and anastomosing lattices of 40-55 nm filaments. This filamentous system is continuous with the plasmalemma and seems to incorporate all formed elements of the cytoplasm. The major filamentous component is actin. It is believed that the cytoskeletal system acts to integrate and organize the complex series of biochemical events that are associated with the granulosa cell.

2. Institutional Programs in Multidisciplinary Reproductive Sciences Research

The RSB supports three types of Institutional Programs in Reproductive Sciences Research: Program Projects, Specialized Reproductive Sciences Research Centers and Reproductive Sciences Research Centers at leading institutions in the United States. Support for these programs is provided to enable such institutions to develop and to conduct reproductive sciences research programs involving such disciplines and such critical masses of highly skilled scientists as may be required for attacks on complex reproductive sciences problems that are beyond solution by the single investigator working alone or in relative isolation in his discipline. A total of 20 Institutional Programs were supported during the period from October 1, 1980 to September 30, 1981.

A. Program Projects

The recipient institutions and principal research areas of the Program Projects supported during the period from October 1980 to October 1981 are: 1) University of Pennsylvania - biochemical and biological events in reproductive processes prior to implantation; 2) University of Texas at Dallas - neuroendocrine and behavioral interrelationships; 3) University of California at La Jolla - reproductive endocrinology; 4) Salk Institute for Biological Studies - neuropeptides in reproduction; and 5) Columbia University - chemistry of human chorionic gonadotropin.

B. Reproductive Sciences Centers

The recipient institutions and principal research areas of Reproductive Sciences Research Centers supported during the same period are: 1) Vanderbilt University - reproductive physiology and endocrinology; 2) Baylor College of Medicine - mechanism of hormone action; 3) Harvard University - reproductive biology and human reproduction; 4) University of Texas at San Antonio - reproductive mechanisms; and 5) University of California at San Francisco - reproductive endocrinology.

C. Specialized Reproductive Sciences Research Centers

The recipient institutions and principal research areas of the Specialized Reproductive Sciences Research Centers supported during the same period are: 1) University of Michigan - reproductive endocrinology from molecular to physiological levels; 2) Case Western Reserve University - reproductive biology of events from ovulation to implantation; 3) University of Texas at Houston - male reproductive function; 4) Mayo Foundation - mechanism of action and molecular biology of reproductive hormones; 5) Salk Institute for Biological Studies - neuroendocrinology of reproduction; 6) Columbia University - reproductive biochemistry and medicine; 7) University of Pittsburgh - primate reproduction; 8) The Oregon Regional Primate

Center - events that control fertility in primates; 9) University of Washington - reproductive medicine and andrology; and 10) The Population Council - reproductive endocrinology.

3. Reproductive Sciences Manpower Development

Because of increased stipend levels for all predoctoral and postdoctoral trainees/fellows who receive support under NIH National Research Service Award training grants and individual fellowships, a curtailment in the funding of new grants took place during the year and only by the retrenchment in the number of trainees and by severe rebudgeting by the institutions affected has it been possible to maintain the current training programs.

A. Institutional Fellowships

The Institute awards grants for maintenance of institutional environments for research training in the reproductive sciences and for the support of both predoctoral and postdoctoral trainees selected for study in these environments. During fiscal year 1981, NICHD supported 24 reproductive sciences research training grants to 23 institutions for a total of approximately $1.6 million. Of the 108 supported trainees, 53 were predoctoral and 55 were postdoctoral fellows.

In addition to these grants, one NRSA Professional Student Short-Term Research Training Grant was supported for 30 predoctoral students. This program is designed to expose talented students in health professional schools to the opportunities inherent in a research career in order to ease the projected shortage of clinical investigators in the future.

B. Individual Postdoctoral Fellowships

In addition to institutional grants for research training, NICHD awards postdoctoral fellowships to individuals for the support of their training in research in the reproductive sciences. During fiscal year 1981, a total of 73 postdoctoral scientists were supported for approximately $1.1 million.

C. Research Career Programs

The Institute awards Research Career Development Awards (RCDA's) to outstanding senior postdoctoral candidates to enable them to devote full time to the development of expertise in research in the reproductive sciences. These awards provide salary to recipients for a maximum of five years support. CPR also supports Research Career Awards (RCA) to established investigators. In fiscal year 1981, 24 RCDAs and one RCA were supported.

D. Clinical Investigator Awards

During 1981, a new type of postdoctoral fellowship was introduced in NICHD, the Clinical Investigator Award. This award affords the opportunity for promising, clinically trained individuals with demonstrated aptitude for research to develop into independent biomedical investigators. It enables candidates to investigate, for up to three years, a well-defined problem with a sponsor competent to provide guidance in a chosen area of research in the reproductive sciences. During FY 1981, three Clinical Investigator Awards were made.

E. Minority Biomedical Support (SO6s)

This program, administered by the Division of Research Resources (DRR), seeks to strengthen the biomedical research and research training capability of ethnic minority institutions in health-related sciences. CPR contributes approximately $127,000 towards this program by supporting four awards in the reproductive sciences.

4. Reproductive Sciences Research Facilitation and Augmentation

A. Conferences, Workshops and Symposia

Conferences, workshops and symposia are sponsored by RSB, CPR to facilitate the exploitation of new knowledge in critical areas of research in the reproductive sciences and to facilitate the rapid dissemination of new and significant information regarding the reproductive sciences. The following meetings were sponsored during the period from October 1, 1980 to September 30, 1981.

A workshop on Functional Correlates of Hormone Receptors in Reproduction was held in Augusta, Georgia, October 1980. The organizer of the workshop was Dr. Virenda B. Mahesh, Medical College of Georgia.

The Annual Meeting of Directors of CPR-supported Reproductive Sciences Centers and Program Projects was held in San Antonio, Texas, May 1981. It was hosted by Dr. Carl Pauerstein, University of Texas.

Following the Directors' Annual Meeting, a workshop on The Primate Model in Reproduction was held in San Antonio. It was sponsored by the University of Texas and organized by Dr. C. Pauerstein.

CENTER FOR POPULATION RESEARCH

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Contraceptive Development Branch

NICHD Annual Report
October 1, 1980 through September 30, 1981
Office of the Director, Center for Research
for Mothers and Children

The Center for Research for Mothers and Children (CRMC) supports research and research training activities related to the special health problems of mothers and children in the biomedical, social and behavioral sciences. Specifically, CRMC develops and supports basic and clinical research and research training in the biological and behavioral aspects of, and environmental influences upon, normal and high-risk pregnancy, embryonic development, fetal growth and maturation, nutritional requirements during pregnancy and infancy, labor and the birth process, postnatal adaptation and maintenance of homeostasis, disorders of infancy, and the effect of perinatal events upon subsequent growth and behavioral development. The goal is to minimize the risk of morbidity and mortality during the perinatal and infancy period of life and to prevent subsequent functional impairment.

The Center also supports studies concerned with the precursors of many adult diseases and disabilities, focusing on congenital defects and developmental immunology, nutrition, metabolism, and endocrinology. These studies extend through adolescence to adulthood. The goal is to prevent chronic disabilities by understanding their genetic, nutritional, metabolic and immunologic antecedents.

The research program of the CRMC includes studies related to the learning process as well as cognitive, personality and social development. Support is given for research that attempts to reveal the nature of the general learning process and certain specific processes such as the acquisition of speech and language and learning to read. Studies of specific learning disorders such as developmental dyslexia are also a major concern. Personality, attitude formation, and social development of young children are investigated by a wide range of different scientists such as developmental and experimental psychologists, sociologists, anthropologists, and pediatricians.

In addition, the CRMC has primary responsibility within the National Institutes of Health for research and research training in the area of mental retardation. It meets this responsibility through support of a wide range of biological, behavioral, and social research directly concerned with determining the causes and appropriate procedures for the prevention and elimination of the problem. Funds are provided through all of the regular NIH research support mechanisms and through core support to 12 Mental Retardation Research Centers located throughout the country..

During this reporting period, Dr. Merrill S. Read continued to serve as the Acting Director of the CRMC, as well as the Chief of the Clinical Nutrition and Early Development Branch. Dr. James F. Kavanagh, the Associate Director of the CRMC also maintained responsibility for the human communication research area in the Human Learning and Behavior Branch.

Dr. Peter M. Vietze, formerly a Research Psychologist with NICHD's Intramural Program, joined the Mental Retardation and Developmental Disabilities Branch in September, 1980. In his new assignment, Dr. Vietze serves as the Head of the Mental Retardation Research Centers program.

On June 29, 1981, Dr. Peter Alterman became the Administrative Officer for the CRMC. A former H.E.W. Management Intern, he comes to the CRMC from the National Center for Health Care Technology.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Clinical Nutrition and Early Development Branch
Center for Research for Mothers and Children

The CNED Branch is the largest Branch in the CRMC. It provides support for approximately 60 percent of the research and training for research conducted under the auspices of the CRMC. The CNED Branch contains the following three Sections:

Genetics and Teratology Section supports research into the underlying mechanisms controlling both normal and abnormal development, and is structured around the following areas: developmental genetics, developmental biology, teratology and developmental immunology. Clinical as well as basic studies are emphasized. The goal of this program is to prevent, alleviate and treat birth defects with a special focus on the structural defects.

Pregnancy and Perinatology Section supports research to advance knowledge on pregnancy and maternal health, fetal growth and maturation, and newborn well-being. Program goals take into account the interrelationships of specific health and developmental problems occurring in the prenatal, perinatal and infant periods of life, and the effects these events may have on the development and well-being of the child. Activities are organized around five maternal-infant health problem areas: high-risk pregnancy, fetal pathophysiology, premature labor and birth, disorders of the newborn and the sudden infant death syndrome.

Nutrition and Endocrinology Section supports research and research training on the roles played by nutrients and hormones in development during fetal life, infancy, childhood, and adolescence. Program goals include the achievement of a better understanding of the relationships between nutrient and hormonal factors during normal growth and development as well as in growth retardation and developmental disorders of the endocrine systems.

Table 1 summarizes the grant and contract programs for each of the three CNED Sections, giving the dollars and numbers of projects active in June, 1981. Detailed reports of the accomplishments of each of the Sections are presented in the following pages.

Table 1.

NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1981
CLINICAL NUTRITION AND EARLY DEVELOPMENT BRANCH

Funds (thousands)

Section	Total		Research Grants								National Research Service Awards		Research Contracts (incl. S06)	
			Total		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	732	$70,406	622	$61,643	548	$47,523	25	$12,333	49	$1,787	72	$3,989	38	$4,774
Genetics and Teratology	263	22,867	228	20,506	197	17,073	7	2,587	24	847	32	2,041	3	320
Pregnancy and Perinatology	282	30,885	235	26,997	205	18,069	15	8,356	15	572	22	1,280	25	2,608
Nutrition and Endocrinology	187	16,654	159	14,140	146	12,381	3	1,391	10	368	18	668	10	1,846

Notes: 1) Excludes scientific evaluation grants.
2) The Genetics and Teratology Section excludes two contracts totaling $144,503 funded from a source other than NICHD.

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The Genetics and Teratology (G&T) Section supports a research program on the biological development of the human organism which focuses on congenital defects as a priority area. During the past year, the Section has extended its studies in developmental biology to gain a better understanding of contributing factors to the human developmental process and to obtain a baseline against which results of teratological studies can be compared. The Genetics and Teratology Section is now structured around the following research areas: developmental genetics, developmental biology, congenital defects and developmental immunology.

DEVELOPMENTAL GENETICS

The role of genetic factors is essential to our understanding of human development and susceptibility to disease. The G&T Section, therefore, supports a genetics research program, both at the clinical and the basic developmental genetics research level.

Human and Clinical Genetics

Studies in human and clinical genetics are concerned with human genetic variation and with genetic determinants of health and disease. Variations in genetic traits are being investigated in classical twin studies of both monozygotic and dizygotic twins. Furthermore, a recently-developed half-sib model is used to estimate the genetic and environmental contributions, as well as the maternal factors relevant to specific diseases, behavioral characteristics and types of twinning. The focus is on medically significant traits such as birth defects, causes of fetal wastage, normal growth and development, genetic control of the immune response, psychological traits, and childhood antecedents of adult disease.

Genetic variability is further investigated with a population genetics model. An assessment is made of selected genetic loci in multi-locus systems, in order to associate measurable genetic traits with disease susceptibility. The results so far suggest that the observed data on the association between the human leukocyte antigen (HLA) system and juvenile onset diabetes are best explained by postulating that the disease is determined by two genetic loci plus environmental factors. Development of two-locus disease models will be continued and an analysis will be made of new data on HLA and disease as they become available.

Other studies supported by the G&T Section in the Clinical Genetics area identify defects in the genetic material determining a number of developmental disabilities and establish the nature of the accompanying biomedical and behavioral disorders. This includes identification of sex chromosomal abnormalities in children with abnormal chromosome counts and establishment of the nature of the abnormal development of the children in physical, hormonal, intellectual, social, and emotional terms. This also encompasses elucidation of the genetic defects underlying the skeletal dysplasias, and identification of new clinical abnormalities (shortened stature and marked deformities) and of biochemical and morphological defects underlying these disorders. Genetic defects are also being investigated in patients with X-linked ichthyosis, and the bio-

Table 1a.

NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1981

GENETICS AND TERATOLOGY SECTION

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts (incl. S06)	
			Total Research		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	263	$22,867	228	$20,506	197	$17,073	7	$2,587	24	$847	32	$2,041	3	$320
Clinical Genetics	13	1,611	12	1,426	6	450	2	823	4	153	-	-	1	185
Basic Developmental Genetics	41	4,001	36	3,790	30	2,825	2	834	4	131	4	122	1	89
Developmental Biology, General	82	6,757	64	5,329	55	5,024	-	-	9	305	17	1,381	1	47
CNS Development	13	1,181	13	1,181	12	897	1	284	-	-	-	-	-	-
Limb Bud Development	4	245	4	245	3	207	-	-	1	38	-	-	-	-
Chondrogenesis	6	888	6	888	6	888	-	-	-	-	-	-	-	-
Myogenesis	15	1,038	13	995	10	888	-	-	3	107	2	42	-	-
Teratology-Biological Causes	11	976	9	697	9	697	-	-	-	-	2	279	-	-
Teratology-Physical & Chemical Causes	21	1,570	19	1,497	19	1,497	-	-	-	-	2	73	-	-
Ontogeny of Immunity	13	1,013	12	948	9	580	1	290	2	78	1	65	-	-
Neonatal Infection	19	1,821	18	1,800	16	1,410	1	356	1	34	1	21	-	-
Immunology of Breast Milk	5	234	4	216	4	216	-	-	-	-	1	18	-	-
Reproductive Immunology	20	1,534	18	1,495	18	1,495	-	-	-	-	2	39	-	-

Notes: 1) Excludes scientific evaluation grant.
2) Excludes two contracts totaling $144,503 funded from a source other than NICHD.

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chemical deficiency in this disorder explored. The results of such investi gations will contribute to an understanding of the consequences of hereditary disorders which will aid in the search for new treatment modalities of hereditary diseases.

Basic Developmental Genetics

Our understanding of human development and genetic diseases has also been advanced through molecular and biochemical genetics. Recent studies provide knowledge of genetic mechanisms that control gene expression, and information on the structure of genes insofar as this may contribute to the genetic regulatory mechanisms.

Genetic control mechanisms for structural genes have been found operative at either the transcriptional or post-transcriptional level. Transcriptional level control allows the differential expression of certain parts of the genome, and this expression is modulated by nearby temporal or spacial regulatory genes to yield stage- or tissue-specific gene products. Structural genes are also modulated by components of the genome other than adjacent sequences of DNA. The mechanisms by which either flanking DNA sequences or various non-histone chromosomal proteins promote selective expression of genes needs continued investigation if genetic control is to be understood.

One of the latest findings in post-transcriptional genetic control is that eukaryotic cells synthesize much larger RNA molecules from DNA in their nucleus than are exported to the cytoplasm for the purpose of translation into gene products. These larger RNA precursor molecules are processed into mature RNA messengers through excision of transcripts of non-coding regions of DNA. The non-coding DNA sequences (introns) interrupt the expressed segments of individual genes; they are not represented in the final gene products but may have a regulatory role in the expression of eukaryotic genes.

The third mechanism serving to control gene expression involves the use of stored cytoplasmic RNA. Messenger RNA is stored in unfertilized eggs in an inactive state. Minutes after fertilization, the rate of protein synthesis increases, encoded by the stored mRNA. The focus of investigations is on mRNA-ribosome interactions, the role of messenger- and tissue-specific initiation factors in stimulating the mRNA-ribosome association, and specific factors that are inhibitory for protein synthesis. These investigations attempt to demonstrate the existence of a translational control mechanism as one of several regulatory processes involved in the expression of genes.

DEVELOPMENTAL BIOLOGY

Studies on the expression of genes during development have to be augmented by investigations on the contributing role of non-genetic factors to the developmental process. The Genetics and Teratology Section, therefore, supports research on the control of morphogenesis by these epigenetic factors as this applies to developing systems in general, and on the development of specific organ systems (e.g., limb bud).

Formation of Tissues and Organs During Embryogenesis

One of the most crucial questions in developmental biology today is the manner in which a single fertilized egg differentiates into highly organized, specialized tissues and organs. An important aspect is the uneven distribution of morphogenetic substances in the egg's cytoplasm that have been synthesized during oogenesis and have a role in the early developmental process. Attempts are being made to establish the identity and function of the cytoplasmic substances (RNAs or proteins) that influence early development, to identify the genes defining these substances, and to determine the mechanism of localization of these epigenetic control substances in the cytoplasmic matrix. These investigations seek answers to the question of whether maternal cytoplasmic substances contain information which specifies organization of the fetus during later developmental stages.

Control of tissue and organ formation during embryogenesis is further investigated through studies of other properties of the developing cells. Of interest are intracellular structures such as the cytoplasmic skeletal support system, especially the microtubules which participate in cell division, cell movement, and the maintanence of cell shape. Of further interest is the extracellular matrix since this influences cellular differentiation, i.e., specific gene expression, and interactions with the cell surface. Components of the cell surface are also under investigation: cell surface components play a role in intercellular adhesions to allow groups of cells to form a structural unit, or form intercellular junctions through which morphogenetic chemical messages can pass that control cell growth and differentiation in the developing embryo. Continued exploration of the architecture and function of intercellular junctions should increase our understanding of how cellular integration gives rise to higher forms of life with capabilities beyond those of individual cells.

Development of the Limb

An example of the development of an organ system that is supported by the Genetics and Teratology Section is the developing limb. Limb development studies explore the interaction of the ectodermal germ layer with the underlying mesoderm in the expression of the mesoderm into limb cartilage and limb muscle cells. Other studies explore the factors that determine which of the morphologically indistinguishable mesoderm cells differentiate to form cartilage and which to form muscle.

Cartilage and muscle differentiation is being investigated in terms of the developmental time when mesoderm cells become determined to express themselves into cartilage and muscle cells. Chondrogenesis and myogenesis is further studied with a view to localization of the muscle and cartilage genes on chromosomes, and identification of muscle and skeletal genes affecting limb shape. Emphasis is placed on the structure of muscle and collagen genes, and on the mechanisms which regulate the gene expression. A most interesting result obtained to date on one of the collagen genes is that the gene contains unexpressed DNA sequences, introns, which may eventually turn out to exert a genetic regulatory function. The various processes by which limb bud cells sort themselves out and interact for differentiation into muscle or cartilage cells at specific developmental times need further investigation. The aim is to understand the events underlying the attainment of normal structure and function of the limb during the embryonic developmental process.

CONGENITAL DEFECTS

A program for prevention and treatment of congenital defects requires studies of deviations from the normal developmental program of the conceptus, embryo, fetus, and child. This requires investigations of disruptions in the normal sequence of developmental events during organogenesis, and the mechanisms by which the causative agents produce structural and/or functional damage.

Causes of developmental defects that are investigated are either genetic, i.e., gene mutations or chromosomal aberrations, or include diverse agents in the internal or external environment of the developing embryo, fetus or child. Mutant genes have been found to produce a wide range of defects including abnormal limb morphogenesis in animals and dwarfism in children while chromosomal aberrations also cause various physical and mental developmental deficits in affected individuals. Congenital malformations in babies are also observed upon their prenatal exposure to viral teratogens. Maternal zinc deficiency is found to cause teratogenic effects in offspring while an excess of certain externally supplied drugs can cause deformities of the limb. More important than the association of teratogenic agents with certain developmental disorders are studies of the developmental mechanisms by which the disorders are produced. Suggested mechanisms range from mutagenic or clastogenic events to mitotic inhibition, altered ionic balances, or changed biosyntheses of cell- or tissue-specific components. Suggested sites of action vary similarly, ranging from intranuclear or intracellular to extra-embryonic. The developmental mechanisms are only starting to be understood for a few teratogenic agents and more research is needed if the goal of prevention and treatment of congenital defects is to be achieved.

DEVELOPMENTAL IMMUNOLOGY

Ontogeny and Phylogeny of Immunity

This Section supports research on the development of the immune system, beginning with the intrauterine period and extending through full adult immunologic maturation. This focus on the ontogeny of the immune system is supplemented with studies of the phylogeny of the system. These investigations provide insights into the normal evolution of the reticuloendothelial system and assist in the selection of lower animal models that allow research not feasible in the human. Other studies in both human and animal models are concerned with the causes of immune deficiencies and the development of new therapies. New research directions in trace element regulation of immunity and infection will be derived from a workshop, organized for September 1981. Present and future immunologic studies should lead to a better understanding of the development of the system and its function, and should identify the aberrations from the normal leading to infection and disease.

Newborn Infections

Of special concern is the immunologic vulnerability of prematures and newborns resulting in increased morbidity and mortality due to infections. Studies attempt to investigate the characteristics found in the premature and newborn that make this developmental period more susceptible to infections. Study of the ontogeny of these events should produce important insights into the prevention and management of intra-uterine infections and their resultant sequelae such as growth dysfunctions, mental retardation, and congenital malformations.

In addition, NICHD is providing partial support with NIAID for a workshop on the role of prophylactic penicillin for control of early onset group B Streptococcal sepsis in the newborn. This is to be held in October, 1981.

Immunology of Breast Milk

The recently discovered nutritional and immunological factors of breast milk require an evaluation of breast feeding for its beneficial effects on infants and especially on the high risk infant in whom either the nutritional and/or immunologic imbalance may be precarious. Studies include the immunologic properties of breast milk, the mother's experience with past infections, the immunological protection of the infant through breast-feeding, and the ingestion of milk and its interaction with the infant's digestive tract. Information on potential adverse effects of breast feeding will also be pursued. A publication, entitled Immunology of Breast Milk (which resulted from an NICHD-sponsored conference), will continue to stimulate research in these areas.

This Section has expanded its research activities on the gastrointestinal tract which is often the portal of entry for agents causing neonatal sepsis. The aim is to better understand regional immunity mediated by locally formed antibody and viable immunologic cells and other factors associated with mucous membranes exposed to the external environment. A broadened knowledge of the local defense mechanisms of the gastrointestinal tract is important in studies of the immunology of breast milk.

Reproductive Immunology

The mammalian fetus survives pregnancy even though it contains some immune characteristics which are foreign to the mother's immune system. The fetus becomes an allograft to the mother during pregnancy and successfully maintains a close association with the mother markedly exceeding the normal rejection time of most allografts. The means by which the fetus is tolerated by the mother is not adequately understood. If the ontogeny of immunity is to be fully appreciated, the fetal period must be properly evaluated in regard to the immune response to this fetoplacental unit and the maintenance of pregnancy.

STAFF ACTIVITIES

CNGT staff participate in various trans-NIH activities and joint efforts with other government agencies. These include membership on the NIH Coordinating Committee For Blood-Related Activities and a new working group on Blood and Its Substitutes established by the Interagency Technical Committee on Heart, Blood Vessel, Lung and Blood Resources. A staff member has been appointed to the NIH Cystic Fibrosis Coordinating Committee which functions to stimulate and plan for research activities across NIH. An international cystic fibrosis conference was planned and jointly funded through this effort. Representation on the NIH Working Group For Reye Syndrome allows coordination of research efforts and has resulted in an NIH Announcement on research needs. The announcement was developed by this Working Group which represents five different Institutes or components within NIH. An NIH Consensus Development meeting on Reye Syndrome was held in March 1981 through collaborative efforts of the Institutes represented by this Working Group. Staff also participates on two planning committees of the Fogarty International Center for the development of two international conferences: (a) International Symposium on the Control of Measles and (b) International Symposium on Increased Control of Poliomyelitis

Feasibility of Eradication. A member of the Section has also been appointed to the Genetics Education Committee of the Health Services Administration (HSA). This committee is developing recommendations for the improvement of genetics education in the HSA funded State Grant Programs providing genetic services across the nation. A workshop on genetics education was developed by this committee and held in September, 1980.

The Pregnancy and Perinatology Section supports and promotes a program of research and research training that is intended to enhance the advancement of knowledge related to pregnancy and maternal health, fetal growth and maturation, and well-being of the newborn. Of special interest are maternal health problems that affect fetal and infant health, problems facing the newborn infant in adapting to extrauterine life, and events in the neonatal period that can influence the growth and development of the child.

RESEARCH ACTIVITIES

Activities of the Section are organized around five maternal-infant problem areas. These areas, and the distribution of research funds among them in FY 1981, are High Risk Pregnancy (33%), Fetal Pathophysiology (22%), Premature Labor and Birth (14%), Disorders of the Newborn (19%), and the Sudden Infant Death Syndrome (12%). The funds were divided among 282 research grants and contracts and amounted to $30.8 million, approximately 27% of the holdings of the Center for Research for Mothers and Children. Research training support accounted for 4.1% of this amount. Table 1b provides a more detailed description of these funds.

High Risk Pregnancy

This research addresses abnormal gestation processes or situations that place the mother, fetus, or both in greater jeopardy of death or disability. Advances from research in this area have contributed significantly to the marked reductions in maternal and infant mortality achieved in the past two decades. A major emphasis in this area is placed on increasing knowledge of the normal course of pregnancy, labor, and delivery, as a basis for better understanding abnormal processes. The abnormal conditions that are of particular interest are pregnancy-induced hypertension and toxemia, diabetes, isoimmunization, malnutrition, infections, anemia, and hemorrhagic phenomena. The role of the placenta in maintaining pregnancy and the fetus is another major focus of research. In recent years increased attention has been given to the pregnant adolescent as a particularly high risk group.

Fetal Pathophysiology

Fetal pathophysiology studies are directed toward factors influencing normal and abnormal embryonic development. Environmental hazards that may be detrimental to the health and growth of the fetus are a major focus of this research. Development of methods and techniques for assessing fetal growth and well-being at various stages of gestation and labor is also of interest. A final common path for fetal injury induced by many pathologic processes of pregnancy or labor is hypoxia. Consequently many projects are addressing the problem of hypoxia, with emphasis on how it causes injury, accurate diagnosis of its presence, and ways to prevent or correct it. Studies of maturation of fetal physiologic functions and mechanisms have led to significant improvements in the treatment and survival of small infants. For example, the demonstration that respiratory distress syndrome in premature infants is caused by lack of pulmonary surfactant, because the enzyme system that triggers its production has not matured before delivery, has made possible the development of prenatal treatment to induce surfactant production when premature delivery cannot be prevented. Numerous developmental studies of this and other systems are supported. The recognition that exposure of the fetus to drugs or chemicals may alter its development and have longlasting or delayed effects has spurred a large number of studies in the field of fetal and neonatal pharmacology. This is a field of growing interest in the Section.

Table 1b.

NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1981
PREGNANCY AND PERINATOLOGY SECTION

Funds (thousands)

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts (incl. S06)	
			Total Research		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	282	$30,885	235	$26,997	205	$18,069	15	$8,356	15	$572	22	$1,280	25	$2,608
High-Risk Pregnancy	94	10,294	79	9,140	70	5,879	6	3,144	3	117	7	308	8	846
Fetal Pathophysiology	58	6,897	52	6,576	43	4,051	4	2,336	5	188	5	285	1	37
Premature Labor and Birth	40	4,164	39	4,155	34	3,041	2	1,005	3	109	-	-	1	9
Disorders of the Newborn	63	5,722	46	4,488	40	3,492	2	838	4	158	10	687	7	547
Sudden Infant Death Syndrome	27	3,809	19	2,638	18	1,606	1	1,033	-	-	-	-	8	1,170

Note: Excludes scientific evaluation grants.

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Premature Labor and Birth

Premature labor and birth constitutes the major problem in maternal and fetal medicine today. Two-thirds of all infant mortality occurs among infants weighing 2500 grams or less at birth, and the nation's high prematurity rate is responsible for our relatively poor performance in infant mortality compared to other countries. Consequently there is a great interest in studies of the normal onset of labor, why labor sometimes begins prematurely, how premature labor might be stopped without detrimental effects, and the mechanism(s) of intrauterine growth retardation. These studies are addressing such topics as endocrine factors that maintain pregnancy and initiate labor, mechanical factors related to premature rupture of the membranes and dilation of the cervix, and indicators of impending labor.

Disorders of the Newborn

Disorders of the newborn are responsible for approximately three-fourths of the the infant deaths in the United States, and produce long term disability for many individuals who are affected by them and survive. Research directed toward reducing the impact of these disorders includes studies of maternal health problems that affect the status of the infant, adaptation of the newborn infant to its environment, and problems in the early weeks of life that influence subsequent development and behavior. Problems of particular importance are neonatal sepsis, intracranial hemorrhage, respiratory distress syndrome, necrotizing enterocolitis, jaundice, and care of the low birthweight infant.

The Sudden Infant Death Syndrome

The sudden Infant Death Syndrome (SIDS) was singled out for special research emphasis by Congress in 1974. The Institute has made particular efforts to encourage research on this problem, and as a result the program has had significant growth. A major consequence of this increased research has been a change in the basic concept of the SIDS infant, who is no longer viewed as having been perfectly healthy prior to death, but rather is believed to have had earlier developmental abnormalities. This revised concept suggests that a profile of infants at particular risk for SIDS might be developed so that intervention and prevention efforts can be targeted to a high risk population. Current research efforts are directed toward specifying these risk factors, identifying the cause(s) of SIDS, developing a more effective apnea monitor, and improving methods for helping families cope with a SIDS death.

MAJOR RESEARCH PROGRAMS

Major Research Programs (MRPs) have been established by the Institute to provide an integrated approach to major unresolved problems in perinatal medicine. These MRPs support multidisciplinary research in areas where knowledge gaps have not been sufficiently addressed by ongoing research, or promising areas in need of special stimulation. The Section supports the seven MRPs funded by the Institute. They are addressing the problems of diabetic pregnancy, prematurity, SIDS, and hypoxia.

RESEARCH ACCOMPLISHMENTS

Selected scientific accomplishments during FY 81 pertinent to the Section's five problem areas are highlighted in the following paragraphs:

Maternal urinary excretion of estriol increases with the progression of gestation. Normal values signify a good fetal outcome, but abnormal ones may or may

not indicate fetal distress. The 24-hour urine collections needed for analysis of estriol level are inconvenient and frequently incomplete and thus misleading. An ongoing study is evaluating the use of unconjugated estriol in maternal plasma, measured by a rapid radioimmunoassay, as an alternative way to screen high risk pregnancies. It appears to be a promising technique.

Pregnancy-induced hypertension often results in intrauterine growth retardation, and may threaten the life of both mother and fetus. Numerous investigations of maternal cardiovascular physiology during pregnancy are under way to clarify regulatory mechanisms and their relationship to maternal hypertension, utero-placental perfusion, and fetal well-being. Results obtained to date have demonstrated that normal pregnant animals respond to estrogen with a significant vasodilation, which has its maximal effect on blood flow to the reproductive tissues including the placenta. It was also found that, in normal pregnancy, the usual vasopressor (constrictive) response to angiotensin II is markedly reduced due to an intrinsic change in response of the vessel wall. Studies are now in progress to determine the mechanism of this alteration, and whether women who develop pregnancy-induced hypertension are unprotected against the actions of angiotensin II.

In order to evaluate the potential risks and benefits to the fetus of chemicals and drugs entering the maternal circulation, knowledge of the mechanisms whereby such compounds may be altered during placental passage is indispensable. Studies are being supported to determine the enzymatic potentialities of the human placenta and its capabilities for bioactivation (conversion of inactive drugs or chemicals to substances with mutagenic and carcinogenic or cytotoxic properties). The studies explore the basic mechanisms whereby such chemicals as diethylstilbestrol, diphenylhydantoin, thalidomide, etc., are handled by the human placenta and fetus. Results have shown that the human placenta contains enzymes that catalyze several bioactivating reactions. Research is in progress to describe and define the biochemical capabilities of placental tissues with respect to altering exogenous compounds and to relate the findings to the practical aspects of the exposure of the unborn child to foreign chemicals.

Sometimes therapeutic successes bring new problems. Such is the case with phenylketonuria (PKU). With newborn screening and diet treatment, patients with PKU now are growing to adulthood with normal intelligence, marrying and having children. However, it has become apparent that children of mothers with PKU are usually retarded, presumably due to brain damage caused by high maternal and fetal phenylalanine (PA) concentrations during pregnancy. Returning the mother to a restricted-phenylalanine diet during pregnancy has given mixed results. Scientists have developed a monkey model of maternal PKU to study this problem. By infusing PA into pregnant monkeys and measuring maternal and fetal blood levels, they demonstrated that there is active transport of PA across the placenta so that fetal levels of PA are markedly higher than maternal levels. Thus maternal PA levels previously considered "safe" (10mg/dl) probably cause damage to the fetal central nervous system, and the maternal level for diet therapy should probably be targeted between 3-6 mg/dl. Ongoing studies are maintaining primate mothers at different PA levels during pregnancy. Their infants will be tested at one year of age to determine what a "safe" maternal PA level during pregnancy might be. These studies will be of a major importance to the more than 100 phenylketonuric women reaching reproductive age each year.

Maternal diabetes remains a major source of infant mortality and morbidity. One interesting study addressing this problem is comparing the pregnancy outcome in normal rats, diabetic rats, and rats whose diabetes has been corrected by transplantation of fetal pancreatic islet cells prior to pregnancy. Preliminary studies indicate that reversal of diabetes in this way provides a maternal environment that is consistent with normal pregnancy. Furthermore, gestation time is normal instead of prolonged, and fetal organomegaly, malformations, and excess mortality are limited. These studies show that pregnancy does not adversely affect the transplanted state, and in fact, transplanted females with normal glucose tolerance tests can undergo multiple pregnancies and remain normal. If gestation time, increased fetal mortality and organomegaly associated with maternal diabetes are complications of the disease, then an adequate reversal of diabetes by transplantation could reverse the risk.

Pregnant adolescents constitute one-fifth of all pregnant women and are a high risk group for abnormal fetal outcome. The biologic reasons for this increased hazard are not completely understood. One group of investigators has suggested that pregnant adolescents and their offspring may be at particularly high risk for cytomegalovirus (CMV) infection. They found that the prevalence of prenatal CMV infection was higher among adolescents than in any other group. Young women who were both sero- and virus-positive had infants with a rate of congenital infection close to 20 percent. One important issue to be resolved is that of possible gestational CMV-reactivation; preliminary data seem to support this possibility.

Fetal distress due to hypoxia during pregnancy and labor is a major obstetric concern, and its causes and means of accurate detection have been a focus of many studies supported by the Section. One group of investigators examined the function of the fetal sympathoadrenal system under basal conditions and during hypoxic stress. They measured basal levels of epinephrine, norepinephrine, and dopamine, and documented their rise during hypoxemia. A strong negative correlation was observed between plasma catecholamine concentrations and the state of fetal oxygenation. Hypoxic episodes occur frequently in the fetus during pregnancy and parturition and contribute to perinatal morbidity and mortality. By achieving a more thorough understanding of the physiologic processes involved in the fetal response to oxygen deprivation, it will be possible to understand the mechanism of action and permit the development of improved means to detect hypoxia so as to institute appropriate treatment.

Studies of the controlling steps in the production of pulmonary surfactant are exploring its relation to active labor. After observing that respiratory distress syndrome (RDS) is more frequent in infants born without labor, investigators recently have shown that labor itself stimulates surfactant production (synthesis and secretion) during immediate newborn period. Present efforts are trying to elucidate the mechanism by which labor exerts this effect. Other studies are addressing the question of why RDS is more common in the infant of the diabetic mother. One possiblity is that insulin may inhibit the activity of cholinephosphate cytidylyltransferase, an enzyme which is stimulated by the glucocorticoids and by estrogen and which may be a rate-regulatory enzyme in surfactant biosynthesis. Related studies are investigating the reasons why the test for fetal lung maturity is less reliable in diabetic patients.

Administration of medications to the mother during pregnancy can have long-term effects on the offspring. The experience of "DES daughters" with vaginal

cancer and reproductive casualties indicates the importance of long-term studies in this area. In one study, investigators administered phenobarbital prenatally, and measured testosterone levels in newborn male rats. The intrauterine exposure resulted in a significant decrease in testosterone level in the brain and plasma, and in the decrease of the testicular synthesis of testosterone. As adults, treated animals exhibited defects in sexual maturation such as a delay in testicular descent, shorter anogenital distance, and infertility. It is known that neuroendocrine differentiation in the rat takes place in response to androgen during a short period just before and after birth. Therefore, phenobarbital administration during the prenatal period was able to alter testicular androgen synthesis secretion, resulting in a modified environment of this hormone during early postnatal period with subsequent sexual dysfunction. The significance of this finding for the human is not known.

Scientists studied the effects of prenatal exposure to the tricyclic antidepressent doxepin HCl on the development of normal function of the catecholamine neuroendocrine and cardiovascular systems in the rat. They found that the doxepin is distributed to and accumulates in fetal tissues in concentrations 5 to 8 times higher than in maternal plasma. Birth weight, infant mortality, and subsequent growth were unaffected by prenatal doxepin. However, reproductive function of female offspring was adversely affected with only 21% having normal estrous cycles and abolition of the normal LH response to estrogen and progesterone. This finding may have far-reaching implications as to the uses of doxepin and other highly lipid soluble drugs during pregnancy, and suggests that the current methods of assessing teratogenicity need to be modified to include assessment of effects on reproductive function.

The factors regulating uterine contractility are important in initiating and maintaining pregnancy and terminating it at the proper time. Investigators have demonstrated that one such factor, the hormone relaxin is elevated in plasma at the time of the first missed period following conception and that it suppresses spontaneous contractile activity of the human myometrium. The studies suggest that relaxin may be an important factor in the promotion of uterine quiescence and hence maintenance of pregnancy.

Another major complication of pregnancy is premature rupture of the membranes which increases the risk of both prematurity and infection. Differences in stress tolerance between term and preterm human chorioamniotic membranes have been examined. Preterm membranes are stronger under normal conditions than tissues at term. These characteristics are being explored in relation to environmental factors associated with their premature rupture. Studies are currently underway of the collagen content of membranes that rupture prematurely and those that do not, and the possible relationship to such factors as their lead content. Ultrasound studies of mechanical volumetric changes in the uterus are also being performed seeking to relate these changes to membrane rupture.

Continuous positive airway pressure (CPAP) respiratory therapy has markedly increased survival of infants with RDS. One complication of respirator therapy for newborns is development of bronchopulmonary dysplasia (BPD) with potential long term lung damage. A study in progress is examining the cytology of aspirates from the trachea of infants receiving endotracheal tube suctioning as part of routine respirator care. The investigators have defined three classes of pulmonary cytology in infants with respiratory distress syndrome. They found that

appearance of a third class of cells indicated development of BPD, and that the cytological changes in pulmonary effluent antedated unequivocal radiologic identification of BPD by 4 days. The finding of inflammatory cell predominance in this class, without evidence of infection, suggests that in human neonates oxidant damage to the lung promotes the influx of cells capable of releasing proteolytic enzymes, such as elastases, that may result in the specific injury of BPD. The cytologic findings may provide an early warning of impending BPD and permit modification of treatment to prevent its development.

Viral and bacterial infections continue to be a threat to perinatal survival. Infected amniotic fluid (amnionitis) is a significant source of maternal and infant morbidity, and a focus of numerous studies. Recently amniotic fluid substances that inhibit bacterial growth have been described. These inhibitory substances tend to be present in non-infected and absent in infected women. With further study, it may be possible to prevent some infections by augmenting or inducing inhibitory activity in amniotic fluid.

Since the sudden infant death syndrome was singled out by Congress for special emphasis in the Sudden Infant Death Syndrome Act of 1974 (P.L. 93-270), continuous efforts by Institute staff have expanded this program. Through research, the initial concept that SIDS victims were completely healthy before their deaths has been replaced by the view that they generally have demonstrated some abnormalities. This concept suggests that the recognition of risk factors could define a "high-risk" population to which preventive measures might be targeted. In addition, epidemiologic and pathologic data have been accumulated that have helped generate a number of etiologic theories.

One set of hypotheses suggests that abnormalities of respiratory control during sleep may be the cause of SIDS. Healthy infants who suffered a "near-miss" episode (resuscitated successfully after sleep apnea) have been monitored in a non-invasive manner during sleep. Breathing during sleep was found to be cyclic; this may be related to a neural control mechanism, and not dependent on sleep state. Infants who had a near-miss episode responded with either underventilation or with increased ventilatory reaction to inhaled CO_2 during sleep, indicating possible abnormalities in the control centers of the brainstem. Siblings of SIDS victims have been studied and as a group had an increased incidence of periodic breathing and a tendency toward lower ventilatory responses to CO_2 breathing. Furthermore, infants who experienced near-miss spells at 1-4 months of age had persistent sleep hypoventilation.

Studies exploring the physiological events preceding arousal in both normal and high-risk infants found that the organization of sleep states is disturbed in high-risk infants, but it is not clear yet if this deficit is related to the potential for failure to arouse from apnea that ends in sudden death.

Studies of fetal breathing movements have shown that respiratory sensitivity to CO_2 is detectable early in the third trimester of gestation. Immediately after birth, this respiratory sensitivity, as reflected by the relation between tidal volume and respiratory period, is haphazard, but it becomes inversely correlated after 48 hours. However, some infants have been observed in whom the haphazard relationship persisted; over half of these later died of SIDS. Expansion of this study is under way to examine this phenomenon closely.

Prematurity and its consequences represent a health problem of major proportion. In order to prevent premature birth, it is necessary to understand mechanisms involved in the initiation of parturition. Research in one of the MRPs is addressing the biochemical events accompanying the onset of labor. Attention has been directed to the metabolism of the human fetal membranes, considering that these structures may play both a metabolic and mechanical role. Measurements of prostaglandin concentrations have shown that they have a specific anatomic distribution in these tissues, giving further credence to their importance in beginning parturition. Another aspect of the research is to clarify the mechanisms of control of the unique endocrine system of the human fetus. One interesting result indicates that when human fetal adrenal is functioning normally, the levels of substrate for fetal steroidogenesis in vivo in the fetal plasma are maintained at low, steady state levels. However, when that function is depressed due to anencephaly, severe maternal hypertension or other conditions leading to fetal growth retardation, the fetal plasma levels are proportionately increased due to lack of fetal adrenal utilization.

Diabetes in pregnancy constitutes a major problem in maternal-child welfare. Presently maternal mortality is similar for both diabetic and non-diabetic women, but maternal morbidity is higher in diabetics. Fetal mortality in diabetic pregnancy has been reduced but not to the level in the general population. An increased incidence of congenital anomalies, macrosomia, late intrauterine death and RDS remain significant problems. Pregnancy per se has a diabetogenic effect, and some women develop abnormalities of carbohydrate metabolism known as gestational diabetes. In both diabetes and gestational diabetes, the intrauterine environment is unfavorable for the developing fetus. Investigators in the four MRPs in this area are attempting to clarify the pathophysiology of this metabolic disorder. The projects are complex multidisciplinary studies in which pregnant women are enrolled during early gestation and followed closely to term. Multiple evaluations are carried out to assess the control of maternal diabetes, the well-being of the fetus, and the adaptation of the newborn infant. A number of interesting findings have emerged from these MRPs. Pregnant women at term have been found to have an accelerated glucose production. An amount of insulin that is sufficient to normalize circulating free fatty acid levels may be insufficient to achieve complete control of fasting plasma glucose in insulin-dependent pregnant diabetic subjects. Furthermore, fasting plasma triglycerides may be elevated abnormally despite minimal elevations in free fatty acid levels and with normal glucose values. Investigators have also observed that individual serum amino acid levels are lower in diabetic pregnancy, and postprandial rises in these levels appear to be of lesser amplitude than in non-diabetics. Minor aberrations in every maternal nutrient appear to correlate significantly with the birthweight of the offspring in gestational diabetics.

Investigators in the newest MRP are studying fetal hypoxia, intrauterine growth retardation and fetal-neonatal depression and their relationship to maternal smoking. The study involves the progeny of women who smoke or who have chronic hypertension. Parallel investigations are being carried out in experimental animals (baboon, dog, sheep) to complement human observations. Systematic examinations of placentas are seeking anatomical correlates of maternal smoking in placental lesions.

Conferences, Staff Activities, and Publications

During FY 1981, the Section provided partial support for the Perinatal Research Society's Annual Conference.

In May, 1981, the Section, in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), sponsored a Research Planning Workshop on Maternal Genitourinary Infections and the Outcome of Pregnancy. The thirty participants discussed the information currently available on the organisms that cause these infections, their effects on growth and development of the fetus and on onset of labor, and the effects of screening and treatment for these infections. Of special interest was data presented relating the presence of phospholipase A_2 in bacteria that cause vaginitis/cervicitis to the onset of premature labor. Workshop participants made a number of recommendations for additional research needed to address this problem. The report from the workshop will be published in a medical journal.

Staff members represented the Institute at a number of meetings of professional organizations, including the Society for Gynecologic Investigation, Society for Pediatric Research, and American Pediatric Society, as well as the American Academy of Pediatrics Committee on Drugs.

Publications by Section staff include:

Wald, N.J., Catz, C., Dayton, D., Alpert, E.: The Quality Control of Alpha-Fetoprotein Reagents and Assay for the Antenatal Screening and Diagnosis of Open Neural-Tube Defects, Clinica Chimica Acta. 105 (1980) 9-24.

Alexander, D.: Risks of Amniocentesis. In Gastel, B., Haddow, J., Fletcher, J., and Neale, A. (Eds.): Maternal Serum Alpha-Fetoprotein: Issues in the Prenatal Screening and Diagnosis of Neural Tube Defects. U.S. Government Printing Office, 1980, pp. 20-24.

Alexander, D. and Ballard, A. Antenatal Diagnosis of birth defects and genetic disease: Past, present, and future. In Kretchmer, N. and Brasel, J. (Eds.): Biomedical and Social Bases of Pediatrics. New York: Masson, 1981, pp. 23-28.

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Contract and Collaborative Research

Contract Number: NO1 HD 9-2828

Contract Title: Non-Human Primate Colony

Contractor: University of California, Davis, California

Money Allocated: $224.371 (September 30, 1981 to September 30, 1982)

Objectives : Under this contract, a colony of Rhesus monkeys (Macaca mulatta) of known medical, reproductive and genealogical history is being kept and developed at the California Primate Research Center, University of California at Davis. This colony serves as a resource of research animals of known quality from a standardized environment for investigators whose research is funded by the NICHD.

Procedures: Animals of different ages including dated pregnancies, embryonic and fetal material, neonates of known gestation, mother-infant pairs, juveniles of known age, as well as certain biopsy material, tissues, and fluids are being made available to selected investigators, all of whom must be directly determined by an advisory committee which also advises on colony and contract management. Animals are shipped to recipient scientists throughout the country; a limited number of investigators can also be accommodated to visit facilities on-site. Biological and behavioral data profiles are compiled and incorporated into the computerized record of each animal. This information is available to recipient investigators.

Significance: Demand for animals from this resource reflects the use of Rhesus monkeys as a model for the human in biomedical research.

Proposed Course: The size of the colony, stabilized at 335 animals, is adequate for the projected demand of 80-100 pregnancies per year plus the need of replacement breeder stock.

Project Officer: Charlotte Catz, M.D.

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Contract Number: NO1 HD 5-2856

Contract Title: Evaluation and Follow-up of Selected Respiratory Cardiac and Neurophysiologic Parameters in Infants as Indicators of Risk for the Sudden Infant Death Syndrome (SIDS)

Contractor: Columbia Presbyterian Medical Center, New York, New York

Money Allocated: $64,000 (July 1, 1981 - December 30, 1981)

Objectives: The objectives of this project are to study cardiorespiratory-neurophysiologic phenomena which may be relevant to understanding the sudden infant death syndrome (SIDS) and identifying infants at risk.

Findings: Healthy infants and infants with "near-miss" episodes were monitored during sleep in a non-invasive manner. Data obtained included recordings of EEG, EOG, EMG, EKG, respiratory rate and respiratory volume. Data collection was completed on 28 near-miss infants, and 21 normal babies. New statistical methods have been developed to determine the interrelationships between cardiac and respiratory parameters. Analysis of the data has shown that breathing during sleep is cyclic, possibly related to neural mechanisms but not dependent on sleep state. Furthermore, infants who recovered from a near-miss episode do not seem to have abnormalities in the mechanisms that control the duration of the respiratory cycle time within the same breath. Aborted SIDS infants with a faster heart rate do have a significantly smaller QT intervals than normal controls. Since the beginning of this contract, there have been 24 completed manuscripts on various methodological and physiological aspects as well as comparative analysis of the population studied.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between the age of one month to one year. This proposal is developing new information that will help to identify infants at risk for SIDS. This study is an integral part of the Institute's SIDS research contract program to develop further knowledge on the causes and underlying mechanisms of SIDS.

Proposed Course: The contracted entered the final six-month period.

Project Officer: Charlotte Catz, M.D.

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Contract Number: N01 HD 7-2839

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic Study of SIDS Risk Factors

Contractor: University of Washington
Seattle, Washington

Money Allocated: $24,524 (January 1, 1981 - November 30, 1981)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving one data coordinating center at the University of Washington (N01 HD 7-2839) and six data collecting centers (University of Washington, N01 HD 7-2839; University of California (Davis), N01 HD 7-2840; Health Research Inc., Albany, New York, N01 HD 7-2841; Medical and Health Research Association of New York City, N01 HD 7-2842; Loyola University (Chicago) N01 HD 7-2843; and St Louis Regional Maternal and Child Health Inc., N01 HD 7-2844.

The purpose of this cooperative study is the development of a risk prediction algorithm to be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of risk associated with any identified factor. The sample will consist of more than 800 possible SIDS cases and 2 matched living controls selected for each possible SIDS case.

Findings: Data collection began on October 9, 1978 with the acquisition of information through interviews with parents (usually mothers) of SIDS infants and of living infants matched with SIDS infants for age, birth weight, and when possible, race (Black/not Black). Pathology data including tissue specimens, gross autopsy, and death investigation reports were obtained from coroners or medical examiners who performed autopsies on the SIDS infants. Entry of subjects into the study ended on December 31, 1979. Also being obtained is a comprehensive set of data about the medical history of study infants and their mothers through abstraction of prenatal, delivery, and postnatal medical records (to be completed by December 1981).

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: All interview data, including neighborhood and housing data on the interviewed families, and birth and death certificate data have been forwarded to the Data Coordinating Center. Pathology specimens and gross autopsy and death investigation reports have been sent to the Pathology Coordinating Laboratory for slide preparation and abstraction of information. Study Centers have participated in the development of data analysis strategies and will participate in the presentation of results during fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 2840

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: University of California
Davis, California

Money Allocated: $81,724 (January 1, 1981 - November 30, 1981)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving one data coordinating center at the University of Washington (N01 HD 7-2839) and six data collecting centers (University of Washington, N01 HD 7-2839; University of California (Davis), N01 HD 7-2840; Health Research Inc., Albany, New York, N01 HD 7-2841; Medical and Health Research Association of New York City, N01 HD 7-2842; Loyola University (Chicago) N01 HD 7-2843; and St Louis Regional Maternal and Child Health Inc., N01 HD 7-2844.

The purpose of this cooperative study is the development of a risk prediction algorithm to be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of risk associated with any identified factor. The sample will consist of more than 800 possible SIDS cases and 2 matched living controls selected for each possible SIDS case.

Findings: Data collection began on October 9, 1978 with the acquisition of information through interviews with parents (usually mothers) of SIDS infants and of living infants matched with SIDS infants for age, birth weight, and when possible, race (Black/not Black). Pathology data including tissue specimens, gross autopsy, and death investigation reports were obtained from coroners or medical examiners who performed autopsies on the SIDS infants. Entry of subjects into the study ended on December 31, 1979. Also being obtained is a comprehensive set of data about the medical history of study infants and their mothers through abstraction of prenatal, delivery, and postnatal medical records (to be completed by December 1981).

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: All interview data, including neighborhood and housing data on the interviewed families, and birth and death certificate data have been forwarded to the Data Coordinating Center. Pathology specimens and gross autopsy and death investigation reports have been sent to the Pathology Coordinating Laboratory for slide preparation and abstraction of information. Study Centers have participated in the development of data analysis strategies and will participate in the presentation of results during fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2841

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Health Research, Inc.
Albany, New York

Money Allocated: $61,678 (January 1, 1981 - November 30, 1981)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving one data coordinating center at the University of Washington (" 2839) and six data collecting centers (Unive- University of California (Davis), N01 HD 7-2840; Health Research Inc., Albany, New York, N01 HD 7-2841; Medical and Health Research Association of New York Cit" N "D 842: Lov versity (Chicago) N01 HD 7-2843; and St Louis Regional Maternal and Child Health Inc., N01 HD 7-2844.

The purpose of this cooperative study is the development of a risk prediction algorithm to be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of risk associated with any identified factor. The sample will consist of more than 800 possible SIDS cases and 2 matched living controls selected for each possible SIDS case.

Findings: Data collection began on October 9, 1978 with the acquisition of information through interviews with parents (usually mothers) of SIDS infants and of living infants matched with SIDS infants for age, birth weight, and when possible, race (Black/not Black). Pathology data including tissue specimens, gross autopsy, and death investigation reports were obtained from coroners or medical examiners who performed autopsies on the SIDS infants. Entry of subjects into the study ended on December 31, 1979. Also being obtained is a comprehensive set of data about the medical history of study infants and their mothers through abstraction of prenatal, delivery, and postnatal medical records (to be completed by December 1981).

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: All interview data, including neighborhood and housing data on the interviewed families, and birth and death certificate data have been forwarded to the Data Coordinating Center. Pathology specimens and gross autopsy and death investigation reports have been sent to the Pathology Coordinating Laboratory for slide preparation and abstraction of information. Study Centers have participated in the development of data analysis strategies and will participate in the presentation of results during fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2842

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Medical and Health Research Association of New York City, Inc. New York, New York

Money Allocated: $83,972 (January 1, 1981 - November 30, 1981)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving one data coordinating center at the University of Washington (N01 HD 7-2839) and six data collecting centers (University of Washington, N01 HD 7-2839; University of California (Davis), N01 HD 7-2840; Health Research Inc., Albany, New York, N01 HD 7-2841; Medical and Health Research Association of New York City, N01 HD 7-2842; Loyola University (Chicago) N01 HD 7-2843; and St Louis Regional Maternal and Child Health Inc., N01 HD 7-2844.

The purpose of this cooperative study is the development of a risk prediction algorithm to be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of risk associated with any identified factor. The sample will consist of more than 800 possible SIDS cases and 2 matched living controls selected for each possible SIDS case.

Findings: Data collection began on October 9, 1978 with the acquisition of information through interviews with parents (usually mothers) of SIDS infants and of living infants matched with SIDS infants for age, birth weight, and when possible, race (Black/not Black). Pathology data including tissue specimens, gross autopsy, and death investigation reports were obtained from coroners or medical examiners who performed autopsies on the SIDS infants. Entry of subjects into the study ended on December 31, 1979. Also being obtained is a comprehensive set of data about the medical history of study infants and their mothers through abstraction of prenatal, delivery, and postnatal medical records (to be completed by December 1981).

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: All interview data, including neighborhood and housing data on the interviewed families, and birth and death certificate data have been forwarded to the Data Coordinating Center. Pathology specimens and gross autopsy and death investigation reports have been sent to the Pathology Coordinating Laboratory for slide preparation and abstraction of information. Study Centers have participated in the development of data analysis strategies and will participate in the presentation of results during fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2843

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: Loyola University of Chicago
Chicago, Illinois

Money Allocated: $95,917 (January 1, 1981 - November 30, 1981)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving one data coordinating center at the University of Washington (N01 HD 7-2839) and six data collecting centers (University of Washington, N01 HD 7-2839; University of California (Davis), N01 HD 7-2840; Health Research Inc , Albany, New York, N01 HD 7-2841; Medical and Health Research Association of New York City, N01 HD 7-2842; Loyola University (Chicago) N01 HD 7-2843; and St Louis Regional Maternal and Child Health Inc., N01 HD 7-2844.

The purpose of this cooperative study is the development of a risk prediction algorithm to be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of risk associated with any identified factor. The sample will consist of more than 800 possible SIDS cases and 2 matched living controls selected for each possible SIDS case.

Findings: Data collection began on October 9, 1978 with the acquisition of information through interviews with parents (usually mothers) of SIDS infants and of living infants matched with SIDS infants for age, birth weight, and when possible, race (Black/not Black). Pathology data including tissue specimens, gross autopsy, and death investigation reports were obtained from coroners or medical examiners who performed autopsies on the SIDS infants. Entry of subjects into the study ended on December 31, 1979. Also being obtained is a comprehensive set of data about the medical history of study infants and their mothers through abstraction of prenatal, delivery, and postnatal medical records (to be completed by December 1981).

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: All interview data, including neighborhood and housing data on the interviewed families, and birth and death certificate data have been forwarded to the Data Coordinating Center. Pathology specimens and gross autopsy and death investigation reports have been sent to the Pathology Coordinating Laboratory for slide preparation and abstraction of information. Study Centers have participated in the development of data analysis strategies and will participate in the presentation of results during fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2844

Contract Title: Data Collection Center for the NICHD Cooperative Epidemiologic SIDS Risk Factors Study

Contractor: St. Louis Regional Maternal and Child Health Inc., St. Louis, Missouri

Money Allocated: $44,561 (January 1, 1981 - November 30, 1981)

Objectives: This contract is an essential component of the NICHD Cooperative Epidemiologic SIDS Risk Factors Study involving one data coordinating center at the University of Washington (N01 HD 7-2839) and six data collecting centers (University of Washington, N01 HD 7-2839; University of California (Davis), N01 HD 7-2840; Health Research Inc., Albany, New York, N01 HD 7-2841; Medical and Health Research Association of New York City, N01 HD 7-2842; Loyola University (Chicago) N01 HD 7-2843; and St Louis Regional Maternal and Child Health Inc., N01 HD 7-2844.

The purpose of this cooperative study is the development of a risk prediction algorithm to be applied in the first week of life. The study design is case-control to be maximally efficient in identification of risk factors; it is also population based (complete case ascertainment within defined study populations) to enable quantification of risk associated with any identified factor. The sample will consist of more than 800 possible SIDS cases and 2 matched living controls selected for each possible SIDS case.

Findings: Data collection began on October 9, 1978 with the acquisition of information through interviews with parents (usually mothers) of SIDS infants and of living infants matched with SIDS infants for age, birth weight, and when possible, race (Black/not Black). Pathology data including tissue specimens, gross autopsy, and death investigation reports were obtained from coroners or medical examiners who performed autopsies on the SIDS infants. Entry of subjects into the study ended on December 31, 1979. Also being obtained is a comprehensive set of data about the medical history of study infants and their mothers through abstraction of prenatal, delivery, and postnatal medical records (to be completed by December 1981).

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of SIDS and to identify infants at risk.

Proposed Course: All interview data, including neighborhood and housing data on the interviewed families. and birth and death certificate data have been forwarded to the Data Coordinating Center. Pathology specimens and gross autopsy and death investigation reports have been sent to the Pathology Coordinating Laboratory for slide preparation and abstraction of information. Study Centers have participated in the development of data analysis strategies and will participate in the presentation of results during fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 7-2839

Contract Title: Data Coordinating Center for the NICHD Cooperative Epidemiologic Study of SIDS Risk Factors

Contractor: University of Washington
Seattle, Washington

Money Allocated: $296,564 (January 1, 1981 - December 31, 1981)

Objectives: This contract serves as the Data Coordinating Center to the NICHD Cooperative Epidemiologic SIDS Risk Factors Study. It is responsible for the processing, management, and analyses of data collected by the participating Study Centers.

Findings: During the current fiscal year, the Data Coordinating Center has continued to clean and edit the interview data. These data include, in addition to interview schedule data, data on the neighborhood and housing of interviewed families and birth and death certificate data. Pathology data from the necropsy checklist, microscopic examination report, and gross autopsy and death investigation reports from the participating coroners medical examiners are being entered as they become available. Three files have been established to receive and store the data from this study.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of the sudden infant death syndrome and to identify infants at risk.

Proposed Course: Cleaning and editing of data by the Data Coordinating Center should be completed by December, 1981. Development of strategies for data analysis began in late 1979 and have continued through the current fiscal year. The data obtained regarding the first 400 cases and their 800 matched controls will be used to examine currently existing hypotheses of SIDS etiology, and to identify refined hypotheses through exploratory analysis on the first half of the data set. Results of the first descriptive analyses of the data set will be reported in fiscal year 1982.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

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Contract Number: N01 HD 8-2845

Contract Title: Pathology Coordinating Laboratory (PCL) for the NICHD Cooperative Epidemiologic Study of SIDS Risk Factors

Contractor: Office of Medical Examiner
City and County of San Francisco
San Francisco, California

Money Allocated: $63,251 (January 1, 1981 through December 31, 1981)

Objectives: This contract serves as the Pathology Coordinating Laboratory to the NICHD Cooperative Epidemiologic Study of SIDS Risk Factors. The Laboratory provides essential services to ensure the accurate diagnosis of SIDS. The necropsy, a common gross dissection protocol, will be followed to dichotomize the dead infants into "definitely not SIDS" and "eligible for further study". The contractor will process tissues submitted by the six Study Centers and will produce a set of stained slides for each infant entered into the study.

Findings: A common gross dissection protocol has been developed. Medical Examiners serving the six Study Centers have been following the protocol since the start of data collection (October 9, 1978). As of June 1, 1980, the PCL has received tissues from 941 infants who have died. Of these deaths, more than 800 are considered to be true SIDS cases; the balance includes quality control cases and some cases where subsequent diagnosis elicited a cause of death. It is expected that the review of all slides by the Pathology Study Panel will be completed by December 31, 1981. Abstraction of Death Investigation Reports and Autopsy Reports should be completed early in 1982.

Significance: The sudden infant death syndrome is the leading cause of death in the United States between one month and one year of life. This study is an integral part of the NICHD SIDS research contract program to understand the causes and underlying mechanisms of the sudden infant death syndrome and to identify infants at risk.

Proposed Course: It is planned to complete the data collection phase which includes the abstraction of all 841 death investigation, gross autopsy, and microscopic review reports and assist the Data Coordinating Center in the editing of these data. It is also planned to set up a resource contract which will make the autopsy material available to investigators.

Project Officer: Eileen G. Hasselmeyer, Ph.D.

The Nutrition and Endocrinology Section (CNNE) supports research and research training in maternal, infant, child, and adolescent nutrition; obesity and nutritional antecedents of adult disease; cultural and behavioral aspects of nutrition; nutritional status; physical growth; and developmental gastroenterology, endocrinology, and physiology.

RESEARCH SUPPORT

In FY 80 the CNNE Section supported 185 projects at a level of $15,354,000. These projects are analyzed according to programmatic mechanism in Table 1c.

Infant Nutrition

The CNNE Sections's largest single program is that of infant nutrition. Emphasis is placed on research aimed at elucidating the roles played by diet in infant development. Research interests also focus on the nutrient requirements of normal, premature, and growth-retarded infants, as well as on analysis of human milk, cow's milk, and synthetic formulae in relation to optimal infant development. Research supported by the CNNE Section has demonstrated that differing rates of infant growth can be obtained, especially among infants born prematurely, by adjusting the nitrogen density of the nutrient medium. The biochemical immaturity of premature infants presents challenging problems that must be solved in order to design appropriate feedings for enteral or parenteral nutrition. Investigators supported by the CNNE are currently working to develop those nutrient mixtures that best meet the metabolic requirements of premature infants but do not violate the delicate mineral, acid-base, glycemic and osmolar balances present in their tiny bodies.

Studies on human milk and milk banking comprise a large part of the program on infant nutrition. Recently, scientists supported by the CNNE Section have discovered digestive enzymes, growth factors and immunocompetent B and T cells in human milk. Research is being supported that addresses the problem of how best to preserve the biological functions of these fragile cells and proteins in the process of collecting and storing human milk and colostrum.

Childhood and Developmental Nutrition

The CNNE Section support nutrition research that emphasizes the roles played by various nutrients in cerebral and somatic development. Of crucial interest are studies that are designed to ascertain the effects, or lack of effects, of general and specific kinds of undernutrition on cognitive development during infancy and childhood.

Table 1c.

NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1981
NUTRITION AND ENDOCRINOLOGY SECTION

Funds (thousands)

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts (incl. S06)	
			Total Research		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	187	$16,654	159	$14,140	146	$12,381	3	$1,391	10	$368	18	$668	10	$1,846
Infant Nutrition	35	4,439	31	3,380	28	2,172	3	1,208	-	-	-	-	4	1,059
Childhood & Developmental Nutrition	19	1,755	16	1,428	16	1,428	-	-	-	-	1	119	2	208
Maternal-Fetal Nutrition	7	638	7	638	7	638	-	-	-	-	-	-	-	-
Developmental Gastroenterology	18	1,188	15	1,125	15	1,125	-	-	-	-	3	64	-	-
Cultural & Behavioral Aspects of Nutrition	14	1,299	11	815	11	815	-	-	-	-	1	20	2	464
Obesity & Childhood Antecedents of Adult Disease	13	1,257	13	1,257	10	1,144	-	-	3	113	-	-	-	-
Nutritional Status	3	318	3	318	2	95	*	182	1	41	-	-	-	-
Adolescent Nutrition	6	390	6	390	4	327	-	-	2	63	-	-	-	-
Developmental Endocrinology	56	4,167	45	3,781	42	3,671	-	-	3	111	9	271	2	115
Developmental Physiology	10	596	6	401	5	361	-	-	1	41	4	194	-	-
Physical Growth	6	607	6	607	6	607	-	-	-	-	-	-	-	-

* Part of a program project counted in another category.
Note: Excludes scientific evaluation grants.

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Despite the complexity of studying the problem of child development in nutritionally and economically impoverished environments, CNNE-supported investigators have shown that nutritional supplementation with extra calories and protein during infancy and early childhood favors superior linear growth rates and superior performance on a battery of psychologic tests and even predicts scholastic success.

Maternal-Fetal Nutrition

One of the most important of the CNNE programs, especially from a preventive point of view, is that of maternal-fetal nutrition. Most of the research supported in this area involves the complex nutritional relationship that exists between the mother and her fetus. Research is also funded on the effects of excessive or deficient amounts of certain nutrients on the morphologic and endocrine development of the fetus.

In FY 80 the NICHD and the USDA co-sponsored a workshop on maternal nutritional status and fetal outcome in order to review the longitudinal studies funded by the NICHD over the past decade that address this complex issue. The proceedings of this workshop were published a supplement to the American Journal of Clinical Nutrition 34, in April, 1981.

Recently, CNNE-supported investigators working on the dynamics of nutrient transport by the placenta have discovered a rapid, efficient placental transport system for retinol from ewe to fetal lamb, and are currently examining the possibility of significant fetal-to-maternal passage of retinol in order to explain what appears to be a greater rate of retinol metabolism in the fetus than in the mother.

Developmental Gastroenterology

The CNNE program of developmental gastroenterology comprises the fastest-growing of the eleven program areas of the CNNE. This burgeoning growth reflects the recent tempo of conceptual and methodological advances achieved in this area, which abounds in clinical disorders such as celiac disease, lactase and other disaccharidase deficiencies, infantile diarrhea, necrotizing enterocolitis and food hypersensitivity. These clinical problems provide the patients and the impetus to pursue basic studies devoted to preventing or ameliorating these conditions . Nutritionally-oriented studies are of special interest because they relate to both the etiology and the therapy of coeliac disease, food hypersensitivity, and other gastrointestinal disorders.

CNNE-supported investigators have recently developed reliable, noninvasive methods to analyze the fate of ingested nutrient substrates. Especially important in this regard has been the development of breath analysis of volatile metabolic products, such as hydrogen and ^{13}C-labelled carbon dioxide. These methods are now being used to assess the contributions of intestinal bacteria to the digestion and conservation of malabsorbed lactose in cases of primary and secondary lactase deficiency.

Cultural and Behavioral Aspects of Nutrition

This program emphasizes research on cultural and behavioral determinants of nutritional individuality. Currently, studies are progressing on: the development of children's concepts and ideas about food; the origin of dietary cravings and rejections during pregnancy; and the impact of television commercials on children's eating habits.

Research in this area also emphasizes anthropological studies of man's interaction with his nutritional environment. For example, a research project is currently underway in Kenya on the effects of cash-cropping and urban migration on nutritional status of traditional agriculturally-based families. Another such project involves study of vitamin D status among Inuit Indians and Eskimos, the majority of whom are lactose-intolerant yet whose main source of vitamin D comes from milk. Examples such as these indicate how isolated or unusual cultural situations provide good models in which to identify determinants of nutritional status.

These research projects demonstrate the primacy of behavioral and cultural variables in determining nutritional status. Research on cultural and historical origins of customary diets and ethnic cuisines represents a vast nearly untapped area of nutritional research, one which promises to elucidate relationships between prehistorical nutritional environments and the genetic basis of nutritional individuality. The relationship between dairying and lactose tolerance among Northern Europeans and that between wheat cultivation and gluten tolerance among European and Middle-Eastern populations serve as examples of this kind of research.

In an effort to stimulate multidisciplinary research on cultural and behavioral aspects of nutrition, CNNE staff have consulted extensively with outside experts in nutritional anthropology, epidemiology, psychology and sociology. As a result of these consultations, two workshops were held, one in June, 1979, in collaboration with the NIA, on Behavior and Nutrition, and the other in June, 1981, on Determinants of Choice and Duration of Infant Feeding Practices. A publication which summarizes the first of these workshops appeared in Appetite 1, 321-331, 1980.

In order to stimulate more research in the multidisciplinary area of behavior and nutrition, the CNNE staff published a Program Announcement entitled Developmental Aspects of Behavior and Nutrition in January, 1980. More than 500 copies of the Program Announcement were mailed to potential applicants, including the entire membership of the Committee on Nutritional Anthropology. Hundreds of inquiries were received in response to this mailing, and so far 35 applications have been received for review. Several of these have been funded, all having to do with animal models of hunger, satiety, foraging and nutritional economy.

Obesity and Childhood Antecedents of Adult Disease

Obesity is a prevalent condition in the industrialized countries of the West. The condition may begin in infancy or childhood and last a lifetime. It appears to be heterogeneous in nature and multifactorial in origin. The aim of the CNNE program is to ascertain, analyze, and evaluate the determinants of obesity. Investigators supported by the CNNE Section are currently addressing gestational, genetic, psychological, and social factors that are associated with obesity in childhood and adolescence. These same contributory factors are also being studied, along with the development of insulin resistance and glucose intolerance, in offspring of diabetic mother.

In addition to such physiologically-oriented research, the CNNE Section supports behaviorally-oriented research on dietary modification, exercise and healthy life styles. One investigator working in this area has recently described the chronic dieter syndrome. He has been able to identify a set of behavioral characteristics that predicts successful adherence to a program of behavioral modification. He has also identified another set of characteristics, that predicts those obese subjects typified by the chronic dieter, whose eating behavior will not be changed successfully by a program of behavioral modification.

Recently CNNE staff has been working with the Chairman of the NIH Nutrition Coordinating Committee and the Executive Secretary of the DHHS Nutrition Coordinating Committee to plan a workshop on conceptual and methodologic issues in regard to the definition and measurement of obesity in the American population. The workshop is scheduled for FY 82.

Nutritional Status

Research in this program emphasizes the development of new methods for assessing nutritional status in pregnancy, infancy, childhood, and adolescence. The chief aim is to develop methods that are noninvasive and pose the least possible risk to the individual while being precise, economical, and convenient. Investigators supported by the CNNE Section have pioneered in the development of anthropometric, biochemical and functional measurements of nutritional status. Recently, an oscillating air displacement technique has been developed to ascertain precisely body volume of infants. In the realm of nutritional biochemistry, new assays have been developed for measuring serum and tissue levels of vitamin E.

CNNE staff devoted much effort in FY 81 to the organization of a large conference on the measurement of nutritional status, held in September 1981, under the auspices of the NIH Nutrition Coordinating Committee along with the FDA and the CDC.

Adolescent Nutrition

The CNNE program of adolescent nutrition emphasizes research in the areas of nutrition and the adolescent growth spurt, obesity in adolescence, and nutrition of the pregnant adolescent. Adolescence is a time of profound physical transformation during which growth rates are attained that are exceeded only by those during fetal life and early infancy. Large differences between boys and girls during their growth spurts in demand for calcium and nitrogen have been documented.

In regard to adolescent obesity, the CNNE began funding an epidemiologic study of 170 adolescents in Berkeley, California, in order to assess various adolescent life-styles, eating habits and exercise patterns and their contributions to obesity in this age group. In this regard it is of interest to note that at least 20% of adolescent boys and 13% of adolescent girls are obese, according to the Ten State Nutrition Survey.

Currently, the three greatest nutritional research challenges in regard to the pregnant adolescent are: to assess accurately her nutritional status; to understand her total nutritional needs and those of her fetus; and to develop nutritional and other interventions to prevent the birth of physically or mentally damaged offspring to mothers who are less than sixteen years old.

Recently the CNNE Section sought to stimulate more research on adolescent nutrition, especially in the area of adolescent eating habits. This aspect of adolescence was emphasized in the Program Announcement entitled Developmental Aspects of Behavior and Nutrition. Research applications dealing with iron intake of adolescents, anorexia nervosa, and adolescent obesity have been received in response to this announcement.

Developmental Endocrinology

This program accounts for nearly one-quarter of the CNNE budget. The field of developmental endocrinology encompasses studies of hormonal influence on growth and development, studies of growth factors, and studies of the development of the hypothalamic-pituitary axis in relation to the thyroid, adrenal glands and the gonads. Much of the research within this program explores the neurohumoral mechanisms involved in the control of the onset of puberty. CNNE-supported investigators have shown in a variety of animal models that pubertal onset begins with rhythmic micro-electrical pulsations that emanate from certain parts of the midbrain. By gaining a better understanding of the central origins of puberty, clinical investigators will be able to provide more effective therapy for the complex neuroendocrine disorders of precocious puberty and pubertal delay.

An important facet of developmental endocrinology concerns the study of growth factors. The CNNE Section currently supports research on epidermal growth factor (EGF), nerve growth factor, somatomedins A and C, mammary stimulating factor, and human growth hormone (hGH). The definitive work on EGF, including analysis of its amino acid sequence, is supported by the CNNE Section. Recently, investigators have learned that the amino acid sequence of somatomedin A appears similar to that of insulin-like growth factor II. Further purification of somatomedin A will soon permit the relationship between these two growth-promoting polypeptides to be clarified. Ultimately, pure somatomedins may be used as a specific therapy for Laron dwarves who respond to neither endogenous nor exogenous hGH.

Developmental Physiology

Most of the projects within this program are concerned with the development of the finely-tuned physiologic feedback pathways by which homeostasis is achieved. Studies of renal maturation and sodium balance provide a good example of this kind of research. One CNNE-supported investigator has observed that the existence of a positive external balance for sodium is an outstanding physiologic characteristic of the young of many mammalian species. He has found that the kidneys of beagle pups conserve sodium, even under conditions of sodium loading and volume expansion. Such basic physiologic research bears directly on understanding the etiology of essential hypertension, a condition that affects 35 million Americans.

Another recent discovery in the area of developmental physiology lies at the interface of physiology and nutrition. It has been found in studies of newborn rabbits that weaning from mother's milk to chow causes a significant acidosis, and that the alkalinogenic properties of mother's milk compensate for the low renal bicarbonate transport capability during infancy. This observation carries important implications in regard to the composition of formula prepared for premature human infants.

Physical Growth

Research in the field of physical growth has progressed under NICHD support from studies of growth trajectories and anthropometric measurements of height, weight, head circumference, skinfold thickness and the like, to sophisticated mathematical analyses and computer-generated models of the dynamic process of volumetric growth in three dimensions. For example, one CNNE-supported investigator has developed a mathematical growth tensor that allows growth at any spatial or temporal point to be characterized by growth along three axes. Such kinematic descriptions of biological growth are based on concepts from the mechanics of continua and permit precise predictions of proportional growth.

Recently CNNE-supported investigators working on skeletal growth have developed a set of equations that accurately predicts in childhood attainment of final height later in life. These equations are based on ascertainment of a child's bone age in relation to his or her chronological age. Bone age in this case is determined by careful radiologic analysis of the ossification centers of the bone of the wrist or the knee.

RESEARCH TRAINING IN NUTRITION AND ENDOCRINOLOGY

Like other Branches and Sections of the NICHD, the CNNE Section has a firm commitment not only to support the best research projects of the current generation of investigators, but also to support the postdoctoral research training of the most promising young investigators of the next generation. In FY 81 the CNNE Section funded one Institutional National Research Service Award (NRSA) in the area of nutritional research. This project currently has enrolled three trainees, all at the post-doctoral level. These fellows are pursuing research projects that emphasize developmental aspects of clinical nutrition in prematurely-born babies and babies born at term. Of special interest to the fellows and preceptors associated with this training award are amino acid requirements and lipid metabolism of the newborn.

In addition to this Institutional NRSA, the CNNE Section funds five individual National Research Service Awards. The post-doctoral fellows supported by these awards are working on projects that vary from studies of newborn intestinal development to anthropological studies of childhood malnutrition in the Louisade Archipelago in New Guinea. In order to encourage more young investigators to submit research grant applications in the area of clinical nutrition, the NICHD joined with several other Institutes represented on the NIH Nutrition Coordinating Committee to issue a Program Announcement for New Investigator Awards in Clinical Nutrition. This announcement appeared in March, 1981. Since then there have been many expressions of interest in the new awards by young investigators seeking to apply.

One year prior to the announcement of this award, the NICHD issued an announcement of the availability of Clinical Investigator Awards. This announcement sought to encourage clinically trained young investigators to apply for research support in the area of clinical nutrition, pediatrics, obstetrics and gynecology, reproductive biology, and andrology.

In the area of developmental endocrinology the CNNE currently supports one Institutional National Research Service Award that includes the training of three post-doctoral fellows. The projects on which these fellows are working are aimed at increasing our understanding of the action of various hormones at the level of the genome, i.e., in determining gene expression for a variety of proteins. In addition to this Institutional Award, the CNNE Section also supports the training of twelve post-doctoral fellows on Individual NRSA fellowships. These fellows are working on mechanisms of pubertal onset, somatomedins and other growth factors, adrenal development, and development of the hypothalamic-pituitary-gondal axis.

LIAISON ACTIVITIES

The professional staff of the CNNE Section represent the NICHD on a number of groups and committees in both the public and private sectors.

Dr. Grave serves as the NICHD Representative on the NIH Nutrition Coordinating Committee, and Dr. Fjellstedt serves as Alternate Representative. Dr. Grave also represents the Institute on the NIH Diabetes Mellitus Coordinating Committee as well as on the interagency Diabetes Mellitus Coordinating Committee. Dr. Grave also represents the Director, NICHD, at meetings of the National Diabetes Advisory Board, and has represented the Director, NICHD, at meetings of the Executive Committee of the USDA-Children's Nutrition Research Center in Houston, Texas. Dr. Grave also represents the NICHD on a new group called the Interagency Collaborative Group on Diet and Behavior.

Dr. Fjellstedt represents the NICHD on the NIH Digestive Diseases Coordinating Committee and also on the U.S.-Japan Cooperative Biomedical Sciences Panel on Malnutrition. Dr. Fjellstedt also serves as liaison representative from the NICHD to the Committee on Nutrition of the Mother and Preschool Child of the Food and Nutrition Board of the National Academy of Sciences. He also serves as liaison representative to the Committee on Nutrition of the American Academy of Pediatrics. Dr. Fjellstedt acts as the NICHD designated representative to the Board of Scientific Counselors of the USDA-Children's Nutrition Research Center in Houston, Texas.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : Evaluation of Breast Milk for Banking
Contract No. : NO1-HD-2828
Contractor : Baylor College of Medicine, Houston, Texas
Buford Nichols, M.D., and Cutberto Garza, M.D., Ph.D.
Money Allocated: FY 81; None as of this writing: FY 80; $298,648:
FY 79; $299,662:

This contract effort is designed to resolve major problems inherent in the collection, storage, distribution and utilization of banked human milk and colostrum in clinical settings. This is a phased operation in which methods of collection and storage are first being studied systematically. In the second phase the variability of human milk and colostrum will be studied in relation to maternal nutritional status, feeding frequency and other factors. In the third phase the Milk Bank will serve the medical research community by providing well-documented samples of human milk and colostrum and fractions thereof for basic and clinical research. This contract is necessary to place the therapeutic uses of human milk and colostrum on a firm footing.

The workscope for the third year of this contract includes the following specific aims; 1) develop methodologies for the preparation of low-lactose lyophilized skim milk with sodium potassium, calcium, chloride, copper, magnesium, phosphorous, zinc, iron, selenium, and maganese content comparable to that of whole human milk and provide this preparation as needed for the following studies. 2) Evaluate the effects of feeding tubes on the net delivery of nutrients and humoral immunologic properties of human milk. 3) Evaluate the nutrient composition and immunologic properties of milk produced by mothers who have been practicing unrestricted lactation for one year or longer. 4) Confirm the deactivation of cytomegalovirus after heating for thirty minutes at 62.5° C. 5) Continue to accumulate the normative data on the nutritional and immunologic content of human milk from mothers exclusively breast feeding their infants for four weeks and six months that are necessary to provide statistical validity to these data. 6) Validate body composition methodology. Prior to initiating the clinical studies proposed, the body composition methodology to be used will be validated in an animal model system. 7) Develop and carry out a pilot clinical study on the use of human milk in the feeding of premature infants. This study shall include approximately seven subjects in the experimental group and an equal number in the control group. Among the outcome measurements to be assessed are nitrogen retention, fat absorption, calcium and phosphorous balance, sodium retention, metabolizeable energy, changes in body composition, metabolic responses and a series of assessments of immunologic development. 8) Identify mothers who are sero-positive and sero-negative to cytomegalovirus as a criteria of eligibility of mothers and their infants for the clinical study. Evaluate sociological and behavioral factors which improve the volume and quality of milk supplied by mothers of hospitalized premature infants in the pilot clinical study.

Contract Title : A Prototype Allergic Disease Prevention Program: Clinical and Immunological Consequences
Contract No. : N01-HD-2832
Contractor : University of California, School of Medicine, San Diego, California, Robert S. Zeiger, M.D., Ph.D.
Money Allocated: FY 81; None: FY 80; None: FY 79; $327,199 for 2 years:

Project Aims: To document by modern immunologic methods that pre- and postnatal dietary and environmental manipulations lead to the reduction of immediate sensitization and subsequent development of allergic disorders secondary to such sensitization.

Progress to Date: It is estimated that approximately three hundred positive allergic families will be enrolled in the treatment group by the end of the second year of the project. Because the families are enrolled while the mother is in early pregnancy the number of infants enrolled is somewhat less. To date, approximately 167 infants are enrolled in the treatment group. Family history data and pertinent medical data on all families involved in the study are completed and these data are computerized.

Because of the complexity of the study, a site visit is planned to evaluate the progress during the first two years and to evaluate studies proposed for the third and later years of this project. The site visit report and recommendation will be presented to the NICHD Contract Review Committee prior to the end of the second year of this project.

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October 1, 1980 through September 30, 1981
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch

Contract Title : Studies on Development of Food Allergy in Infants and Children
Contract No. : NO1-HD-2833
Contractor : University of California, School of Medicine, San Francisco, California, Oscar L. Frick, M.D., Ph.D.
Money Allocated: FY 81; None: FY 80; None: FY 79; $294,348 for 3 years:

Project Aims: To study the natural history of the development of food allergy in infants in order to discover what circumstances generate allergic sensitization to foods.

In pre-school children allergy to certain foods accounts for one-third of all allergic problems, and one percent of all babies born experience allergic reaction to cows' milk. Allergy to cows' milk is associated with a four-fold increase in the incidence of asthma and allergic disease later in life. In order to determine the natural history of food allergy and the frequency of immunological reactions associated with its development, the contractors will follow from birth a group of 24 infants born into bilaterally allergic families. It is expected that at least half of these infants will develop food allergies within their first year of life.

Progress to Date: In the prospective study, approximately fifteen new born infants of allergic parents are now enrolled. Of the older eight children five have developed mild to moderate eczema, two have chronic rhinitis, two have serousotitis, and one has had several wheezing episodes. Positive RAST and lymphocyte transformation and leukocyte inhabition assays (LIF) to cows' milk, soy, and/or egg have been observed on four of five children along with positive RAST to home dust in three and to dog in one child. All five children had upper respiratory infections prior to the first positive immunologic test for food or inhalant sensitivity. Thus, the association between virus infection and onset of allergic sensitization that was reported in 1979 is being confirmed in this group of allergy-prone infants.

In addition, a paper was presented at the American Academy of Allergy on the demonstration of circulating immune complexes by Raji and Clq-binding tests in the large majority of children with delayed onset food allergy. A manuscript on this work has been prepared for submission to a refereed journal.

With the advent of commercially available monoclonol antibodies to T-Cell subset markers, they are now also measuring percent distribution of T-helper (OKT-4) and T-suppressor (OKT-8) cells in the infants in the study. They are measuring such T-Cell subset ratios in the infants before, during, and after virus infection to see if the ratio of T-Cell subsets are disturbed thus supporting the concept of "allergic break-through" induced by virus infection in regard to allergic sensitization to foods.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : Mechanisms of Food Protein Intolerance
Contract No. : N01-HD-2834
Contractor : University of Texas Medical Branch, Galveston, Texas
Geraldine K. Powell, M.D.
Money Allocated: FY 81; None: FY 80; None: FY 79; $282,564 for 3 years:

Project Aims: 1) to quantitate macromolecular absorption and examine some mechanisms of its control; 2) to examine the role of sensitized lymphocytes in food intolerance.

Progress to Date: A protocol is now being developed to analyze data development of the secretory IgA system in normal human infants and compare these data with the development of the same system in patients with soy-milk induced enterocolitis to see if there are differences between these two groups. The data on lymphocyte transformation has been analyzed and an abstract submitted to the annual meeting of the Society for Pediatric Research.

Laboratory analysis of the ELISA methods show that when antibodies are present in low amounts, addition of further antibodies within a certain range still does not cause significant interference with serum antigen analysis. The investigators did not find serum inhibition if ovalbumin antibodies were not detected in the baseline serum or in low quantities. They were able to detect ovalbumin in the serum of fourteen infants at one hour post-ingestion of the standardized oral dose of egg white. They are also evaluating twelve-hour urinary excretion of ovalbumin during the ingestion of the same dose of egg white.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : Dietary Cravings and Aversions During Pregnancy
Contract No. : N01-HD-2837
Contractor : Albany Medical College, Ernest B. Hook, M.D.
Money Allocated: FY 81; None: FY 80; None: FY 79; $173,490 for three years:

Project Aims: An epidemiologic study designed to characterize several factors relating to dietary cravings and aversions during pregnancy. Among these are: 1) the type and frequency of cravings and aversions; 2) the reaction to food as a function of dietary habits prior to pregnancy; 3) the association of cravings and aversions with other biological and demographic factors; 4) the time during gestation when cravings and aversions occur and establishment of recognizable trends over the course of gestation; 5) the changes in maternal diet and in the rate of exposure to embryo toxins such as tobacco smoke and alcohol associated with cravings and aversions; and 6) the association of cravings and aversions with fetal outcome.

Progress to Date: A. Tables illustrating the effect of pre-pregnancy food habits on the subsequent development of specific cravings and aversions during pregnancy had been completed. B. The results of cross-tabular analysis of associations among specific cravings and aversions and maternal variables had been completed and are presently under review in order to determine inherent patterns and the magnitude of associations involved. C. The intake levels of specific nutrients and calories consumed during two periods of pregnancy (twelfth week and thirtieth week of gestation), which were calculated from twenty-four hour dietary recalls, have been compared in a cross-tabular manner with specific cravings and aversions. D. Fetal outcome variables, such as infant birth weight, fetal viability, head circumference, fetal growth index, gestational length and infant sex, have been analyzed in a univariate manner by testing for associations with specific cravings and aversions. Stated differently, the data will be analyzed with respect to cravings or aversions for specific items that may be associated with any of the series of accepted measures of fetal outcome. E. Cross-tabular associations among fetal outcome variables and the intake of specific nutrients and calories consumed during pregnancy have been determined. F. The effect of intake levels of specific nutrients and calories (obtained for the two periods during pregnancy stated above) on infant birth weight (as an indicator of fetal outcome) are presently being analyzed by multiple regression techniques. G. As with item F above, the effect of continuous maternal variables (such as weight gain during specific periods during gestation, pre-pregnant weight, educational level, days of nausea and/or vomiting during pregnancy, gestational length) on infant birth weight are also undergoing initial analysis by multiple regression techniques.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Section on Nutrition and Endocrinology
Clinical Nutrition and Early Development Branch
Contract and Collaborative Research

Contract Title : The Prevalence of Lactose (Milk) Intolerance and its Impact on Vitamin-D Status in Canadian Indian and Inuit Children
Contract No. : N01-HD-2844
Contractor : University of Western Ontario, London, Ontario
J. J. Ellestad-Sayed, Ph.D.
Money Allocated: FY 81; None: FY 80; None: FY 79; $183,770 for 3 years:

Project Aims: Lactose malabsorption is common among the Inuit (Eskimos) and Indians of Canada (73% and 60%, respectively). This study proposes to determine the prevalence of milk intolerance in these groups and relate it to the effectiveness of the vitamin D milk fortification program in Canada.

Progress to Date: Throughout the winter, field work has been carried out in the Artic region. The communities of Cape Dorset, Broughton Island, Pond Inlet, Cambridge Bay, Spence Bay, and Rankin Inlet have been studied. Each community has taken about three weeks. Sampling among the Inuit population will begin during the summer. All four Indian communities scheduled for sampling this summer have agreed to participate--2 in Quebec and 2 in the Maritimes. Data from seven communities have been key-punched and a preliminary analysis completed. In addition, data from another community has been key-punched and the collection sheets on the fifth Inuit community are being analyzed.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children

Mental retardation ranks foremost among all conditions of chronic disability and is one of the major unsolved problems confronting scientists and citizens of this country. It is estimated that about 3 percent of our population--6.6 million adults and children--are so afflicted; and the number increases by 126,000 new cases each year. The causes are multiple, the effects manifold, and the knowledge needed for prevention and treatment is insufficient. The disabling consequences of intellectual and social impairment are felt in many ways. To the handicapped individual, it is a potential source of personal unhappiness and prolonged dependence. To some families in our society, it is a human tragedy of the utmost magnitude, while to the underprivileged groups, from whom most of the retarded come, it is one element among many in the daily struggle for survival. To the Nation, it represents huge outlays for care and even greater economic losses because of the underproduction and underachievement of its retarded citizens.

Considerable evidence exists today that the causes of mental retardation are biological, psychological, and social in origin and frequently occur in combination in a single individual. Genetic factors, prematurity, metabolic disorders, and other disturbances during pregnancy are only a few of its biological determinants. Infection or injury at birth or in early childhood may also underlie mental retardation. In addition, the impact of lack of stimulation, inadequate educational opportunities, and generally deprived living conditions cannot be overlooked as causal or contributory factors of retarded intellectual and social growth. Knowledge is needed from almost every branch of science in order to understand the interaction of these elements in the development and behavior of children and adults.

The NICHD, within the NIH, has primary responsibility for research and research training concerned with mental retardation. This interest is expressed categorically through the Institute's Mental Retardation and Developmental Disabilities Branch (MRDD) of its Center for Research for Mothers and Children (CRMC). The MRDD Branch supports research into the biological, behavioral and social processes which contribute to, or influence the development of, retarding disorders. Of primary concern are studies concerned with finding causes and means for preventing mental retardation. Support for research pertaining to prevention of mental retardation also derives from activities of other branches of the CRMC. The Institute employs research grant mechanisms, supports special research facilities as the Mental Retardation Research Centers (MRRC's), disseminates scientific and public information, and provides contract support for research to accomplish its goals. The Institute's research programs and resources provide research knowledge and understanding applicable not only to mental retardation but to other closely related developmental disabilities as well.

Research into the causes, means of prevention, and methods for the amelioration of mental retardation serve many of the research requirements of developmental disabilities including autism, epilepsy, cerebral palsy and special

TABLE 1

NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1981
MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES BRANCH

Funds (thousands)

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts	
			Total Research		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	146	$29,705	125	$27,237	83	$7,853	38	$19,241	4	$143	18	$1,729	3	$739
Etiology/Pathophysiology	77	10,273	66	9,202	53	4,276	11	4,856	2	71	10	839	1	232
Epidemiology	4	732	3	607	3	607	-	-	-	-	1	125	-	-
Diagnosis and/or Evaluation	39	6,124	30	4,852	21	2,175	7	2,605	2	72	7	765	2	507
Prevention	3	892	3	892	2	306	1	586	-	-	-	-	-	-
Amelioration--Education	4	1,422	4	1,422	1	123	3	1,299	-	-	-	-	-	-
Amelioration--Medical Treatment	4	996	4	996	3	367	1	630	-	-	-	-	-	-
Other (includes Implementation Grants)	15	9,265	15	9,265	-	-	15	9,265	-	-	-	-	-	-

Note: Excludes scientific evaluation grants.

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July 15, 1981

learning disabilities. These developmental disabilities are a frequent consequence of reproductive casualty resulting from genetic defects, disorders of pregnancy, complications of birth, and maternal ill health. The Institute, because of its assigned responsibility for research in maternal and child health, has a major research concern for these disorders.

RESEARCH ACTIVITIES

As of June, 1981, the Mental Retardation and Developmental Disabilities Branch (MRDD) supported 146 grants and contracts in the amount of $29,705,000. Table 1 shows the allocation of grants and funds in the major areas of mental retardation research activity which include: etiology/pathophysiology; epidemiology; diagnosis and/or evaluation; prevention; and amelioration. Basic studies concerned with the etiology and pathophysiology of mental retardation syndromes are supported at a level of $9.2 million and represent a major concentration of Branch effort. Studies of the epidemiology of mental retardation accounted for but $607,000 of Branch outlays indicating need to expand research efforts in this important area.

Contract activities represent a small but important part of Branch research activity. These contracts were supported this year in the amount of $739,000 or about 2.5 percent of Branch funds (Table 1). All were directed toward research resource development objectives. Details of these contracts are provided at the close of this report.

RESEARCH TRAINING

Eighteen institutional research training grant awards totaling $1,729,000 were supported by the Branch in this fiscal year (Table 1). These training grant awards are evenly distributed between the biomedical and the behavioral and social sciences, and some give emphasis to interdisciplinary training.

MENTAL RETARDATION RESEARCH CENTERS (MRRC's)

The Branch has administrative responsibility for the Mental Retardation Research Centers--12 in number--constructed under the authority of P.L. 88-164. The MRRC's were constructed to conduct research and research training in mental retardation and related aspects of human development. Under the provisions of the Act, the centers contracted to use the facilities for their intended purpose for a minimum of 20 years. On average, the centers have now completed about 12 years of this obligation.

The MRRC's form our nation's major research resource for the investigation of the problem of mental retardation in all its aspects. In keeping with the multiple and diverse causes and the complex nature of the problem, the centers bring to this research and research training enterprise a strong capability for multidisciplinary and collaborative research between the biomedical, behavioral and social sciences in laboratory and field settings. Through these efforts, the centers have contributed increasingly to an understanding of the etiology and pathogenesis of mental retardation and related central nervous system disorders and to programs of prevention, treatment and amelioration.

Although a significant portion of the research portfolios in the centers consists of basic studies, fundamental to an understanding of biological and behavioral processes in animal and human organisms, considerable attention is directed toward seeking solutions to practical issues and problems. Thus, investigators in the centers are exploring the impact on retarded development of deinstitutionalization, normalization, mainstreaming, and various forms of community placement. These efforts are expected to provide an empirical base for large-scale intervention and amelioration programs in the years ahead.

Research in these areas is made possible by the vigorous outreach activities of center scientists, administrators, and communication specialists to community education, health and social service systems. In addition, most of the centers have very close working relationships with public or private residential facilities for the mentally retarded which permit access to subject populations and the development and evaluation of enriched environmental settings. As a consequence of these studies and others in community-based preschool and day care programs, models for effective intervention are being developed and applied in many communities and school systems throughout the country.

The range of research studies being conducted in the MRRC's encompasses every known major dimension of the problem. This concentration of activity is supplemented by the work of investigators located in other universities, agencies, and research settings. The activities described in the section which follows represent a few of the research accomplishments and highlights from the Branch portfolio during this fiscal year.

RESEARCH HIGHLIGHTS

From its inception, the MRDD Branch has placed emphasis on developing a strong program of genetics research. Added impetus to this development occurred in FY 1978 when both the House and Senate Appropriations Committees included language in their reports urging the Institute to increase its research in genetics, specifically stipulating research in Down syndrome. Data supportive of continued emphasis on genetics/Down syndrome research are compelling and advances realized and expected from Branch contract and grant supported research are encouraging.

Down Syndrome

Down syndrome (trisomy 21) is the most prevalent among the many organically caused mental retardation syndromes. Based on six separate surveys conducted between 1969 and 1975, an overall frequency of one baby with Down syndrome in 800 live births was obtained in 57,000 consecutive newborn babies studied. More recent evidence suggests that the incidence of Down syndrome is decreasing. The observed reduction has been attributed to two factors: a progressively smaller proportion of women 35 years or older who are having babies, and prenatal diagnosis followed by selective abortion of affected fetuses.

Because proportionately more infants with trisomy 21 are born to women over 35, it has been postulated that the mother was the more likely source of the extra chromosome. With the advent of new staining techniques, it is now

possible to determine, in about 80 percent of cases, the parental source of the extra chromosome. Institute-supported investigators have reported that the father was the source of the extra chromosome in 23 percent of children with Down syndrome. This observation has resulted in a major shift in the approach to genetic counseling and in designing research studies to determine the cause of the syndrome.

Ethical restraints on research using human subjects, particularly those who cannot provide informed consent, have stimulated the search for suitable animal models for conditions that afflict humans. Institute-supported investigators are selectively breeding mice which produce litters that are trisomic or monosomic for specific chromosomes. The investigators have demonstrated that three of the genes that are known to be located on chromosome 21 in man are located on mouse chromosome 16, suggesting a genetic homology between the two chromosomes. The investigators are also developing techniques to freeze viable mouse embryos which can be reimplanted in pseudopregnant mice to produce litters with specific chromosomal defects. Experiments that are difficult or unethical to perform on human subjects can be carried out using the animal model. Plans are currently underway to develop a mechanism to supply the mouse model to the scientific community.

Fragile X Syndrome

Social and behavioral variables traditionally have been cited to explain why approximately 50 percent more mentally retarded males than females are found among institutionalized and community-based subjects. Several investigators are now pursuing biological hypotheses to explain this phenomenon.

The Fragile X syndrome, named after the apparent instability of one portion of the X chromosome, is considered to be the most common cause of mental retardation in males and second only to Down syndrome in the association of mental retardation with a chromosome abnormality. Institute-supported investigators are trying to characterize the clinical features of the syndrome. The main features are a variable degree of mental retardation, characteristic faces with large jaws and ears which are frequently low-set, enlarged head in the absence of hydrocephalus, normal bodily proportions, large testes and a compulsive repetitive, jocular form of speech.

Data on the frequency of the Fragile X syndrome in different populations are not available. Thus, the proportion of non-specific X-linked mental retardation that is attributable to the Fragile X syndrome is not known. However, it must be responsible for a very significant proportion of mental retardation not only in males but also in females. In families in which the Fragile X is segregated, a number of heterozygous females have been noted to be mildy retarded.

NICHD-supported investigators are looking for the optimal tissue culture conditions needed to detect the abnormal X chromosome. They have demonstrated the chromosome in cultured skin samples obtained from carrier females and affected males. The findings will provide a basis for reliable diagnosis of carriers and for prenatal diagnosis and genetic counseling relating to a potentially preventable form of mental retardation.

Phenylketonuria (PKU)

Phenylketonuria is an inborn error of metabolism which is associated with severe mental retardation, behavior problems, epilepsy and other signs of neurological impairment. With an incidence of 1 in 14,000 births, the condition is one of the most common metabolic disorders which, when untreated, invariably requires long-term institutional care.

A collaborative study of children treated for PKU is being conducted in 15 medical centers across the country. The study has demonstrated that, when treated with a diet containing restricted amounts of phenylalanine (an amino acid which is essential for normal growth and development) within 120 days after birth and up to six years of age, the growth pattern is normal for height, weight, and head circumference. The occurrence of congenital or neurological defects and EEG abnormalities among the treated PKU patients is comparable to that among control subjects. Measures of intellectual development show that treated PKU children achieve scores that are comparable to the normal population. The intellectual achievement of children diagnosed and treated within the first 30 days of life is significantly above that of children whose treatment is delayed.

The question as to whether it is safe to discontinue the phenylalanine-restricted diet is being addressed by the investigators. Upon reaching his or her sixth birthday, each child was randomly assigned either to continue or discontinue the diet, subject to parental informed consent. IQ data at six and seven years of age are available on 120 subjects. Sixty-two children who remained on the phenylalanine-restricted diet had a mean IQ of 100 at age seven in contrast to a mean IQ of 97 for 58 children who discontinued the diet. This represents no change in IQ for continuers and a 2-point decrease for discontinuers. This decline is not statistically significant. Analysis of covariance, using the 6 year Stanford Binet IQ as covariate, produced adjusted IQ averages at 8 years on the three scales of the Wechsler Intelligence Scale for Children which varied by three to four points between the groups.

WISC Scale Group	Adjusted Mean WISC IQ 8 Years
Verbal Scale	
Continuers	100
Discontinuers	97
Peformance Scale	
Continuers	100
Discontinuers	98
Full Scale	
Continuers	101
Discontinuers	97

The difference in the scores between the two groups of children in the verbal and performance scales are not statistically significant but the four-point difference in the Full Scale IQ is statistically significant (P. .05).

At eight years of age, the school achievement of 35 continuers and 48 discontinuers was evaluated. Both groups scored above grade placement in reading and spelling. Continuers scored slightly above grade level on arithmetic, and discontinuers slightly below.

No evidence has been produced by this study to document the concern that continuation of the diet into the school years produces significant psychological difficulties.

The study is still in progress and the sample sizes at this time are small, particularly for those children reaching the age of 10 years, upon which a definitive conclusion can be based.

Maternal PKU

In the past, virtually all phenylketonuric women of childbearing age were mentally retarded and bore few, if any, children. Routine newborn screening and early treatment of PKU over the past 18 years have resulted in normal physical and intellectual development among phenylketonuric girls, many of whom have normal capacity for conception. A woman with PKU who eats a regular diet during pregnancy is likely to bear a child with mental retardation, congenital heart defects, intrauterine growth retardation or microcephaly even though the child does not have PKU. In an effort to learn what the mechanism is for these fetal effects and if they can be avoided by dietary restriction of phenylalanine intake, the Institute is supporting a maternal PKU project using rhesus monkeys as an experimental model. High blood levels of phenylalanine are induced in the pregnant monkeys with a diet high in phenylalanine and a chemical which blocks the action of the metabolizing enzyme. To facilitate fetal blood sampling, an in-dwelling catheter is placed into the common carotid artery of the fetus in utero. Maternal and fetal blood levels of phenylalanine, tyrosine and other amino acids are determined periodically. The newborns are examined for congenital malformations and various developmental parameters. Initial findings show that the phenylalanine level in fetal blood is about 1.5 to 2 times higher than that in maternal blood, indicating an active transport of phenylalanine across the placenta. This suggests that the maternal phenylalanine level which is considered safe for the mother's health may be detrimental to the developing fetus.

Reading Disability

A multifaceted study is in progress which will characterize the clinical and genetic variations of specific reading disability. The study is being carried out with extensive collaboration with schools in the Baltimore area specializing in children with reading disorders. A follow-up study, consisting of a large sample of boys with reading disability who attended a boarding school near Buffalo between 1940 and 1977, has been recently added to the project. The study consists of three projects: 1. the familial aspects of specific reading disability; 2. a study of educational and occupational outcomes,

reading habits and attitudes of alumni of a school for dyslexic boys; and 3. a study of aspects of language development in dyslexic children and controls. A battery of experimental language tasks has been compiled to assess a variety of language skills in individuals ranging from eight years of age through adulthood.

Preliminary analyses of data suggest that there are highly significant overall group differences among the different groups of subjects with reading disability. The experimental tools used in this study are capable of measuring subtle differences in language skill. The investigators are now attempting to identify subgroups of disabled readers. The long-range goal is to identify one or more homogenous language-based subgroups of individuals with reading disability on the assumption that this might be a genetically-based form of the disorder.

Cytomegalovirus (CMV) Infection

An Institute-supported project is investigating the pathogenesis and model of transmission of cytomegalovirus infection. CMV is the most common cause of congenital infection of the human fetus; 0.6 to 2.5 percent of newborns have been reported to excrete the virus. Congenital CMV infections may result from primary maternal infection with transplacental viral transmissions or from reactivation of latent maternal infection. The consequences for the developing fetus can be mental retardation, hearing loss or low birthweight but, more commonly, congenital infections are subclinical.

The investigators have noted that stillbirth and widespread viral infection in newborn guinea pigs, the animal model used for the study, occurred more frequently if dams were infected in late gestation than in the first trimester. Neonates of dams infected during early gestation were not found to have infectious virus. Tissue destruction was observed in kidneys, lungs and/or brain of 40 percent of newborns examined, however, even without detectable virus in these tissues.

To prevent congenital CMV infection, the female guinea pigs were vaccinated with either live virus attenuated through several passages in tissue culture or with vaccines prepared from antigens obtained from the surface membranes of the virus. Both vaccines offered protection against transplacental transmission of CMV and reduced the severity of primary infection during pregnancy. The live attenuated virus vaccine gave the greater protection against maternal and fetal infection.

It is not yet known whether the attenuated guinea pig or human CMV vaccines reactivate during pregnancy. The investigators will continue the study on guinea pigs to determine if live attenuated vaccines reactivate during pregnancy, revert to virulence, or result in transplacental transmission of the vaccine virus to offspring. Prevention of CMV infection by a vaccine will ultimately serve to reduce the incidence of mental retardation.

Low Level Lead Exposure

An on-going study is exploring behavior and body lead burden in children with

no antecedent history of lead intoxication. Teachers' ratings, parents' ratings, and cognitive performance are being compared in children with high and low lead burdens. The index of body lead burden is based on the concentration of lead found in dentine of shed deciduous teeth. Dentine lead levels are measured, rather than blood levels, because blood concentration is a marker of recent exposure which can return to normal levels even when exposure is excessive.

Using an observational method designed to assess distractibility on children in the third through sixth grades, the investigators found that the high-lead group of children engaged in a variety of "off-task" behaviors significantly more often than the low-lead group. Furthermore, for a significant number of the observational categories, the children's scores were dose-related to lead. On a questionnaire containing 11 items related to classroom behavior, teachers gave high-lead children a greater number of negative ratings on nine of the items. In general, these scores were also dose-related to lead. Observational ratings conducted by the investigators in the classroom confirmed the teachers' scores of children's behavior. On the Behavioral Problem Checklist, the frequency with which teachers reported "conduct problems" for the children also was dose-related to lead. The group scores on the other subscales of this instrument did not differ.

Detailed examinations in a neuropsychological laboratory setting showed that children with high levels of lead in dentine were found to be significantly inferior on IQ, verbal IQ, attention and auditory processing compared to children with lower dentine levels. The investigators concluded that lead exposure, at doses below those producing symptoms severe enough to be diagnosed clinically, can be associated with neuropsychologic deficits that may interfere with classroom performance.

Prevention of Psycho-social Mental Retardation

The question of modifiability of intelligence is one which has been debated for decades. A large proportion of those children identified as retarded in school show no clear signs of biological cause of the retardation. In many cases these children are from the low-income segment of the community and it is assumed that the retardation is the result of a variety of factors including environmental causes as yet unspecified. Almost a decade ago, a project was begun which sought to identify a group of children at risk for this form of mental retardation and prevent its emergence. A well-planned educational program was designed and infants were enrolled randomly from a group considered to be at risk for psychosocial retardation. These children and a matched comparison group have been studied to discover the effects of the intervention effort in preventing retardation and to uncover some of the factors which might be related to the onset of retardation. Presently, these children are in grade school and have been studied continuously for the past eight years.

There are a number of important findings which have been reported from this project in the area of family interaction, language development and the development of intelligence. First, it is clear that the children who were randomly assigned to the educational day care program have performed at significantly higher levels on a variety of standardized tests when compared with the group

who were not in the program. The comparison children have shown gradual decrement in performance on intelligence tests, indicating that without the preventive intervention, they begin to be identified as borderline or mildly retarded. Furthermore, whereas the relationship between mother's intelligence and child's intelligence is usually high in this high risk group, the children who were involved in the intervention program do not show such high correlations. This latter finding may add strength to the notion that under some circumstances, the investigators in this project have successfully demonstrated that children at risk for psycho-social retardation can be identified during the first year of life. This is well before there is evidence of measurable intellectual decline. This has great implications for prevention since the use of an instrument such as the one developed by these investigators might improve prevention efforts considerably in other sites. Further testing of the screening device for infants at risk for psycho-social retardation is necessary to validate this finding.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-8-2858

Contract Title : Development of An Animal Model for Genetic Disorders with Emphasis on Trisomy 21 (Down Syndrome)

Contractor : University of California, San Francisco

Money Allocated: FY 1981 $75,000

A contract was awarded to the University of California, San Francisco, in 1978 to develop an animal model for genetic disorders with special emphasis on trisomy 21 (Down syndrome). The development of such an animal model will allow the performance of many types of investigations which will either be difficult or impractical to carry out in human subjects due to ethical or scientific considerations. The animal model will permit a detailed developmental analysis of the pathogenesis of congenital defects; in vivo and in vitro investigation of cells, tissues, and the central nervous system; studies of pre- and post-implantation stages; gene dosage studies; and effects of genetic and environmental factors on the aneuploid phenotype.

The San Francisco investigators, in collaboration with scientists from Lubeck, West Germany, have succeeded in isolating mice with metacentric chromosomes, breeding mice to produce selected trisomic or monosomic embryos and mapping three genes (superoxide dismutase-1, interferon receptor, phosphoribosylglycinamide synthetase) that are known to be on human chromosome 21 on mouse chromosome 16. This finding strongly suggests a genetic homology between human chromosome 21 and mouse chromosome 16. Regional mapping of these gene products on mouse chromosome 16 is underway.

One of the major differences in the phenotype observed in human trisomy 21 and murine trisomy 16 is the early postnatal death in the latter. It would be desirable, therefore, to construct experimental situations in vivo and in vitro which permit the study of the effects of mouse trisomy 16 on cellular functions. One major approach, the construction of trisomy/disomy chimeras, has been initiated and two types of chimeras, radiation and aggregate, have been produced.

The embryo freezing methodology is now established. Although the technique works with reasonable efficiency, further experimentation is required before it will be ready for routine use.

Plans are underway to develop a mechanism for supplying the mouse model to qualified investigators.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-8-2855

Contract Title : Field Testing for Chromosome Preparation and Karyotyping Device

Contractor : Northwestern University

Money Allocated: FY 1981 - None - Unexpended funds in the 1980 budget will be used to complete the research and development aspects of the contract.

The objectives of the contract are to carry out a comprehensive evaluation and testing of an automated karyotyping device which was developed at the Jet Propulsion Laboratory, and a semi-automated system for chromosome preparation which was developed at the City of Hope Medical Center. The automated systems were transferred to the Northwestern University in 1978. The contract with Northwestern is divided into three phases. The first phase was to evaluate both machines and conduct preliminary trials to test all aspects of the automated system. Clinical and research stress tests were initiated during the second phase. Minor modifications of the system were initiated in phase three. In addition, the systems will continue to be evaluated during the current contract period. Budgetary constraints made it difficult to up-date the original hardware which was "frozen" using 1973 technology. Since the installation of the piston pumps which replaced the peristaltic pumps, the automated specimen preparation system has been functioning smoothly with minimal human intervention. The system has the capacity to handle a large volume of specimens for clinical, epidemiologic, and research purposes. The equipment is time-saving, cost-effective, flexible, and capable of yielding high-quality metaphase spreads. It will minimize operator boredom and has the distinct advantage over the manual methods in being able to culture several samples from the same or different subjects using one to four 12-well culture trays that are subjected to the same environmental and culture conditions. Thus, specimens from patients and controls (or known karyotype) can be cultured concurrently in the same "run".

The remainder of the contract will be devoted toward the development of the operator's manual and a summary of the results of parameter trials, i.e. culture times, volumes, mitotic indices, and number of analyzable metaphases per slide. An orderly documentation of the electro-mechanical system, including drawings, diagrams, schematics, and circuitry will be completed.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Mental Retardation and Developmental Disabilities Branch
Center for Research for Mothers and Children
Collaborative Research and Contracts
Summary of Reports

Contract Number: NO1-HD-32713

Contract Title : Coordination of Regional Cytogenetic Registries

Contractor : University of Oregon Health Sciences Center

Money Allocated: FY 1981 - $233,000

This contract was initiated to develop a common format for the collection of clinical and cytogenetic information; to develop a manual of procedures to standardize the selection of material for analysis and for maintenance of quality control; and to develop a phenotype coding system. The registry is designed to maintain a record of genetic and clinical information which will help provide better health care services, and to provide a data base for research and epidemiologic studies. Initially awarded to the University of Oregon to demonstrate the feasibility of coordinating an existing cytogenetic registry in Portland and a new registry in Denver, the contract now includes laboratories in Memphis, Albany, Indianapolis, New Haven and Portland, thus providing a greater geographic area for population sampling.

Data have been collected on about 36,000 patients, including over 9,000 seen for amniocentesis. The data base is kept in a computerized sequential file format. Data are recorded in each of the participating laboratories and sent to the coordinating center in Portland where they are keypunched and verified on tape and batch-entered. Routine editing programs permit data correction. Confidentiality is maintained by deleting names and other identifying information. Three different methods are used to assure quality control of data.

Six research projects have been completed, using registry data, which demonstrated the feasibility of retrieving, collating, and analyzing data stored in the registry. The six projects dealt with the following issues: frequency, type and significance of 'spurious cells' in amniotic cell culture; chromosomal rearrangements; frequency of mental retardation in Turner syndrome; maternal and paternal ages in Turner syndrome; phenotype of patients with structural rearrangements of the X chromosome; and rates of cytogenetic abnormalities diagnosed prenatally.

Preliminary development of the data entry system using the Apple microcomputer has been completed. Because one of the collaborating laboratories (Tennessee) presently has an Apple microcomputer available on site, the master diskettes developed in Oregon will be tried on a sample of current data in Memphis.

An announcement describing the registry and the availability of registry data was published in March 1981 in the NIH Guide for Grants and Contracts.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Human Learning and Behavior Branch
Center for Research for Mothers and Children

The program of the Human Learning and Behavior Branch (HLB) encompasses the development of human behavior from the perinatal period through infancy, childhood and adolescence, into early maturity. Its major concern is to determine how the interaction of biological, psychological, social, and environmental factors result in normal behavioral development, and to identify those factors which interfere with such development.

Five major areas are subsumed under this program area: (1) developmental behavioral biology, including the study of brain-behavior relationships, the biochemical, physiological, and hormonal bases of behavior, sensory-motor processes, and the reciprocal relationship between biological disease states and psychosocial factors; (2) learning and cognitive development, including perception, memory, reasoning, and comprehension; (3) the development of communicative abilities (speech, language, reading); (4) social and affective development, including parent-child relations, family dynamics, peer relations, social learning and assumption of social roles, and the development of temperament and affect; and (5) health-related behaviors, including the behavioral aspects of children's adaptation to physical illness, disease, or disability, and the psychosocial factors which lead to health endangering or health-fostering attitudes and behaviors in children.

Processes and factors specific to each stage of development are studied. These include studies of the behavioral development of children born at biological risk and studies of learning problems, delayed or impaired speech and language development, and specific reading disability, or dyslexia.

A table is appended to this report, summarizing the grant and contract program of the Branch for the fiscal year 1981.

DEVELOPMENTAL BEHAVIORAL BIOLOGY

The Developmental Behavioral Biology (DBB) program has as its major goal the support of research designed to provide an understanding of the biological bases of behavioral development. The program is divided into six areas as follows: (a) Developmental Behavioral Genetics; (b) Developmental Behavioral Endocrinology; (c) Developmental Behavioral Neurobiology; (d) Sensory and Psychomotor Development; (e) Pre- and Perinatal Effects on Behavioral Development; (f) Animal Models of Learning and Social Development.

Developmental Behavioral Genetics

This aspect of the program has as its major goal the support of research designed to acquire empirically based information on the role of genetic factors in the development of normal behavior and learning disability. Two types of studies are involved in this category of research support, animal models and human studies. The animal studies employ experimental paradigms with prospective designs to ascertain the role played by genetic factors in behavioral development. The Branch currently supports five projects in this domain. While the support of research in this field is small compared to other aspects of the program, the work is

NICHD GRANTS AND CONTRACTS ACTIVE DURING JUNE 1981
HUMAN LEARNING AND BEHAVIOR BRANCH

Funds (thousands)

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts (incl. S06)	
			Total Research		Research Projects		Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	140	$13,469	122	$11,995	109	$8,466	8	$3,340	5	$189	14	$859	4	$616
Developmental Behavioral Biology	32	2,444	28	2,341	25	2,014	1	253	2	74	4	102	-	-
Human Learning, Cognition, Perception, Memory	44	3,770	37	3,047	32	1,905	3	1,065	2	77	6	684	1	39
Social and Affective Development	14	1,149	13	1,130	12	1,093	-	-	1	37	1	19	-	-
Human Communicative Processes	31	3,718	26	3,462	22	1,440	4	2,023	-	-	3	53	2	203
Health-Related Behaviors	19	2,388	18	2,015	18	2,015	-	-	-	-	-	-	1	374

Note: Excludes scientific evaluation grants.

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July 14, 1981

yielding important results. Dr. John DeFries of the University of Colorado, for example, is using a prospective longitudinal design in which adopted and control children are being studied to elucidate the relative contributions of genetic endowment and environment as factors which underpin the development of cognitive capacities and personality. Tests are administered to assess these variables in both the children and their natural and/or adoptive parents. A preliminary analysis of the data indicates that there is: (1) a great similarity with respect to demographic profile, test reliability, and cognitive test score variance among the biological, adoptive, and control parents; (2) a similarity between cognitive ability of parents and offspring in all sets of parent/child comparisons studied; (3) a genetic influence on rate of communicative development during the first year of life; (4) a lack of relationship between environmental variables and personality during the first year of life.

In general, this study has the potential, due to its innovative design and subject population, to become a landmark study in the field of child development.

Developmental Behavioral Endocrinology

This component is the largest one in both number of grants and dollar amount of the Developmental Behavioral Biology program. Its major goal is the support of studies designed to determine the role of the endocrine system in the development of behavior and affect. The experiments carried out in this area of inquiry generally employ prospective designs in which a variety of different types of animal subjects are used to ascertain how specific hormones influence behavioral development from birth through maturity. Progress has been achieved in this aspect of the DBB program in two different areas of research.

In one series of experiments, Dr. Seymour Levine of Stanford University is studying how separation of infant/mother dyads affects bonding and hormonal response in Rhesus monkeys. Results indicate that mothers separated from their infants for two weeks showed a specific pattern of behavioral and pituitary-adrenal response to their own infant but not to other separated infants. Further, mothers not separated from their infants (housed with their offspring) responded to the sight of any distressed infant. Non-mother females showed no change in plasma cortisol levels in the presence of separated infants. Thus, hormonal stress pattern in adult female Rhesus monkeys is related to their status as mothers and attachment to their own infant. Data on the separated infants in these studies reveal that during the first 24-hour period they exhibited a brief elevation in plasma cortisol levels. Such adrenal cortical responses were correlated with observations of increased locomotion and distress. These data suggest a time-locked relationship between the behavior and a biological substrate related to the stress of separation.

Dr. Masakasu Konishi of the California Institute of Technology is studying the role of testosterone in the development of the avian brain. The results of the study are exciting because they document the involvement of this hormone in the specialization of brain loci which control the onset of species-specific male song behavior.

Developmental Behavioral Neurobiology

The objective of this program area is to determine the role played by the developing brain in the onset of normal learning and behavior, as well as an understanding of how abnormal central nervous system development may contribute to the develop-

ment of learning and language difficulties in children. The program, as currently constituted, supports both human studies and investigations which employ animal models. Several recently funded applications are utilizing the evoked response method to elucidate how reflexes, sensory processing, and information processing develop in normal children. The program also emphasizes experiments on hemispheric lateralization and the relevance of such brain specialization for the development of learning, behavior, information processing and language acquisition in children.

Dr. David Galin of the University of California, San Francisco, is studying (under contract) the neuropsychological correlates of dyslexia. This project, which is now in its final year of funding, has compiled some important new data on the neuroelectric (EEG and evoked potential) events associated with this major reading disability. A finding of great interest is the observation that dyslexic children cannot be distinguished from controls on measures of vestibular ocular-motor function. These data are important because they do not support the findings of other workers who have previously suggested that vestibular and ocular motor dysfunction may be an underlying mechanism for reading difficulties observed among diagnosed dyslexics.

Sensory and Psychomotor Development

This program has as its major objective the study of how sensory/behavioral and psychomotor control develop from birth to maturity. Particular emphasis in this area of research is placed upon understanding such development in both human neonates and animal models. The Branch hopes to develop this program area during the coming year by holding a workshop on human and animal psychophysics of sensory systems (vision and audition) development. The primary emphasis is to be upon methods for measuring sensory capacity in the human neonate.

Pre- and Perinatal Factors in Behavioral Development

The objective of this program area is to achieve an understanding of the role played by intra-uterine factors and those which occur during the first month of life on the course of normal behavioral development. Particular emphasis is placed upon identification of variables which lead to the birth of children who are at biological risk (e.g., below 2500 grams) and who consequently exhibit delayed or non-optimal biobehavioral development. During FY 1981, a potential landmark study was funded by the Branch. The research, conducted by Dr. June Reinisch of Rutgers, the State University, is investigating the long-term behavioral, psychological, and physical development consequences of fetal exposure to therapeutically administered hormones. The study is utilizing a sample from a cohort of Danish children born between 1958-1961 on whom extensive records exist in the form of a biographic and health registry.

Animal Models of Learning and Social Development

The objective of this aspect of the program is to study basic behavioral processes, primarily in primates and other mammals, which underlie and have high relevance for human development. Major foci of the currently supported work involve methodological and substantive research on socialization and affective development during infancy and studies of behavioral mechanisms (e.g., habituation, discrimination, generalization) involved in early learning.

LEARNING AND COGNITIVE DEVELOPMENT

Cognitive Development in Infancy and Early Childhood

A large portion of the Human Learning and Behavior Branch program in cognitive development is concerned with the abilities and the early development of newborns and infants under a year old. This work encompasses the condition and growth of infants' perceptual abilities, ability to process information, to learn and to develop concepts. In its totality this research represents an area of major concentration for the Branch both in terms of the number of active projects and the funding level.

The greatest research emphasis is on understanding normal developmental processes, but several projects also include, as subjects, high-risk infants who are premature and/or have suffered perinatal trauma. Most of the studies supported concern the development of visual perception, but there also are a substantial number of studies of the auditory system, including research on the perception of speech and non-speech sounds, and some work on other sensory systems. The study of visual perception includes perception of patterns, depth, and movement, and perception of various types of forms, sizes, and textures. Recent emphases have been on the development of increasingly efficient visual scanning patterns. In one promising investigation, Dr. William Kessen of Yale University has found large and persistent differences between infants in their ability to scan visual stimuli well. The study includes normal-term, premature, and small-for-gestational-age babies, and seeks to establish whether differences in scanning patterns can be used as predictors of later cognitive deficit.

Several other grantees have investigated the development of infants' and young children's ability to integrate information from several modalities, e.g., visual and auditory, or visual and tactual.

Dr. Holly Ruff of the Albert Einstein College of Medicine has been studying individual differences in infants' exploration of objects, that is, whether the infant explores objects visually and/or tactually, or mouths objects, and the effects of these exploratory patterns upon the information the infant receives.

New advances in techniques for measuring physiological responses, eye movements and other behavioral responses have made it possible for researchers supported by the Institute to investigate the earliest indications of infants' rudimentary cognitive abilities. They have asked questions, for example, about infants' recognition of pictured objects, understanding of contingencies in their environment, and the systems which infants use to categorize similar and discrepant stimuli and events. Several grantees have searched for early signs of cognitive deficit and early predictors of later disability in premature infants or infants and toddlers with cerebral palsy.

Cognitive Development in Later Childhood and Adolescence

Although the Human Learning and Behavior Branch has had a strong program of research in infant development, it is equally interested in how perception, attention, information processing, learning, and memory processes develop and change as the child matures, gains experience, and is aided by input from language. Thus, the Branch supports research on the development of all of these processes throughout the preschool years, middle childhood, and adolescence, and into maturity.

Several Institute-supported research projects are investigating the development of spatial concepts in preschool children, elementary school children and adolescents. Dr. Lila Braine of Barnard College has traced the process by which young children learn the meaning of the terms "right" and "left." The accuracy with which children use these concepts of directionality depends upon their ability to abstract spatial relationships, their language ability, and the nature of the stimulus itself.

Dr. James Herman of Washington University, St. Louis, is studying children's judgment of distance and the contribution of cues from familiar or unfamiliar environments. In one condition the usual environmental cues are eliminated by requesting the child to judge the distance he/she has traversed within a tunnel. In this condition children base their distance judgment upon time spent going through the tunnel.

Still other researchers supported by the Branch have focused upon the ways in which perceptions are organized so that attention becomes more and more selective with age; that is, the child attends to those features of the stimulus or event which are particularly important in distinguishing between possible interpretations of what has been seen.

Similarly, children's memory becomes organized and selective so that the essential features are those which are remembered. Language development is also crucial to the development of effective memory skills. Several researchers, for example, are studying methods children use to remember an array of objects. One grantee observed the rehearsal strategies which children use for memorizing material. Still another researcher (Dr. Marion Perlmutter, University of Minnesota) is studying the ways in which particular verbal cues can trigger recall.

From a developmental perspective, Dr. Robert Siegler of the University of Chicago is studying the process of scientific reasoning in children 3 years of age to adulthood. In the first year of the study, subjects' concepts of time were assessed in a series of Piagetian-type problems involving toy electric trains on parallel tracks departing and stopping at different points, specifically to determine how subjects structured the information (encoding) and what inference strategies they used to solve the problem. When feedback training was given, the most effective instructional approach was to call the child's attention to key dimensions of the problem, and then, either explicitly or implicitly, indicate the algorithm (rule) by which the dimensions could be combined.

Experience in Head Start programs appears to have a long-lasting effect on the academic and social development of children from disadvantaged backgrounds, according to several follow-up studies now underway. The Institute is currently supporting Dr. Louise Miller of the University of Louisville, who is following up pre-school children from low-income black families who attended one of four different programs in the late 1960's. The Montessori program, though initially not very effective, now appears to have had the strongest "graduates" in the 7th and 8th grades. This "sleeper" effect, also reported in other studies, suggests that even though IQ and achievement gains made by the Head Start children show a decrease in the elementary school years, a gain occurs again by junior and senior high school. The NICHD investigator's data on the 11th and 12th grade Head Start children will further test this trend.

HUMAN COMMUNICATIVE PROCESSES

Why are the initial consonants of the syllables /di/ and /du/ perceived as the same phoneme (speech sound), 'd', even though the acoustic cue (sound spectrum) for this consonant is different in the two syllables? Are the complexities in classifying (distinguishing) one consonant from another the result of experience with one's parental language, i.e., learned behavior, or are the mechanisms which are responsible for this process a part of the system that infants bring to the language acquisition process, i.e., innate? Is the phenomenon of categorical perception mediated by a phonetic or an auditory (psychophysical) mechanism?

What is the relationship between a child's non-language skills (symbolic play) and his language development? Can we use a child's meanings for words to assess his conceptual development? Is there a developmental order for a child's application of word meanings? To what extent has the overall form and organization of language been determined by the articulatory and perceptual modality in which it has been developed (its transmission system) and to what extent does its form and organization represent more fundamental aspects of the human mind, the intellect and cognitive processes?

Why do some dyslexics (poor readers) call the letter b a d or respond saw when they see the word was? Should reading instruction and remediation vary with the sex of the child?

The Human Communicative Processes (HCP) program of the HLB Branch has provided research support to scientists who are attempting to answer these and other questions related to the acquisition and development of speech, language, reading, and associated communicative functions. In FY 1980, $3,814,000, or almost one-third (31.5%) of the funds expended by the HLB Branch, was provided for support of fundamental research and training for research in human communication.

The specific research projects currently receiving support are evenly distributed over seven broad areas: infant vocalization; language learning; verbal skills and speech behavior; reading and dyslexia; language structure and psycholinguistics; sign language and nonhuman patterns; and research training.

Since the last Annual Report was prepared, two major human communication conference proceedings have been published. The first, Orthography, Reading and Dyslexia, was edited by J. Kavanagh and R. Venezky and distributed by the University Park Press. This book discusses some of the intriguing and perplexing questions about the interaction of language, writing systems and reading skill. The second publication is a two-volume set entitled Child Phonology, edited by G. Yeni-Komshian, J. Kavanagh, and C. Ferguson. The main focus of Volume 1 is on production, while Volume 2 features perception. Normal acquisition is emphasized in these books; however, some aspects of deviation are also addressed.

During this reporting period, Branch-supported scientists have made some progress toward answers to the illustrative questions presented at the beginning of this discussion of the Human Communicative Processes program.

Dr. Dennis Molfese of Southern Illinois University has reported for the first time the identification of neuroelectrical responses which reflect the processing of phonemes within the cerebral cortex. He has provided the first evidence that consonant phonemes (e.g., /d/) are processed in the brain as distinct and indepen-

dent phonemes. Although the acoustic frequencies for consonant transitions change when the consonant is combined with different vowels, two components of the brain's electrocortical response were found to distinguish between phoneme classes rather than between acoustic differences. It would appear, therefore, that at some level within the cortex of man the phoneme may be the basic perceptual unit of language.

Dr. Janellen Huttenlocher of the University of Chicago has been investigating two-year old children's conceptual development as related to their understanding and use of words that describe action. While adults' ideas of action combine two different types of experience -- one's own goals and feelings of moving, and seeing other people moving -- Dr. Huttenlocher's research suggests that young children's word meanings at first cover only their own experiences. A group of children were observed in situations in which they used words like jump and get. By examining these situations Dr. Huttenlocher discovered that young children use these words almost entirely when they themselves were acting and not to describe others' actions. Furthermore, when she showed the children short movies of others carrying out these same actions, they could not identify many of them. This research will provide normative information about linguistic/conceptual development which is essential for improved assessment of children whose development is aberrant.

Dr. Ursula Bellugi of the Salk Institute has been studying a language which has developed in modalities other than auditory-vocal: American Sign Language (ASL), the system of hand signs developed by deaf people and passed down from one generation to the next. Study of the system shows that it is a true, autonomous language with its own inner form, using a visual-manual system of communication rather than the auditory-vocal system of hearing individuals. This finding suggests that the human capacity for constructing and using a complex language system is independent of the specific modality in which the language originated, making it possible to address fundamental issues about the human capacity for language, the general form of language, and the forces determining its structural properties.

Another grantee, Dr. Frank Vellutino of the State University of New York at Albany, has produced research evidence that certain classic symptoms, traditionally believed to be a sign of visual perceptual problems in dyslexics, may actually be a manifestation of subtle language deficits that result in naming and labeling difficulties in such children. Specifically, Dr. Vellutino has found, in carefully conducted studies using elementary school children, that when a poor reader sees the letter b and calls it d, or the word was and calls it saw, it isn't because he literally sees these items "backwards," as some hypothesize. Nor is it because he is spatially confused and doesn't always scan from left to right. Instead, it may be because he has difficulty remembering their names, a process that seems to have a high correlation with language ability.

Dr. Isabelle Liberman of the University of Connecticut has reported that the difficulty of most children who have problems learning to read is basically linguistic--not visual, auditory, or motor. Although more boys than girls experience reading disability, Dr. Liberman's research suggests that the critical problems underlying reading disability may be the same for both boys and girls. Therefore, reading instruction and remediation should not be planned according to the child's sex but rather by the individual needs of the particular child.

SOCIAL AND AFFECTIVE DEVELOPMENT

The program of the Branch in social and affective development covers a broad range

of important research issues. It is concerned with the process of socialization of the child, both within and without the family, from infancy, through early and middle childhood, to adolescence and early adulthood. Also included within the program are studies on the relation of affect, temperament and personality to social and biological factors during development. Although the smallest of the five program areas of the Branch, an interesting array of problems, nevertheless, is being studied, with Institute support, from earliest infancy through adolescence.

In one recently funded project, Dr. Christoph Heinicke of the University of California of Los Angeles is measuring prospective parents' adjustment to pregnancy and their expectations before the birth of a child. The parents and their children will be followed during four home visits in the first year and, again, when the children are two- and three-years old. Home observations and developmental tests will be used. Parent adjustment and quality of their interactions with their children will be assessed and related to children's later development.

Dr. Eleanor Maccoby of Stanford University is continuing to follow several cohorts of children, studying the complex relationship between children's gender, birth order and inter-pregnancy-interval, levels of sex hormones taken from the unbilical cord at birth, and temperament and behavior in the preschool years. The researchers focus upon sex differences in children's interactions with their peers and in response to their parents. Sex differences in activity, proximity-seeking, conformity and obedience, and typical types of play are of particular interest.

Two related issues are of interest to several of the researchers supported by the Branch program. One is the development of the infant's capacity for emotional expressiveness during the first two years of life, and the other is the relationship between the mother's social approaches to the infant and the infant's response. Dr. John Watson of the University of California, Berkeley has found that in the first few months of life the child's smiles and vocalizations are contingent on the mother's smiles and vocalizations to the child. As infants develop, their vocalizations become less contingent upon the mother's approach; however, their smiles remain contingent upon the mother's social stimulation.

A rapidly developing field of research is the study of children's development of prosocial behaviors, such as helping and sharing. In one study by Dr. Harriet Rheingold of the University of North Carolina, parents are enlisted as confederates of the researcher, and children's responses to parent's instructions, verbal prompting, requests for help, and praise are observed.

In a second project, children's beliefs about help-giving and generosity are being studied by Dr. Donna Gelfant of the University of Utah and their beliefs are then related to their rates of altruistic behavior when they are with their peers. Children from several age groups are studied, from kindergarten through first, third, and sixth grade, and in college.

Children's personality characteristics, of course, also contribute to their cognitive and intellectual development. An interesting approach used by Dr. Susan Harter of the University of Colorado has been to study individual differences in children's mastery motivation. The researcher has constructed a "mastery-motivation" scale for children from third to ninth grade and the child's score on this scale is then related to his/her preference for easy or difficult tasks and reliance on his/her own judgment versus the teacher's judgment.

In an effort to understand how aggressive patterns of behavior develop in childhood and adolescence, Dr. Robert Cairns of the University of North Carolina has begun a series of studies comparing the behavior of children identified as having problems of aggression control with that of matched normals. Behavioral observations, interviews with children and parents, teacher ratings, and peer nominations are being used. Observations of coercive interchanges between peers in everyday life and in laboratory settings will help answer such questions as: What processes, within and outside the child, regulate the instigation, expression, and termination of aggressive acts and how do these processes change with age?

In a large-scale collaborative study involving five countries, Dr. Urie Bronfenbrenner of Cornell University is examining the environmental factors that contribute to family stress and the support systems that families use in such situations. From the preliminary analysis of data collected on the U.S. sample of lower- and middle-class families (as supported by the NICHD), it appears that the amount of stress in families is higher in lower-class families, especially single-parent households, and increases with the number of children in the family. Furthermore, parents in lower-income families, especially single parents, report less shared activity with their children, and the trend increases with family size. It is interesting that these trends are more pronounced when the studied child is male rather than female. If confirmed, this finding would support other evidence in the literature on the greater vulnerability of males in terms of such diverse indices as mortality, morbidity, and measures of behavioral adjustment under stress. In regard to support networks, the data from the U.S. suggest that single parents are more likely to turn to non-kin and married women to relatives for support. Furthermore, network size for non-ethnic mothers increases with income level, whereas network size for ethnic mothers is unaffected by income.

The Branch plans to expand its program in social development in the future by placing increased emphasis on such topics as: the reciprocal relations between parents and children born at risk; the development of prosocial and gender-specific behaviors; the impact on children of family disruption and differences in family composition; and the role of peer relations during development. Greater emphasis will be given to studies of development during middle childhood, and during early and late adolescence, as well as to the role of the family during different phases of development.

HEALTH-RELATED BEHAVIOR

As part of its mandate to encourage research that improves the health of children and ameliorates disease, the Institute has continually encouraged research in children's health behavior, but only recently has the program been given prominence as a separate category within the HLB Branch. Already this area accounts for 18 grants and 1 contract at a total cost of $2.4 million for FY 81. The growth in this program area reflects the growing awareness among both behavioral and biomedical scientists of the importance of behavioral factors in the etiology of illness and the promotion of a healthy lifestyle, further attested by the establishment of three new professional associations: the Academy of Behavioral Medicine, the Society of Behavioral Medicine and the Division of Health Psychology within the American Psychological Association.

The area of health behavior within the Human Learning and Behavior Branch currently focuses on four major areas:

1. Behavioral aspects of children's illness and disease (both acute and chronic)
2. Promotion of healthy lifestyles in children
3. Risk-taking behavior in children and adolescents
4. Parental health behavior that affects the child's health and psychosocial development

The psychosocial adaption of the child and its family to being ill, hospitalized, in pain, or under stress is a research topic of interest to several NICHD-supported investigators. In a recently completed study, Dr. David Grove of the Good Samaritan Hospital in Portland, Oregon analyzed the impact of the chronically ill (epileptic) child on the family, based on a series of longitudinal naturalistic observations made of the child and family members in the home setting. Predictable "behavior chains" of child and family actions were identified, particularly behavior immediately preceding and following the child's epileptic seizure. Dr. Sheila Ross of the Palo Alto Medical Research Foundation has been assessing the pain experiences of chronically ill and hospitalized children in order to determine the psychological correlates of pain and children's coping strategies. The findings will help clinicians make more informed decisions concerning pain management.

In an attempt to understand the development of healthy behavior in children and their families, Dr. John Bruhn of the University of Texas at Galveston is studying how healthy attitudes and practices develop in nursery school children in relation to personal cleanliness, nutrition, dental care, and safety. He has designed an innovative health education curriculum for four-year old children involving a variety of learning strategies, including puppetry, songs, poems, crafts, and role playing. The impact of this curriculum on the children and family members' health behavior and values is now being evaluated, both in terms of immediate learning and after a one-year follow-up when the children are in kindergarten.

The Institute supports studies designed to further our understanding of behaviors which are harmful to health. In this domain are behaviors associated with the use of cigarettes, eating disorders, accident proneness and excessive risk taking. The Institute is particularly interested in fostering research to identify those predisposing factors, either intra-personal or inter-personal, which enhance the probability that a child will engage in such health endangering behavior. Of importance also is research which focuses on how such behaviors are acquired, what maintains them, and how children can be helped to cease these potentially self-injurious behaviors.

This category of research is the largest one in the area of health behavior, and by far the greatest number of studies ask the question: "How do children begin the process of ciagarette smoking?" Institute-supported scientists are studying the role of parents, siblings, peers, and the media in the initiation process. Also, much research concerns both a description of the acquisition of the behavior itself and the development of methods to intervene early in order to help adolescents break the chain of behaviors which leads inevitably to becoming an habitual cigarette smoker.

NICHD investigators who are studying children's smoking behavior have found it useful to share methodological strategies and preliminary findings at both professional meetings and Institute workshops. As a result of the first workshop held in 1979, investigators were encouraged to develop common definitions of smoking levels, including comparable self-report questionnaire items, and most

importantly, to validate the accuracy of such self-reports by using biochemical tests, particularly the saliva thiocyanate test. The second workshop, held in mid-August 1981, helped researchers clarify data analysis techniques and examine trends for future research directives.

In the smoking area, several noteworthy findings have emerged from the research supported thus far. Preliminary findings from a contract-supported study headed by Dr. Maurice Mittelmark of the University of Minnesota suggest that when both biochemical tests and self-report measures are used, the incidence of cigarette smoking among adolescents is considerably higher than that reported in other studies, notably the 1971 National Institute of Education survey. In fact, it would appear that smoking levels among adolescents are not declining; among 12-16 year olds, smoking levels were found to be 1.7 to nearly 4 times as high as that reported elsewhere. Another study by Dr. Anthony Bigler of the Oregon Research Institute has sought to identify the socio-psychological factors that are associated with adolescents becoming regular smokers by asking a group of adolescent smokers to self-monitor the situations in which they smoke cigarettes. It was hypothesized that smoking begins as a social activity but generalizes to nonsocial situations as addiction takes hold. However, the evidence from this group of smokers is that non-social smoking did not increase as smoking rate increased, unlike other measures of addiction.

In many instances the health behavior of parents may have a long-lasting effect on the health and psychosocial development of their children. The adverse impact of maternal smoking during pregnancy on the developing fetus is now well documented (see 1979 Surgeon General's Report), but much less is known of the long-term effects of maternal smoking on the behavior and cognitive development of the infant and child. Dr. David Rush of Columbia University, who has access to extensive biomedical and behavioral data on 17,000 children who were born in Britain during one week in 1970 (with follow-up data at 5 and 10 years), is testing for differences between children born to mothers who smoked during pregnancy and those who did not, taking into account SES and other factors. Also needed is research to develop smoking cessation strategies among pregnant women; thus far, the Institute has funded no studies in this area.

For the future, the Institute plans to stimulate further research in this area by holding a conference, "Children's Health Behavior: State of the Art," in FY 1982. The participants, drawn from the disciplines of behavioral medicine, health psychology, pediatrics, nursing, neonatology, sociology, and anthropology, will critically review such topics as: response of children and their families to illness (chronic and acute), inpatient illness behavior in hospitalized children, the development of health behavior among children and adolescents, risk-taking behavior in children and adolescents, patient compliance, and methodological and measurement issues in health behavior.

RESEARCH TRAINING AND CAREER DEVELOPMENT

The Human Learning and Behavior Branch has a very modest research training program, providing two types of support: Individual postdoctoral fellowships and institutional research training grants, including stipends for pre- and post-doctoral students. At the present time, the Branch is providing support for six training programs at major universities. All but one are for support of generic developmental psychology, with special emphasis in such areas as learning, human communication, and socialization.

Eight individual postdoctoral fellowship awards have also been provided for advanced research training in such areas as infant learning, sensorimotor and perceptual development, language dominance, and the use of genetic markers in behavioral analysis. Five research career development awards were also active during the year.

STAFF REPORT

Dr. Norman Krasnegor, Ph.D., joined the Branch on October 1, 1980, as a Health Scientist Administrator, with responsibility for the development and administration of the Branch's program in Developmental Behavioral Biology. On September 1, 1981, Dr. Krasnegor was appointed Chief of the Human Learning and Behavior Branch, replacing Mr. Philip Sapir, who is retiring from government service. Dr. Krasnegor is a research psychologist, having served in various research positions at the Walter Reed Army Institute of Research, and, from 1972 to 1980, the National Institute on Drug Abuse.

Staff Activities

Members of the staff, as part of their responsibilities, attended the annual meetings of several of the major professional organizations relevant to the program of the Human Learning and Behavior Branch, including presentation of papers and participation in symposia. Meetings attended include the American Psychological Association, the American Speech-Language-Reading Association, and the Society for Research in Child Development. In addition to continuing liaison activities with other government agencies and relevant scientific and professional organizations, the staff took part in the following activities:

Dr. Arasteh served as Planner and Coordinator of Workshop II: Smoking Behavior in Children and Adolescents, August 13, 1981. The focus of the workshop was on problems of data analysis and future research needs. Participation was limited to scientists actively engaged in research in this area, including 12 NICHD-supported investigators.

Dr. Berman served as Coordinator for a major interdisciplinary research conference entitled Women: A Developmental Perspective, November 20-21, 1980, sponsored by the NICHD, with the cooperation of NIMH and NIA. The conference addressed a broad range of research issues concerning the physical and psychological development of women. Dr. Berman is editing the conference papers and proceedings for publication. Dr. Berman also served as NICHD delegate to the Women's Advisory Committee, which advises the Director, NIH, about issues of special concern to women, and presented several papers at national and local meetings on women's issues, including role conflicts of women and the relation of parenting to the development of children's gender roles.

Dr. Berman also served as Planner and Coordinator for a workshop sponsored by the Branch on Gender Roles: Conceptual and Methodological Issues, which served to review the current status of research in the field and develop promising strategies for research in the future.

Dr. Kavanagh served as co-chairman of the NIH Extramural Associates Program. He Also assumed duties as a member of the editorial board of Topics in Language Disorders, a new language journal.

As a member of the Planning Group for a Research Forum, sponsored by the Interagency Panels for Early Childhood Research and Development and for Research and Development on Adolescence, Mr. Sapir participated in the planning and conduct of a Health Research Forum, May 18-19, 1981, preliminary to the 1981 White House Conference on Children, Youth and Parents (since cancelled). The Forum consisted of state-of-the-art presentations by scientific experts on sixteen health topics of major relevance and concern to the health and well-being of children and adolescents, which are being edited for distribution to a number of agencies and organizations, including all State-sponsored Conferences to be held this year on Children, Youth and Parents. Also, as a member of the Interdisciplinary Affairs Committee of the Society for Research in Child Development, Mr. Sapir helped to develop a full day's series of symposia on the relation between nutrition and behavior, held at the annual meeting of the Society.

Honors and Awards

Dr. Krasnegor was elected to the Collegium of Distinguished Alumni of Boston University, for his scientific and administrative contributions to the fields of physiological psychology, behavioral pharmacology, and neurobiology. He also received the Distinguished Service Award of the Division of Psychopharmacology of the American Psychological Association, for his contributions to the field of behavioral pharmacology.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1 HD-1-2420

Contract Title: Research Materials and Assistance for Studies of Language Development in Children

Contractor: Haskins Laboratories, New Haven, Connecticut
Alvin M. Liberman, Ph.D.

Money Allocated: $99,051.00

The purpose of this contract is to continue to provide expert assistance and specialized facilities to research scientists at other institutions who are engaged in studies relevant to an understanding of the development of spoken language and reading in children.

In general, three kinds of assistance have been provided to researchers working on problems related to the acquisition of speech and reading.

1. Research Materials. Stimulus tapes for a wide variety of experiments continue to be the primary assistance that the Laboratories have provided. Several methods of generating recordings suitable for research on speech are available. The techniques can be grouped either by type of stimulus (natural speech, synthetic speech, or nonspeech tones and noises) or by type of recording (single track or dual track). All combinations of these tapes have been used for one research project or another.

2. Specialized Assistance. The professional and technical staff of Haskins Laboratories makes its knowledge and skill available to the user groups. At the very least this involves having someone from the technical staff teach the user group how to operate the computer. But it often involves, beyond that, getting various kinds of help and advice about the more scientific aspects of the research from members of the Laboratories' professional staff. In the end, the user group generates its own tape recordings.

3. Education about Speech Research. A rather high percentage of the user groups are fairly new to the field of speech. Consequently, as a by-product of their visits to the Laboratories, they have an opportunity to become better acquainted with the entire program of research underway there. Many have taken advantage of that opportunity.

With a team of senior scientists the Institute visited the Haskins Laboratories in September of 1979 to examine the activities supported by this contract. Without exception these reviewers recommended approval of the request for three years of additional support. There was general agreement that the service and assistance provided by the contract have been helpful to many investigators, most of whom would not have been able to otherwise conduct their proposed research. Moreover, there was general agreement that there will remain for some years a strong need for the type of research assistance provided through this contract. Accordingly, a renewal of this contract for an additional three years has been approved by the Institute.

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Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1-HD-8-2824

Contract Title: Neuropsychological Studies of Reading Disabilities

Contractor: David Galin, M.D., and Charles D. Yingling, Ph.D.
University of California San Francisco, San Francisco, California

Money Allocated: No FY 81 funds

This contract research program is one of several activities in a program of research on reading disabilities and developmental dyslexia. The immediate objective of this contract research program is to evaluate information processing characteristics in a group of reading disabled children and matched controls, utilizing quantitative methods of brain electrophysiology, neuropsychological tests and psychometric tests. The ultimate objective of this research program is to develop a parsimonious neuropsychological test protocol for the early detection and identification of potential reading disabilities in the pre-school and/or pre-verbal child.

A site visit held in June of 1981 revealed that vestibular oculomotor tests do not differentiate the dyslexic children from controls. The contractors have begun testing the second cohort of subjects and should complete this process by the end of the fiscal year. This contract research program was initiated on September 29, 1978 and will be completed on March 31, 1982.

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Human Learning and Behavior Branch
Contract and Collaborative Research

Contract Number: NO1-HD-9-2831

Contract Title: Childhood Antecedents of Habitual Cigarette Smoking

Contractor: University of Minnesota, Minneapolis, Minnesota
Maurice Mittelmark, Ph.D.

Money Allocated: No FY 81 Funds

This contract is attempting to identify the factors that lead to habitual cigarette smoking and those that lead to continued non-smoking among adolescents, with biochemical measures (carbon monoxide, saliva thiocyanate and cotinine tests) used as validators of self-reported smoking levels. Findings from this research will be useful for developing programs to reduce the incidence of smoking among adolescents.

In its second year of study the contract determined basic prevalence rates for age-sex groupings, examined smoking patterns in relation to parental, friends', and teachers', smoking levels, compared saliva thiocyanate and carbon monoxide data in relation to students' self reports of smoking; and initiated the longitudinal analyses in order to predict changes in smoking levels over a course of one year.

The third and final year of the study will be devoted to further follow-up surveys of students in senior high school, an analysis of longitudinal smoking patterns, and preparation of the final report.

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Office of the Director
Epidemiology and Biometry Research Program

The Epidemiology and Biometry Research Program (EBRP) conducts epidemiological and biostatistical investigations of disorders of mothers and children, of perinatal mortality and morbidity, of infant nutrition and abnormalities of human growth and development. In addition, the Program provides expert biostatistical advice to intramural scientists and to the extramural programs of the Institute. Epidemiological consultation is provided upon request to programs of the Center for Research for Mothers and Children, the Center for Population Research, and the Office of the Director, NICHD.

Several staffing changes have occurred during the past year. Mr. Nathaniel White, Jr. left to accept a position with the National Institute of Environmental Health Sciences. Ms. Marcia Withiam joined in October, 1980, to provide the day to day coordination of the Diabetes in Early Pregnancy Study. Ms. Barbara Scott joined in January, 1981, to coordinate the D. C. Perinatal Project. In addition, Ms. Beverly Trainor joined in November, 1980, to provide clerical support to the Diabetes in Early Pregnancy Study.

Dr. James Schlesselman resigned his position as Chief of the Biometry Branch to accept a position with the Uniformed Services of the University of Health Sciences in Bethesda, Maryland. Mr. Howard Hoffman has been selected to take over the responsibility as Chief of the Biometry Branch. The freeze of outside employment imposed by the current administration has delayed filling the position of the Chief for the Epidemiology Branch.

Several research projects are in progress. A case-control study of factors associated with the birth of low birth weight children in the District of Columbia (also called the D. C. Perinatal Study) has progressed to the pilot phase at Howard University to pretest and evaluate the protocol for this project. The project has been approved by the various hospitals which are to participate in this project after the pre-test is completed.

The Diabetes in Early Pregnancy Study is progressing as planned in collaboration with several university hospitals. The EPA has expressed an interest in providing at least partial support for the study to enlarge the group of control women. EPA is interested in getting a more precise measure of the size of early fetal loss in a normal population.

Ongoing projects using existing data systems include an analysis of the effects of spermicidal agents around conception on the risk of congenital malformation among the offspring, patterns of umbilical cord growth, the effect of maternal and fetal pathologic conditions on umbilical cord length and the risk of low birth weight among young teenage mothers.

Dr. Berendes, upon the request of the Director of NICHD, chaired a ad hoc review panel to evaluate the current status of the Sudden Infant Death Collaborative Study conducted by the NICHD and advise the Director on its future course. Evaluation was completed in June, 1981, with a Summary Report and recommendation forwarded

to the Director at that time. Dr. Berendes served on the Steering Committee of the NICHD to develop a five-year plan for research. He also serves on the NIH Clinical Trials Committee.

Dr. Berendes also assumed responsibility for the development of a congressionally mandated study of the long-term effects of Neo-Mull Soy of children who experienced alkalosis while consuming this product in 1979. He serves on the Steering Committee of the Health Study of American Men, a project on the long-term effects of vasectomy coordinated by the University of California in Los Angeles. He also continues to serve as a consultant to the Food and Drug Administration's Advisory Committee on Fertility and Maternal Health Drugs and was recently appointed a consultant to the Environmental Protection Agency's Program on Toxic Effects of the Health of Children. He continues to serve on the Advisory Committee of the WHO Special Programme for Research in Reproduction.

Together with Dr. Forman, he visited Ben Gurion University in Beer Sheva, Israel in November, 1980, to set up a collaborative study of "The Effects of Exposure to Westernization on Infant Feeding Patterns Among the Negav Bedouins." He visited China from June 27 through July 21, 1981, as a member of the Health Services Research Task Force to participate in a workshop at the Shanghai First Medical College. He specifically reviewed data from Shanghai County and from hospitals regarding the rate of abnormal pregnancy outcome, particularly low birth weight, perinatal mortality, as well as maternal complications during pregnancy and also data on family planning and contraceptive use in Shanghai County. In discussion with Chinese colleagues from the Shanghai First Medical College, School of Public Health, he outlined a study of pregnancy outcome to be conducted in Shanghai County and also discussed this project subsequently with the Ministry of Public Health in Beijing. A study of pregnancy outcome was one of several recommendations of the Health Services Research Group and was forwarded to the Ministry for consideration.

Dr. Berendes presented a paper on "Health Risks Associated with the Use of Oral Contraceptives" to the Maryland Medical Society in April, 1981, and talked on the "Epidemiology of Prematurity" at Shanghai First Medical College on July 3, 1981.

Dr. James Mills presented a paper at the Society for Epidemiologic Research in Snowbird, Utah, June, 1981, entitled "Is Coitus Late in Pregnancy Dangerous?" He lectured at the Johns Hopkins School of Public Health, Department of Epidemiology in Baltimore in April, 1981, about "Design Issues in the Study of Malformations in Infants of Diabetic Mothers.

Computer Sciences Section

The Section on Systems Design and Data Processing continued its mission of developing and implementing systems for handling the many studies in the Epidemiology and Biometry Research Program area. System staff members continue to provide technical consultation in the discussion of and to collaborate in the design of studies, questionnaire development, data organization, data collection, and to do preliminary programming to answer initial questions arising from study development.

Support in the form of data base maintenance, tabulations and graphing is being provided for the following ongoing studies:

1. A clinical trial that makes use of phototherapy in the treatment of neonatal hyperbilirubinemia. A data base of over 18,000 records to date have

been created;

2. A study of breast feeding in relation to the child's health was performed utilizing data obtained from a Pima Indian Population;

3. A study of high infant mortality in a nearby metropolitan area designed to determine the relationship of environmental factors to the cause of death for infants who died in the first year of life;

4. Studies using information from linked birth and death certificates for years 1967 through 1978 for several states in the U.S.;

5. A study to determine obesity in early childhood;

6. A study of the tendency to repeat birth weight and gestational age in successive births; and

7. An investigation of crown-heel length in physical growth.

The past year has been especially productive for the Section on Systems Design and Data Processing. A major commitment of systems staff time and effort in technical consultation in the development of questionnaires, data collection, data organization as well as pilot study analysis is being provided for the following new studies:

1. Infant mortality in the District of Columbia;

2. Safety and efficacy of cysteamine in the treatment of nephorpathic cystinosis;

3. The effects of exposure to westernization on infant feeding patterns among the Negav Bedouins;

4. A prospective study of the frequency and duration of infant feeding practices;

5. Diabetes in Early Pregnancy; and

6. Fetal growth study.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Office of the Director
Epidemiology and Biometry Research Program

Contract and Collaborative Research

Project Title : Malformations and Fetal Losses in Pregnancy in Women With Juvenile Onset Diabetes Mellitus

Contractors : The Boston Hospital for Women, Cornell University Medical Center, The University of Pittsburgh, Northwestern University Medical Center, and The University of Washington

Money Allocated:

Boston Hospital for Women	$138,666
Northwestern University	156,778
University of Pittsburgh	119,807
Cornell University	123,573
University of Washington	207,135

Objectives: Performing a prospective collaborative study

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;

2. To compare early fetal loss rates in women with diabetes and control subjects.

Significance to Biomedical Research and the Program of the Institute: The incidence of malformations in offspring of juvenile diabetic women is believed to be at least twice that of normal women. The early fetal loss rate in diabetic women has not been determined. This study will search for a teratogenic mechanism in diabetes and attempt to determine whether good diabetic control during organogenesis can prevent these excess malformations. In addition, it will be determined whether or not diabetic women have increased losses early in pregnancy.

Proposed Course: The study is now in the field. NICHD will continue to direct the conduct of the study. Specific objectives include: stimulating recruiting efforts, maintaining strict quality control and accurate data reporting and preparing for the analysis phase of the study. The Steering Committee and Advisory Committee will meet jointly in October, 1981, to assess progress, deal with problems and to plan analysis and reporting of the data.

Project Officer: James L. Mills, M.D., M.S.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Office of the Director
Epidemiology and Biometry Research Program

Publications

Mills, J., Harlap, S., Harley, E.: Should coitus late in pregnancy be discouraged? Lancet 1:136, 1981

Mills, J., Stolley, P., Davies, J., and Moshang, T.: Premature thelarche: Natural history and etiologic investigation. Amer. J. of Diseases of Children. In press.

Stanley, C., Mills, J., and Baker, L.: Intragastric feeding in Type I Glycogen Storage Disease: Factors affecting the control of lactic acidemia. Pediatric Research. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00314-2 EBRP

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

HLA Types and Malformations in Infants of Diabetic Mothers Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Marvin Cornblath	Spec. Assistant to Sceintific Director	IRP	NICHD
Heinz W. Berendes	Director	EBRP	NICHD
Joanna Koppe	Neonatologist, University of Amsterdam		

COOPERATING UNITS (if any)

University of Amsterdam, The Netherlands

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Diabetes Mellitus is known to be associated with certain HLA types. It is also known to cause malformations in infants of diabetic mothers. This study will examine the relationship between HLA "markers" for diabetes and malformations.

The pilot study undertaken last year demonstrated that diabetic women with malformed offspring have an extremely high rate of DR3 and DR4 HLA types. Because of this interesting finding, the major part of the study has started. HLA typing is now being performed on diabetic women with normal offspring to determine whether the HLA markers are equally common in that group. If they are not, it will indicate that HLA type may be a useful indicator for risk of producing malformed children.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00315-2 EBRP

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Malformations in Infants of Diabetic Mothers in Jerusalem

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Susan Harlap	Hebrew University		
Ernest Harley	Supervisory Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)

Hebrew University, Jerusalem, Israel

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.35	.15

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of the study is to demonstrate that infants of diabetic mothers have an increased risk of congenital anomalies. This study is unique in that it uses control subjects from the same time and place, and it covers almost the entire population of a defined geographic area. Data from the Jerusalem Perinatal Project will be used in a case-control format.

Data is currently being collected on diabetic women and their offspring.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00319-1 EBRP

PERIOD COVERED
October1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Umbilical Cord Growth

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Heinz W. Berendes	Director	EBRP	NICHD
Ernest E. Harley	Supervisory Computer Specialist	EBRP	NICHD
Adrian Moessinger	Asst. Prof. of Pathology College of Physicians & Surgeons		
William Blanc	Professor of Pathology College of Physicians & Surgeons		

COOPERATING UNITS (if any)

College of Physicians and Surgeons, Columbia University

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Prenatal growth of the human umbilical cord is being assessed using data from the Collaborative Perinatal Project. Standards for normal growth are being derived from these data.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00320-1 EBRP

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Factors Influencing Umbilical Cord Length

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Heinz W. Berendes	Director	EBRP	NICHD
Ernest E. Harley	Supervisory Computer Specialist	EBRP	NICHD
Adrian Moessinger	Asst. Prof. of Pathology College of Physicians & Surgeons		
William Blanc	Professor of Pathology College of Physicians & Surgeons		

COOPERATING UNITS (if any)

College of Physicians and Surgeons, Columbia University

LAB/BRANCH

EBRP

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Factors which increase fetal movement or change amniotic fluid volume are believed to influence umbilical cord lengths. This study will use data from the Collaborative Perinatal Project to relate various pathological processes to umbilical cord length.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00321-1 EBRP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Teratogenic Effects of Spermicides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow	EBRP	NICHD
Heinz W. Berendes	Director	EBRP	NICHD
Erenst E. Harley	Supervisory Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data from the Walnut Creek Contraceptive Study is being examined to determine whether or not spermicide use is associated with congenital malformations. Malformation rates in women exposed to spermicides either before or after conception are being compared with rates in users of other (or no) contraceptives.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00322-1 EBRP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Retinopathy and Tight Diabetic Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

James L. Mills	Staff Fellow EBRP NICHD
Lois Jovanovic	Assistant Professor, Cornell University
Andrew Drexeler	Post-Doctoral Fellow, Rockefeller University
Charles Petersen	Associate Professor, Rockefeller University
Stanley Chang	Assistant Professor, Cornell University

COOPERATING UNITS (if any)

Cornell University, Rockefeller University

LAB/BRANCH
EBRP

SECTION

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.0	10.00	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

NICHD (Dr. Mills) will provide epidemiologic expertise in this proposed study of the effect of tight control of diabetes on the progression of retinal disease. If funded, the cooperative study will prospectively evaluate the effect of tight vs. routine control of diabetes on background retinopathy. Cornell University, Rockefeller University and NICHD will collaborate.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00323-1 EBRP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

D. C. Perinatal Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	Heinz W. Berendes	Director	EBRP	NICHD
	Barbara Scott	Research Nurse	EBRP	NICHD
	Ernest Harley	Supervisory Computer Specialist	EBRP	NICHD
	James L. Mills	Staff Fellow	EBRP	NICHD
	Nathaniel B. White	Statistician	BB	NIEHS
	George Nolan	Prof. OB/GYN Howard University		

COOPERATING UNITS (if any)

Howard University Hospital

LAB/BRANCH
EBRP, Office of the Director

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	.8	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Case-control study of factors associated with the birth of low birth weight children delivered in Washington, D.C. Pilot study to pretest protocol initiated August 1, 1981, at Howard University.

PHS-6040
(Rev. 2-81)

NICHD Annual Report

October 1, 1980 through September 30, 1981

Biometry Branch

The Biometry Branch continued activities 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 and biostatistics; and (3) support of clinical trials initiated by the NICHD. While the Branch maintained its traditional strong ties to both the Intramural and Extramural programs of the Institute, this year international activities were also important.

In May of 1981, Prof. Leiv Bakketeig, M.D., Department of Community Medicine, University of Trondheim, Norway, came as a guest research scientist for two weeks to work in collaboration with H. Hoffman. During his stay two seminars were given to the staff of the Epidemiology and Biometry Research Program. The first seminar was a report on Dr. Bakketeig's trip in March, 1981, to China as one of three Expert Consultants to the World Health Organization to evaluate needs in the perinatal field for a United Nations sponsored project. The second seminar described an investigation of the clinical management of all perinatal deaths in five Norwegian counties during a one year period, 1979-1980. Dr. Bakketeig is the Principal Investigator on this Cooperative Study which came about as a result of some earlier studies commissioned by the Norwegian Board of Health into the maternity services provided to Norwegian mothers throughout the country.

While working in Bethesda, Dr. Bakketeig and H. Hoffman also reviewed progress of the NICHD contract to the University of Trondheim on the "Outcome of Successive Pregnancies for Norwegian Women, 1967-1976." The final report from this contract will be presented for publication as a Supplement to the Acta Obstetricia et Gynecologia Scandinavica. In addition to this final report, five publications have already resulted from this contract, and three more manuscripts are in press or in preparation.

In August of 1981, H. Hoffman spent two weeks in Norway and one week in Scotland. In Edinburgh, Scotland, he attended the IX Scientific Meeting of the International Epidemiological Association, and presented papers on international comparisons of perinatal mortality as well as on the epidemiology of small-for-date births in successive pregnancy outcomes. In Norway, H. Hoffman spent one week as a Visiting Research Scientist in the Department of Community Medicine, University of Trondheim, working in collaboration with Dr. Bakketeig, Chairman, Department of Community Medicine, and Associate Dean of the Medical Faculty. In the second week, H. Hoffman met with Anders Ericson, Director of the Swedish Medical Birth Registry in Stockholm and with Professor Tor Bjerkedal, Chairman, Department of Hygiene, University of Oslo, and also Director of the Norwegian Medical Birth Registry. In Bergen, H. Hoffman met with the staff of the Norwegian Medical Birth Registry and also with Professor Per Bergsjo, Chairman, Department of Obstetrics and Gynecology, University of Bergen. Scientific discussions were held with these academic and governmental officials on research studies in perinatal health.

Several joint research endeavors have occurred within the Epidemiology and Biometry Research Program. The Diabetes in Early Pregnancy Study, initiated by J. Mills of the Epidemiology Branch, requires the continuous involvement of D. Bryla, as study coordinator, and G. F. Reed, as statistician. This is a prospective attempt to compare incidence of fetal loss and fetal malformation between diabetic and non-diabetic women, as well as to determine if diabetic control is a factor in fetal loss or malformation rates. Patients began to enter the study in January, 1981, and there are no findings yet to report.

H. Hoffman has given advice on the analysis of a study of trends in breast feeding among Pima and Papago Indians over a 30 year period, conducted by M. Forman. Also, H. Hoffman has worked with M. Forman on two other studies: (1) decision to breast feed by middleclass women, and (2) cultural changes and breast feeding in nomadic groups. H. Hoffman has also provided statistical advice to R. Nugent and C. Keller regarding the Baltimore City Study of infant mortality.

D. Bryla has been involved in the analysis of teenage pregnancies from the National Collaborative Perinatal Study with H. Berendes.

A major committment of staff time and effort continued to support follow-up and analysis of a randomized clinical trial of the safety and efficacy of phototherapy in comparison with exchange transfusion for the treatment of hyperbilirubinemia. D. Bryla has devoted virtually all of her time to the analysis and data management of this project. The field phase, funded by contract with the Intramural Program and administered by the Scientific Director, NICHD, entered the sixth year of follow-up of children enrolled in the study. Since the sixth year examination is comprehensive, including physical, neurological, psychological and audiology data, a major effort has been devoted to the development of coding instructions and computer edits. The analysis of the one year data is continuing.

A second clinical trial supported by the Branch concerns an investigation of the safety and efficacy of cysteamine in the treatment of nephropathic cystinosis. This study is being done in collaboration with Dr. Joseph D. Schulman, Human Biochemical and Developmental Genetics Section, NICHD, and with investigators at medical schools at the University of Southern California and the University of Michigan. The Biometry Branch will provide statistical analysis of the accumulating clinical trial data, with data processing support from E. Harley in EBRP.

Work on a statistical characterization of the natural history of cystinosis also continued. In the past year G. F. Reed has been developing statistical and graphical techniques for appropriately summarizing and presenting the age-specific characteristics of cystinotic patients enrolled in a previous clinical trial. The project is a collaborative effort with Dr. Joseph D. Schulman of the Human Biochemical and Developmental Genectics Sections, NICHD, and publication of the results is expected to be broadly useful in research and clinical applications.

Although the preceding three studies represented a major component of Biometry Branch support for intramural projects at NIH, a number of consultations were made during the past year, a summary of several being as follows.

During the past year Dr. Reuben Steinherz of the Human Biochemical and Developmental Genetics Section, NICHD has engaged the statistical support of G. F. Reed in investigating a hypothesized association between certain HLA types and severity of cystinosis. Publication of results, based on a small number of cystinotic patients who underwent renal transplants, is now being contemplated.

D. Denman provided advice for further analyses and the use of statistical packages on the computer for Dr. Richard Branchflower, Laboratory of Chemical Pharmacology, NHLBI, in regard to data on the partial separation of multiple forms of liver microsomal cytochrome P-450.

H. Hoffman has consulted with F. Comite, Endocrinology and Reproduction Research Branch, NICHD, regarding the analysis of her data on hormonal determinations for children manifesting precocious puberty. Both H. Hoffman and D. Denman have provided statistical advice to M. A. Brock, Clinical Physiology Branch, Gerontology Research Center, N.I.A., regarding time series analysis for data reflecting seasonal patterns in the immune defense system.

Work with NICHD staff in the Extramural Program ranged from participation in ad hoc contract review committees, to site visits, to serving as co-project officer on NICHD contracts. Biometry Branch staff have worked closely with staff of the Center for Research for Mothers and Children (CRMC). H. Hoffman is serving as assistant project officer for the NICHD Cooperative Study of SIDS Risk Factors, funded by a contract with the Clinical Nutrition and Early Development Branch, NICHD. He is a member of the steering committee, has participated in site visits to all of the study centers and has been active in the sub-committees advising on the analysis of data. He is also collaborating in reporting findings from a study of developmental neurophysiology and SIDS, funded by contract with CRMC. G. W. Reed has provided statistical and computer programming assistance in the analysis of the data for this project.

Staff of the Biometry Branch serve as consultants to outside investigators. H. Hoffman has consulted with Dr. Glenn Bartlett, Pediatric Outpatient Services, Hershey Medical School, Pennsylvania, and with Dr. Jose Villar, Department of Obstetrics and Gynecology, Johns Hopkins University, on indices of maturity at birth and the associated rates of perinatal mortality from the National Collaborative Perinatal Study. He has continued work with Dr. Josephine Weatherall, Medical Statistician, Registrar Generals Office, England and Mr. Peter Goldblatt of the Office of Population Censuses and Surveys, England on the analysis of British data on birth weight and gestational age in relation to major congenital malformations. He has continued collaboration with Dr. Don Gibson and Dr. Leonard Jakubczak, National Institute of Aging, on the lifespan characteristics of mice and rats derived from NIA contract data. Together with D. Denman he has consulted with Dr. Thomas Wehr and Dr. Norman Rosenthal, Clinical Psychology Branch, NIMH on time series analysis of long-term measures (temperature, mood ratings, sleep amount, activity, etc.) in patients with manic-depressive illness and the basic rest-activity cycle. He has been called upon to advise Dr. Bryan McCarthy, Center for Disease Control and the Georgia State Health Department, on a longitudinal study of consecutive births to white and black mothers in Georgia 1972-1976. He has also provided advice on the possible association between triple vaccine (DPT) and SIDS to Dr. Charles Manclark, Bureau of Biologics, FDA.

J. Schlesselman has served as a statistical advisor to Dr. Howard Ory, Center for Disease Control, on a case-control study of oral contraceptive use and breast, endometrial and ovarian cancer, funded under contract by the CPR, NICHD. He has presented a seminar, for Dr. Ory's staff, on the application of logistic regression and log linear models to the analysis of case-control data, and has reviewed contract proposals from organizations competing to serve as the data processing center for the study. As the study progresses he will become fully involved in the data analysis.

G. F. Reed represents NICHD on the NIH Working Group on the Census-National Death Index Study which is being proposed by the Epidemiology Branch of NHLBI. It is proposed that a sample of respondents to the 1980 Census be matched annually against new deaths as recorded on the National Death Index, recently installed by the National Center for Health Statistics. The purpose is to gain a prospective view of the antecendents of mortality as provided by the census data. The task of the working group is to assess the feasibility of the study via a pilot study and to determine its usefulness to the participating institutes.

G. F. Reed attended the 16th Annual Epidemiology Summer Session at the University of Minnesota for a three-week course of study in the design, conduct, and analysis of clinical trials and in topics in advanced statistical methods in epidemiology.

Other activities of Biometry Branch staff include serving as referees for papers submitted to the Journal of the American Statistical Association, American Journal of Epidemiology, American Journal of Obstetrics and Gynecology, Biometrics, Blood, and Obstetrics and Gynecology.

Departures from the staff of the Biometry Branch in the past year included Dr. James J. Schlesselman (Branch Chief) and Ms. Erica Brittain (Mathematical Statistician). Ms. Andrea Hines (Clerk/Typist) was recruited during the year and Mr. Daniel Denman (Mathematical Statistician) was converted to a permanent position. Summer workers this year were Ms. Leigh Baker and Mr. George W. Reed.

A summary of publications, talks and research projects follows.

NICHD Annual Report

October 1, 1980 through September 30, 1981

Biometry Branch

Publications:

Bakketeig, L.S., and Hoffman, H.J.: Interpreting Survey Data. In Chalmers, I., McIlwaine, G. (Eds.): Perinatal Audit and Surveillance. London, U.K., Royal College of Obstetricians and Gynaecologists, 1980, pp. 249-262.

Bakketeig, L.S., and Hoffman, H.J.: Epidemiology of preterm birth: Results from a Longitudinal Study of Births in Norway. In Hendricks, C.H., Elder, M.G. (Eds.): Butterworths International Medical Reviews, Obstetrics and Gynaecology, Volume 1 - Preterm Labour. London, U.K., Butterworths, 1981, pp. 17-46.

Brittain, E., Schlesselman, J.J., and Stadel, B.V.: Costs of case-control studies. Biometrics (In press).

Corash, L., Spielberg, S., Bartsocas, C., Boxer, L., Steinberg, R., Sheetz, M., Egan, M., Schlesselman, J., and Schulman, J.D.: Reduced chronic hemolysis during high dose vitamin E administration in Mediterranean type glucose-6-phosphate dehydrogenase deficiency. N. Engl. J. Med. 303:416-420, 1980.

Cox, E.A., and Reed, G.F.: High performance liquid chromatographic determination of intermediates and two reaction by-products in FD&C Red No. 40. Journal of the Association of Official Analytic Chemists. 64:324-331, 1981.

Dixon, G., Schlesselman, J.J., Ory, H.W., and Blye, R.P.: Ethynylestradiol and conjugated estrogens as postcoital contraceptives. J. Am. Med. Assoc. 244:1336-1339, 1980.

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Schlesselman, J.J.: Case-Control Studies: Design, Conduct, Analysis. New York, N.Y., Oxford University Press, 1981. (In press).

Smith, H.J., Newman, J.D., Hoffman, H.J., and Fetterly, K.: Statistical discrimination between vocalizations of individual squirrel monkeys. Folio Primatologia (In press).

Talks:

Bakketeig, L.S., Hoffman, H.J.: International comparison of fetal and infant mortality rates. First Nordic Symposium on Health Services Research. Tampere, Finland, November, 1980.

Bakketeig, L.S., Hoffman, H.J.: The tendency to repeat small-for-gestational age births. International Epidemiological Association, IX Scientific Meeting, Edinburgh, Scotland, August, 1981.

Brittain, E., Schlesselman, J.J., Stadel, B.V.: Costs of case-control studies. Society for Epidemiological Research. Snowbird, Utah, June 15, 1981.

Forman, M.R., Hoffman, H.J., Harley, E.E., Cross, J., Bennett, P.: The PIMA infant feeding study: The role of sociodemographic and attitudinal factors in the trend in breast and bottle feeding. Larry Frank Symposium, Society for Research in Child Development, Boston, Massachusetts, April, 1981.

Hoffman, H.J., Bakketeig, L.S.: International comparisons of perinatal mortality: Adjustments for indices of maturity at birth. International Epidemiological Association, IX Scientific Meeting, Edinburgh, Scotland, August, 1981.

1981 Annual Report
Biometry Branch

Project Numbers	Project Title	Principal Investigator
Z01-HD-00801-06 BB	Studies based on the Medical Birth Registry of Norway, 1967-1973.....	H. Hoffman
Z01-HD-00802-06 BB	Study of Linked Information on Infant Death Certificates and Live Birth Certificates for Selected U.S. States....	H. Hoffman
Z01-HD-00806-04 BB	Case-Control Studies.....	J. Schlesselman
Z01-HD-00811-02 BB	National Collaborative Cysteamine Study.....	G. Reed
Z01-HD-00812-01 BB	Interval Estimation of the Attributable Risk for Multiple Exposure Levels.....	D. Denman
Z01-HD-00850-05 BB	Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia.....	D. Bryla
Z01-HD-00860-01 BB	Analysis of Hormonal Time Series Data.....	H. Hoffman

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00801-06

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies based on the Medical Birth Registry of Norway, 1967-1973

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:			
Howard J. Hoffman	Mathematical Statistician	BB	NICHD
Other Investigators:			
Ernest Harley	Supervisory Computer Spec.	EBRP	NICHD
Karen Fetterly	Computer Specialist	EBRP	NICHD
May Chiu	Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)
Institute of Hygiene and Social Medicine, University of Bergen, Norway
Department of Community Medicine, Univ. of Trondheim, Norway

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway, (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.

PHS-6040
(Rev. 2-81)

Methods Employed: Methods which have been used to analyze these data include life-table techniques, correlation and examination of bivariate distributions (based on contour diagrams), indirect standardization for rates, and other procedures applicable to cross-tabulations of data. The epidemiologic approach is that of a "prospective" population-based design which permits analyses by case-control, cross-sectional or longitudinal (cohort) means.

Major Findings: Several important findings have emerged from these studies. Norwegian 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. Recent studies have explored the relation of perinatal mortality to birth order and size of sibship. 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 gms.) follow the same pattern generally as that of perinatal mortality. The incidence of preterm delivery (less than 36 weeks gestational age) also follows the same pattern. These associations may help account for the decline in perinatal mortality as birth rank increases.

The epidemiology of preterm birth has been the subject of a special inquiry. Many of the previously-known risk factors (such as low maternal age, unwed mother, lower social class status, occurrence of previous low birth 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 clearly not associated with the risk of a preterm birth. The present study was based on longitudinal data in which the first three consecutive single births 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.

A recent report of findings has shown 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 low weight second births ($\leq$ 2,500 gms.) is twice the perinatal mortality rate for low weight births 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 study has revealed the importance of crown-heel length measurements, in addition to birth weight, for predicting adverse perinatal outcomes.

Significance to Biomedical Research and the Program of the Institute: These studies have provided information on several issues pertinent to programs of the Institute. The "repeater" studies have helped to elucidate some of the etiologic problems of prematurity. Studies of fetal and infant mortality, and especially comparisons with available U.S. data sets, will aid understanding of the cause-specific mortality categories in which the U.S. rates may be higher than Scandinavian rates.

Proposed Course: Approximately 17 research publications have resulted from this collaborative effort with Dr. Bakketeig utilizing the data from the Norwegian Medical Birth Registry. Four additional papers are either in manuscript form, or have been submitted for publication. These studies are completing a collaboration which began while Dr. Bakketeig was a Visiting Scientist, EBRP, NICHD.

Publications:

1. Bakketeig, L.S., and Hoffman, H.J.: Interpreting Survey Data. In Chalmers, I., McIlwaine, G. (Eds.): Perinatal Audit and Surveillance. London, U.K., Royal College of Obstetricians and Gynaecologists, 1980, pp. 249-262.

2. Bakketeig, L.S., and Hoffman, H.J.: Epidemiology of preterm birth: Results from a Longitudinal Study of Births in Norway. In Hendricks, C.H., Elder, M.G. (Eds.): Butterworths International Medical Reviews, Obstetrics and Gynaecology, Volume I - Preterm Labour. London, U.K., Butterworths, 1981, pp. 17-46.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00802-06

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Linked Information on Infant Death Certificates and Live Birth Certificates for Selected U.S. States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:			
Howard J. Hoffman	Mathematical Statistician	BB	NICHD
Other Investigators:			
Ernest Harley	Supervisory Computer Spec.	EBRP	NICHD
Karen Fetterly	Computer Specialist	EBRP	NICHD
May Chiu	Computer Specialist	EBRP	NICHD

COOPERATING UNITS (if any)
Departments of Health in the following states: California, Colorado, Illinois, Minnesota, Missouri, New York, (state and city), North Carolina, Rhode Island and Washington

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

The objective is to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g. birthweight, gestational age, maternal age and race, parity, etc.). These studies will be compared with similar studies on a 1950 and 1960 cohort of U.S. births. Additional comparisons will be made to linked data from Canada (1971), Great Britain (1970), Norway (1967-1976), and Sweden (1975-1978).

PHS-6040
(Rev. 2-81)

Methods Employed: Since linked data from birth certificates and infant death certificates is not available from the National Center for Health Statistics, we have dealt directly with individual State Centers for Health Statistics. We have chosen states which have routinely linked this information and are willing to cooperate in this venture. The data is received on magnetic tape from the individual states and merged into a common format. The job of editing these tapes and merging the information into a common file is accomplished by the computer programmers within the Office of the Director, EBRP. When the study data sets have been edited and merged in to a common file, the data will be analyzed using a variety of statistical techniques ranging from simple cross-tabulations to the making of sophisticated contour diagrams.

Major Findings: Perinatal mortality has been analyzed as a function of maternal age for birthweight-specific categories using 500-gram intervals. Maternal age has been stratified by single years through age 19, and by five year age groups thereafter. We have found that among white births, infants born to 19 year old mothers experience higher rates of "early neonatal mortality" (deaths occurring in the first six days of life) compared to births of mothers aged 20-24 years, 25-29 years, 30-34 years for each 500-gram birthweight interval. Similarly, 18, 17, 16 and 15 year old mothers have successively higher rates of early neonatal mortality for each 500-gram birthweight category. Since these mortality rates are birthweight-specific, it is unlikely that the known association of preterm birth with younger maternal age can account for these results. Additional studies comparing fetal and still-birth mortality rates by maternal age groups and birthweight-specific categories are underway.

A recently completed manuscript for publication compared the birth weight-specific perinatal mortality rates for U.S. Blacks, U.S. Whites and Norwegian births. 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 gm.) were affected by the incidence of low-weight births. Thus, Norwegian births have a higher birthweight-specific perinatal mortality rate among low weight births than do the U.S. Blacks. The interpretation of birth weight-specific perinatal mortality rates should therefore be altered to reflect the need for further 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 the crude perinatal mortality rate for the years 1972-73. 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.

Significance to Biomedical Research and the Program of the Institute: This study will provide a unique resource for the statistical and demographic study of infant mortality and some associated conditions (prematurity, teenage pregnancy, illegitimate births, etc.). Also, cause-specific infant mortality can be compared between the states participating in the study. The data will be available for the period 1968 through 1974 and will also permit an examination of time trends during this period. Some additional years of data for selected states are being added to the data set, particularly for the years 1978-1980.

Proposed Course: Analyses of these data have been presented at an invited address for the X Birth Defects Symposium in Albany, New York, and also at a

joint meeting of the Royal Statistical Society (Medical Section) and the British Society for Population Studies in London. An additional presentation has been prepared for IX Scientific Meeting of the International Epidemiologic Association in Edinburgh, Scotland. A manuscript based on these presentations has been submitted for publication and other manuscripts are in preparation.

Publications:

Hoffman, H.J., and Bakketeig, L.S.: Human Fetal Death Rates—Relation to Indices of Maturity at Birth and Maternal Demographic Factors. In Hook, E.B., Porter, I.H. (Eds.): Human Embryonic and Fetal Death. New York, N.Y., Academic Press, 1980, p. 19.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00806-04

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Case-Control Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators:

James Schlesselman	Chief	BB	NICHD
Daniel Denman	Statistician	BB	NICHD

Other Investigators:

Paul D. Stolley	Professor	Univ. Penn Medical School	

COOPERATING UNITS (if any)

University of Pennsylvania Medical School, Department of Research Medicine

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.3	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A book on case-control studies has been written which details the logic of the study design, provide explicit recommendations for its application, and describe a variety of methods for the analysis of case-control data. The book is planned as a reference for researchers and advanced students in medicine, public health, epidemiology, and statistics.

PHS-6040
(Rev. 2-81)

Project Description and Progress to Date: The entire book has been written and edited and submitted to the publisher. Galley proofs are currently being checked. Chapters dealing with the following topics have been written: Advantages and Disadvantages of Experimental and Observational Studies, Approaches to the Assessment of Risk, Planning and Conducting a Case-Control Study, Use of Matching, Sources of Study Bias, Planning the Size of the Study, Basic Statistical Methods of Analysis, Multivariate Methods of Analysis, and Approaches to the Interpretation of Study Findings.

Significance to Biomedical Research and Program of the Institute: Case-control studies are extensively used to determine relatively rare adverse effects of drug or environmental exposure. For example, most of the adverse effects associated with use of oral contraceptives have been investigated using the case-control approach. Other current examples concern the discovery of an association between the development of vaginal cancer in young women with a previous maternal exposure to diethylstilbestrol during pregnancy, and studies of a potential link between bladder cancers and saccharin exposure in humans.

Proposed Course: The completed manuscript was submitted to Oxford University Press by December of 1980, with publication expected in November 1981. The book will appear as the first in a new series in biostatistics and epidemiology, edited by Dr. Abraham Lilienfeld of the John Hopkins University.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00811-02

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

National Collaborative Cysteamine Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

George F. Reed	Statistician	BB	NICHD
Daniel Denman	Statistician	BB	NICHD
Ernest Harley	Computer Spec.	EBRP	NICHD
Maria Keyser	Computer Tech.	EBRP	NICHD
Elva Nelson	Statistical Asst.	EBRP	NICHD
James Schlesselman	Chief	BB	NICHD
Joseph Schulman	Chief	HBDG	NICHD

COOPERATING UNITS (if any)

University of California, San Diego
School of Medicine

University of Michigan
Medical School

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

This study is a clinical trial to determine the safety and efficacy of cysteamine in the treatment of nephropathic cystinosis, a metabolic disease which usually leads to end-stage renal disease before 10 years of age. All children enrolled in the trial will receive cysteamine. Control information is provided by data collected on 30 patients who were randomized to placebo in a previous trial evaluating the efficacy of Vitamin C for the treatment of this disease. Approximately 60 children will eventually be enrolled in the current trial, which is anticipated to last about three years. Evaluation of the drug's effectiveness will be chiefly determined by the creatinine clearance values of the treated children as compared with those of the historical controls.

PHS-6040
(Rev. 2-81)

Objective: Determine the benefits and risks associated with treating cystinotic children with cysteamine.

Method Employed: This study is a clinical trial designed to determine the safety and efficacy of cysteamine in treatment of cystinosis. The trial will compare children treated with cysteamine with data collected previously on untreated children who were enrolled in the Vitamin C study. The Biometry Branch is responsible for key-punching data from examinations of approximately 60 children. Data obtained in follow-up examinations scheduled to occur three times a year will also be keyed. The Biometry Branch is responsible for analysis of the data, with computer support being provided by the EBRP data processing unit.

Major Findings: Tentative analyses based on early follow-ups of 39 cysteamine treated patients suggest a treatment related improvement in renal function among younger patients. Conclusive results, however, must await longer follow-up information on more patients.

Significance to Biomedical Research and the Program of the Institute: Despite symptomatic treatment, children with cystinosis typically develop end-stage renal disease by age 10. It is believed that cysteamine may be effective in arresting the development of the disease, since it has been shown to significantly lower the free cystine content in fibroblasts cultured from cystinotic patients. This study will assess the efficacy and safety of cysteamine.

Proposed Course: To key data into the computer as it becomes available. Updated summaries of the data collected will be provided on a regular basis. Analyses which address emergent issues will be performed at regular intervals at the time sufficient data accumulates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00812-01

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Interval Estimation of the Attributable Risk for Multiple Exposure Levels

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators:

Daniel Denman	Statistician	BB	NICHD
James Schlesselman	Chief	BB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.1	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project was to develop a method for producing confidence intervals for the attributable risk in case-control studies when exposure occurs at multiple levels and to demonstrate that the method has satisfactory properties for practical applications.

PHS-6040
(Rev. 2-81)

Method Employed: Using well known mathematical and probabilistic techniques and approximations, a method for generating confidence intervals was derived on theoretical grounds. Monte Carlo simulations of the method were run on the computer, simulating a variety of situations likely to be found in case-control studies. In the simulations actual performance of the intervals was compared with the performance expected from theory.

Major Findings: The theoretical performance was found to be in good agreement with the actual behavior found in the Monte Carlo study.

Significance to Biomedical Research and the Program of the Institute: This study provided a practical approach to interval estimation in a situation previously unanalysed. This has relevance to case-control studies which seek to ascertain the impact on public health of a suspected risk factor which occurs at several different levels in the population.

Proposed Course: The derivation of the interval and the results of the Monte Carlo study were presented to the Branch in a seminar. A more detailed presentation has been submitted to Biometrics for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00850-05

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Dolores A. Bryla		Statistician	BB	NICHD
Karen L. Fetterly		Computer Specialist	EBRP	NICHD
Leigh Baker	(Summer)	Mathematical Aide	BB	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 MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study 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 will be followed through six years of age.

Objectives: Determine short and long-term benefits and risks of phototherapy for treatment of newborn hyperbilirubinemia.

Methods Employed: 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 serves as a data center for this study, and is the focal point for receipt of examinations 1,339 newborns and approximately 1,000 follow-up examinations each year until the child is six years of age. These forms are checked for accuracy, and precoded in each center. The master files for each year's follow-up are edited for keypunch and coding errors, and for internal consistency. The Branch is also responsible for the analysis of the data.

Major Findings: Since the clinical trial is continuing, final results are not yet available. An intensive analysis of the newborn data is completed and the findings related to the newborn data will be published in a supplement to Pediatrics. The one-year follow-up data, which includes physical, neurological, ophthalmological, audiological and laboratory information, are being analyzed. Coding instructions and edits were developed for the six-year follow-up examination. This examination is gathering socio-economic, physical, neurological, audiological and psychological data.

Significance to Biomedical Research and the Program of the Institute: There is a well documented relation between hyperbilirubinemia during neonatal life and an increased risk of death or injury to the central nervous system from bilirubin encephalopathy, possibly resulting in impaired mental and neuro-muscular abilities and deafness. Phototherapy has been widely accepted as a simple and effective treatment to reduce high bilirubin levels. In 1971, 51% of a sample of 300 U.S. hospitals were using phototherapy as a treatment procedure. In this study, phototherapy will be appraised for its immediate safety and efficacy, and for its possible long-term implications.

Proposed Course: To complete the analysis of the one-year follow-up data, with particular emphasis on the neurological, mental and physical data. To complete the six year follow-up examinations at the participating institutions. As the forms are received at the Branch, to continue coding and editing of the data for future analysis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00860-01

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Analysis of Hormonal Time Series Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:			
Howard J. Hoffman	Mathematical Statistician	BB	NICHD
Other Investigators:			
Daniel Denman	Mathematical Statistician	BB	NICHD
Florence Comite	Clinical Associate	DEB	NICHD
Barry Bercu	Clinical Associate	NPMB	NICHD
Nancy Vieira	Biologist	NPMB	NICHD

COOPERATING UNITS (if any) James B. Brown, Professor, Department of Obstetrics and Gynecology, University of Melbourne, Australia. Griff T. Ross, Deputy Director, Clinical Center, National Institutes of Health. James W. Hansen, Associate Director, Pediatric Nutrition, Mead Johnson Company.

LAB/BRANCH
Biometry Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 radioimmuno assay methods for measuring urinary luteinizing hormone, urinary follicle stimulating hormone, and urinary estradiol, estriol and estrone hormones, (2) To accomplish an analysis of the circadian rhythm of various hormone levels measured hourly in serum from normal control and irradiated monkeys. (3) 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.

PHS-6040
(Rev. 2-81)

Methods Employed: Spectrum analysis is the standard statistical procedure for the analysis of equally-spaced, moderately long series of data points. Several related statistical quantities can be calculated using the Fast Fourier Transform algorithm including: autospectrum, cross-spectrum, coherence and phase spectrum, and even lagged auto- or cross-correlation functions. Additional methods used in the analysis of these time series data are digital filtering (via complex demodulation) and autoregressive modeling or filtering. Among the nonlinear techniques which have recently been developed, the most useful in these applications has been the robust smoothing procedures based on the running median smoother.

Major Findings: The presence of approximate 30-day cycles has been shown in peri-pubertal girls, even prior to menarche. These cycles were investigated in a sample of 11 peripubertal girls based on 60 consecutive days of nightly-voided urine samples. Both LH and FSH were analyzed separately by techniques of statistical time series analysis. Independent occurrence of cyclic peaks in the two hormonal measurements strengthened the evidence for the nearly 30-day cycle.

In addition to the analysis of measurements for 60 consecutive days, one pre-pubertal subject (Tanner Stage I or II) continued to collect urine specimens for an entire year. This longer series of measurements has been analyzed by spectrum analysis. Evidence was found for both a 13-day cycle (follicular growth cycle) as well as an approximate 30-day cycle. The significance of the 13-day cycle, particularly in relation to the timing of the 30-day cycle, is the subject of further analyses.

Significance to Biomedical Research and the Program of the Institute: These data on developmental endocrinology are related to the mission of the NICHD to seek a better understanding of normal growth processes, and of the derangements accompanying various disorders of growth. The statistical aspects of the data analysis have led to publication of new procedures for the analysis of moderate length time series data. In turn, this methodology is now available to other biomedical researchers who have similar problems of data analysis and interpretation.

Proposed Course: Portions of the urine samples from the subject who collected her specimens for one year have been sent to Dr. Brown, University of Melbourne, Australia, to assay estrone, estradiol and estriol. A pilot study, already accomplished, confirmed that Dr. Brown's assay techniques were sufficiently sensitive to measure the low levels of estrogens in the urine of pre-pubertal girls. Discussions of how to accomplish the analysis of the urine specimens (60 consecutive days) from 12 peripubertal boys have been initiated with Dr. Sidbury, Scientific Director, NICHD.

ANNUAL REPORT

October 1, 1980 through September 30, 1981

Epidemiology Branch
EBRP/NICHD

During the past year two changes occurred in the Branch staff. Dr. Keller resigned his position as Acting Branch Chief to take a position with the Biometry and Epidemiology Branch of NIEHS, and Ms. Thomas left for a secretarial position with Dr. Malone in the OD/NIH. Dr. Berendes assumed the position of Acting Branch Chief while recruitment for that position continued. The professional full-time staff of the Branch currently consists of Dr. Forman and Mr. Nugent. Ms. Wetherill was hired in December 1980 as the Branch secretary.

Projects in which members of the Branch have or have had primary responsibility during the past year include:

1. The Seasonality of Infant Mortality From linked Birth-Death Records in North Carolina and Minnesota, 1967-73.

2. Infant Feeding Among The Pima Indians: Time-Trend Factors Associated with Infant Feeding and Its Effect on Child Health.

3. The Epidemiology of Infant Mortality in Baltimore, MD.

4. The Effects of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins.

5. The Geographic Variation of Fetal and Infant Mortality and Low Birth Weight in the United States, 1969-74.

6. The Relationship of Body Density to Other Methods of Assessing Obesity In Eight Year Old Children and the Association of Obesity With Present and Past Nutritional and Activity Factors and Parental Attitudes.

7. Breast and Bottle Feeding in the United States: The 1979-80 National Natality Survey.

8. National Natality Follow-Back Survey and National Fetal Mortality Survey.

9. A Prospective Study of the Frequency and Duration of Infant Feeding Practices.

A brief description of these projects is included in the next section.

Additional areas of concern to the Branch staff include the effects of ascertaining infections during pregnancy on premature delivery. Additional contract requests may be developed in these areas.

Other professional activities of the staff included presentations at the University of Maryland and at national meetings. Staff members have also been involved in several committees of national significance such as the DHHS Nutrition Coordinating Committee on which Dr. Forman participated as an Ad hoc member. Dr. Forman has also been involved as a member of the Review Panel of the Four Country Infant Feeding Study sponsored by the Department of State's Agency for International Development. In accordance with the Nutrition and Endocrinology Branch, CRMC, Dr. Forman planned, organized and co-chaired a Workshop entitled "Determinants of Choice and Duration of Infant Feeding Practices." The main objective of the workshop will be to produce a handbook for research workers who are involved in gathering data that relate to a model of the determinants of the duration of breast and bottle feeding. The handbook will address theoretical aspects of the model as well as methodologic pitfalls that might be encountered in gathering data, e.g., sampling problems, operationalizing variables, etc. The handbook will also contain a useful current bibliography.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00305-04 EB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Geographic Variation of Fetal and Infant Mortality and Low Birth Weight in the United States, 1969-1974

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	: Carl A. Keller	Acting Chief	EB/EBRP/NICHD
Other	: Karen Fetterly	Computer Specialist	CS/EBRP/NICHD
	Nathaniel White	Math Statistician	EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.35	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The assembly of a large number of variables, including pregnancy outcome, has provided data on the marked variability in infant and fetal mortality among different populations in the U.S. The correlation of adverse pregnancy outcome rates with environmental variables will make it possible to assess the relative importance of the various factors associated with these rates in a large population. These data will also provide a basis for the assessment of changes in infant and fetal mortality which may be occurring in some populations.

PHS-6040
(Rev. 2-81)

1. Project Description:

Approximately 80 variables have been assembled for each of the 3,078 counties in the United States and include data from the 1970 census and other sources pertaining to environmental and geographical characteristics of each county. The file also includes births, fetal and infant deaths, mother's age, parity, and race.

Analysis included multivariate statistical procedures and a description of various factors associated with high and low rates of adverse pregnancy outcomes.

The results of these analyses including maps and text material were provided for use by the Council on Environmental Quality in their publication "Chemical Hazards to Human Reproduction" Council on Environmental Quality, January 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00306-04 EB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Seasonality of Infant Mortality From Linked Birth-Death Records in North Carolina and Minnesota, 1967-1973

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Robert P. Nugent	Research Assistant	EB/EBRP/NICHD
Others :	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
	Ernest Harley	Computer Specialist	CS/EBRP/NICHD
	Karen Fetterly	Computer Specialist	CS/EBRP/NICHD
	Maria Keyser	Computer Aide	CS/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.67	.67	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The observed seasonality of neonatal deaths in the U.S. has implications as to the role of environmental agents, e.g., infectious organisms, during pregnancy in influencing pregnancy outcomes. Since seasonality of conceptions may generate seasonality of neonatal death rates, linked birth-death records from North Carolina and Minnesota are being utilized to examine this relationship to that seasonality of death rates can be assessed independently of any seasonal variation in conceptions.

PHS-6040
(Rev. 2-81)

1. Project Description:

The data needed for this project have already been obtained through the LINK program and are part of Branch resources.

The neonatal mortality rate shows an excess of deaths during the first week of life each year in the late spring in the United States. An investigation of the nature of this peak is being made using linked birth-death records so that an account may be made for seasonal variations in conceptions, gestation length, and fetal deaths. Any seasonal variation in infant deaths not due to these factors has implications for variations in infant mortality from environmental causes such as infections.

Analysis is currently nearing completion, and preliminary results were presented in October, 1978, at a national meeting. Of considerable interest is the finding that there is a significant increase in the probability of a preterm birth and of an early neonatal or late fetal death during July, August, and September of each year in both Minnesota and North Carolina. This seasonality is most dramatic for causes of perinatal death related to infections in mother, fetus, or infant. An evaluation of these findings along with evidence in the literature for the past 20 years is highly suggestive that ascending infections during pregnancy may play a significant role in premature delivery and associated perinatal mortality.

Results of the seasonality analysis will be submitted for publication in FY 82.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00310-04 EB/Inter-agency Clinical Investig. No.78-CH-102 w/NIAMDD

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Infant Feeding Among The Pima Indians: Time-Trend Factors Associated With Infant Feeding and Its Effect on Child Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Epidemiologist	EB/EBRP/NICHD
Others :	Ernest Harley	Computer Specialist	CS/EBRP/NICHD
	Peter Bennett	Chief	EFB/NIAMDD
	Robert Beren	Med. Records Abstractor	EB/EBRP/NICHD
	Howard Hoffman	Biostatistician	EBRP/NICHD

COOPERATING UNITS (if any)

NIAMDD

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The decrease in breast feeding in infants in the U.S. over the past 30 years has been reflected in Native American populations in a shorter period of time. This study is intended to document changes in breast feeding among the Pima Indians living on a reservation in Arizona and to compare the health effects of breast and bottle fed infants over the past 20 years. The availability of centralized health records at the Sacaton Hospital of the Indian Health Service and the relative lack of mobility of the population makes this an excellent source for the study of this issue of current national concern.

PHS-6040
(Rev. 2-81)

1. Project Description:

The objectives of this study are to determine the trend in breast and bottle feeding over time and the reasons for this trend among the Pima Indians and to determine whether breast feeding compared to bottle feeding is associated with reduced infections, reduced infant mortality, weight gains, and cholesterol levels in children.

The methods being used to meet these objectives include interviewing a sample of 257 women on the Gila River Reservation in Arizona and reviewing medical records kept in the Indian Health Service Hospital. Both the survey and review and abstraction of the medical records has been completed and analyses are being done at present.

The following results have been submitted for publication:

Between 1949 and 1977, there was a significant decline in the proportion of women who breast fed. This decline was evident among women age 35-44 in 1978, while women age 30-34 experienced an increase in breast feeding, across socioeconomic strata. Between 1949 and 1963, women of 50-100% Pima Indian descent breast fed significantly less than those of other tribal affiliations, however the influence of tribal descent was reduced thereafter. Parity had a positive association with bottle feeding over time, while family size had a noticeable effect on the association between parity and bottle feeding. The highest proportion of bottle feeding occurred among women with small families before 1963 and among women with large families after 1963 across parity.

Further analyses revealed a limited influence of pre- and post-natal care on the trend in and determinants of breast and bottle feeding. Similarly whether a woman read literature on or received free samples of infant formula was not associated with her decision to breast and/or bottle feed her last child.

An indepth examination of the association between maternal age and breast and bottle feeding was undertaken. Attitudinal differences did not explain this association, however attitudinal differences did show up between bottle feeders and all other feeding groups and attitudes did change within each feeding group over time. Whereas mother and infant oriented reasons dominated in the selection of breast or bottle feeding the first child, work had a strong influence on the decision making process for feeding the last child.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00311-02 EB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Breast and Bottle Feeding In The United States: The 1979-80 National Natality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	: Michele R. Forman	Epidemiologist	EB/EBRP/NICHD
Others	: Ernest Harley	Computer Specialist	CS/EBRP/NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Participation in the National Natality Survey of the NCHS will provide data on current breast feeding practices in all segments of the population. Comparison with data from the 1969 Survey will provide data for the assessment of changes in breast and bottle feeding over the last decade.

PHS-6040
(Rev. 2-81)

1. Project Description:

Three questions on the attitudes about and social influences surrounding breast feeding practices have been submitted and are included in the current National Natality Survey. This survey, currently in progress, will follow up 6,000 births at approximately 3 months of age with a mailed questionnaire to a 1 in 500 sample of mothers and will include demographic and some medical information on each birth. Data will be analyzed to assess which factors are associated with breast feeding and to estimate the current status of breast feeding in the United States.

The National Natality Survey is being conducted in 1979-80, rather than 1978-79 as was expected. Data will be sent to NICHD by May 1982. Analyses will begin at that time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00312-02 EB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
National Natality Follow-Back Survey and National Fetal Mortality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Project Officer:	Carl A. Keller	Acting Chief	EB/EBRP/NICHD
P.I.	: Paul Placek		NCHS

COOPERATING UNITS (if any)
National Center for Health Statistics

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.01	.01	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The National Natality Follow-Back Survey is a mailed survey to mothers, physicians and hospitals for a 1 in 500 sample of legitimate births in the U.S. during calendar 1980. The National Fetal Mortality Survey is a similar survey to a 1 in 4 sample of all stillbirths over 20 weeks gestation.

PHS-6040
(Rev. 2-81)

1. Project Description:

The NICHD submitted a number of questions concerning drugs used during pregnancy to be included on the NNFS and the NFMS which is currently in the field. Data collection will be completed during FY 81 and we will receive a copy of the data tape after it has been cleaned and edited. Analysis of these data will begin upon receipt of the tapes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-HD-00318-01 EB

PERIOD COVERED October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Prospective Study of the Frequency and Duration of Infant Feeding Practices

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. :	Michele R. Forman	Epidemiologist	EB/EBRP/NICHD
Others :	Natalie Truran	Field Researcher Nutritionist	EB/EBRP/NICHD
	Ernest Harley	Computer Specialist	CS/EBRP/NICHD
	Howard Hoffman	Math.Statistician	BB/EBRP/NICHD
	Allan Weingold	Professor	George Wash. Univ.
	Judith Gussler	Anthropologist	Ross Labs.

COOPERATING UNITS (if any)

George Washington University Medical Center

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.40	.40

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Statements like "my milk dried up" and "I had too little milk" are frequent reasons why women stop breast feeding before six months. Since breast feeding has been the norm for infant feeding for centuries, it would seem that milk insufficiency may be less of a physiological inability and more of a socio-cultural condition. Thus it is the objective of this study to examine the role of psychosocial as well as physiological factors in the termination of breast feeding.

PHS-6040
(Rev. 2-81)

1. Project Description:

The purposes of this study are to determine the frequency of breast and bottle feeding at several intervals up to six months and to describe the influence of certain factors on the duration of breast and bottle feeding. The conditions and factors which may interfere or promote breast or bottle feeding include: 1) pre- and post-natal medical services; 2) the mother's perception of the supportiveness of her peers, family, and medical environments; 3) the confidence, enthusiasm and attitudes of the mother toward breast feeding; 4) sociodemographic factors such as maternal employment status and mother's educational level; 5) the physiological conditions of the mother and infant which may interfere with her breast feeding; and finally, 6) the initial infant feeding pattern as well as the change(s) in the pattern over time. Eight hundred mothers, 600 of whom are breast feeders and 200 bottle feeders, and their newborns will be followed from hospital discharge through the first 6 months and administered questionnaires at 4 time intervals in order to examine the independent and joint effects of various factors and conditions, mentioned above, on the frequency and duration of breast and bottle feeding.

A pilot study of 150 women is currently under way to pretest the questionnaires at four intervals during the first six months; that is, at hospital discharge, week 2 and at 3 and 6 months, respectively. In addition, analyses will focus on determining the frequency of breast and bottle feeding at each interval and the feasability of a six month follow-up. The study should begin in the spring, 1982.

NICHD ANNUAL REPORT

October 1, 1980 through September 30, 1981

Epidemiology Branch
Contract and Collaborative Research

Contract Title : The Epidemiology of Infant Mortality in Baltimore, MD

Contractor : Johns Hopkins University School of Public Health

Money Allocated : None

Objectives: To elucidate the biological, social, and environmental variables which influence the high rate of infant mortality in the Baltimore metropolitan area.

Major Findings: Analyses of data have only recently begun; therefore, as of this date, there are no major findings to be reported.

Significance of Biomedical Research and Programs of the Institute: An important part of this study will be identification of factors which effect the infant mortality rate in an urban metropolitan area where infant mortality is often 1.5 to 2 times the national average. Identification of those factors may provide the opportunity to intervene on these variables and significantly reduce the infant mortality rate in such high risk populations.

Proposed Course: No additional funding is anticipated. Collaborative analysis, using NIH computing staff and facilities, is expected to continue for two years with interim publication of any significant findings.

Project Officer: Carl A. Keller, D.V.M., Ph.D.

NICHD ANNUAL REPORT

October 1, 1980 through September 30, 1981

Epidemiology Branch
Contract and Collaborative Research

Contract Title : The Relationship of Body Density To Other Methods of Assessing Obesity in Eight Year Old Children and The Association of Obesity With Present and Past Nutritional and Activity Factors and Parental Attitudes

Contractor : University of California, Berkeley School of Public Health

Money Allocated : None

Objectives: The objectives of this study are to develop a valid method for the measurement of body fat in 8 to 9 year old (pre-puberty) children. Practical suggestions for the use of anthropometric measurement of pre-pubescent obesity which can be accomplished with available clinical instruments will be determined. In addition, these measurements will be related to previous eating and activity practices as well as previous anthropometric measurements on the same children to assess the effects of early feeding and activity and the predictive values of early measures on pre-pubescent body fat content.

Major Findings: One of the preliminary findings of immediate interest is that the triceps skinfold thickness is most highly correlated with body fat determinations of all standard anthropometric measures in this young group of children, as is the core with post-pubescent children and adults.

Significance to Biomedical Research and Programs of the Institute: The results of this study should provide data for more accurate clinical measures of childhood obesity and early predictors for these measures.

Proposed Course: All data tapes have been received with documentation and are currently being analyzed. A final report on this subject will be completed during FY 1982.

Project Officer: Carl A. Keller, D.V.M., Ph.D.

NICHD ANNUAL REPORT

October 1, 1980 through September 30, 1981

Epidemiology Branch
Contract and Collaborative Research

Contract Title : The Effect of Exposure to Westernization on Infant Feeding Patterns Among the Negev Bedouins

Contractor : Epidemiology and Health Services Evaluation Unit, Center for Health Sciences, Ben Gurion University on the Negev, Beer' Sheva, Israel

Money Allocated: $290,659.00

Objectives:

1. To document the present trend of infant feeding practices (breast and bottle feeding) among the three groups of Negev Bedouins: The semi-nomadic, the transitional, and the sedentary. This will be achieved by a cross sectional study, interviewing a stratified sample of 900 women who gave birth 5 to 8 months prior to the interview.

2. To describe in this cohort a set of characteristics distinguishing breast from bottle feeders in each of the three groups. To identify factors which influence women to choose to breast feed or bottle feed and determine whether these factors are indicative of exposure to "westernization."

3. To further use the information from this cohort of 900 women to describe whether Bedouin women have changed their infant feeding practices in the last few years, by getting information about older children, born earlier in calendar time to these same women.

4. To prospectively compare whether attitudinal statements of the Bedouin women after delivery, and past behavior (with regard to infant feeding practices of older children), correlate with their behavior 5 to 8 months later. This will be done by consecutive interviews of 1,500 mothers after delivery in the hospital and a second interview 5 to 8 months later.

5. To determine whether breast fed children in the prospective cohort have fewer hospital admissions and shorter average duration of hospital stay in the first 5 to 8 months of life regardless of category and other indices of westernization

Findings: During FY 81, questionnaires, interviewer and coder manuals were developed. Four interviewers fluent in Arabic, Hebrew, and English were trained. A pilot study was undertaken with 150 mothers of 5 to 8 month olds and 50 mothers of newborns interviewed. Data analysis of the pilot study is currently under way and revision of the questionnaire will follow. The study is expected to begin in the Fall 1981.

Significance of Biomedical Research and Programs of the Institute: An important part of this study will be identification of factors which influence the breast and bottle rate in a population undergoing rapid social change, where commonly bottle feeding is on the increase. Identification of those factors may provide the opportunity to intervene on these variables and significantly reduce the bottle feeding rate in such high risk populations.

Proposed Course: No additional funding is anticipated. Collaborative analysis, using NIH computing staff and facilities, is expected to continue for three years with interim publication of any significant findings.

Project Officer: Michele R. Forman, Ph.D.

NICHD ANNUAL REPORT

October 1, 1980 through September 30, 1981

Epidemiology Branch
Publications and Presentations

Publications:

Keller, C.A.: Epidemiological characteristics of pre-term births. In S. Friedman and M. Sigman (Eds.): Pre-term Births and Psychological Development. Academic Press, New York, February, 1981, pp. 3-15.

Presentations:

Forman, M.R.: Pima Infant Feeding Study: The Influence of Socio-Cultural and Prenatal Factors on the Decision to Breast and Bottle Feed. Presented at the Department of Epidemiology, University of Maryland School of Medicine, Baltimore, MD, December 1980.

Forman, M.R.: The Pima Infant Feeding Study: The Role of Sociodemographic and Attitudinal Factors in the Trend in Breast and Bottle Feeding. Presented at the annual meeting of the Society for Research in Child Development, Larry Frank Symposium, Boston, Mass., April, 1981.

Workshops:

Forman, M.R.: Co-chair, NICHD Infant Feeding 3-day Workshop entitled, Determinants of Choice and Duration of Infant Feeding Practices. Chantilly, Virginia, June 9-11, 1981.

ANNUAL REPORT

October 1, 1980 to September 30, 1981

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY, IRP

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

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Z01 HD 00095-11 LDN
Regulation of neuroendocrine metabolism: Transsynaptic mechanisms in the pineal gland

Z01 HD 00096-11 LDN
Regulation of neuroendocrine metabolism: Intracellular mechanisms

Z01 HD 00097-11 LDN
Regulation of neuroendocrine metabolism: Melatonin physiology (rat, hamster, rhesus monkey)

Z01 HD 00700-04 LDN
Cell interactions in synaptogenesis

Z01 HD 00702-01 LDN
Genetic of primate vocal behavior

Z01 HD 00703-01 LDN
Effect of long chain fatty acids on developing neurons in cell culture

Z01 HD 00704-01 LDN
Physiologic effects of tetanus toxin on nerve cells

NICHD ANNUAL REPORT

Laboratory of Developmental Neurobiology

October 1, 1980 to September 30, 1981

This laboratory continues to focus on the development of the nervous system as our major overall concern. The range of studies is broad, including social interactions between primates, but the major experimental interest is at the cell, membrane and molecular level of organization. The activities of the laboratory are divided among five sections.

I. Section on Neurobiology

Several probes of neuronal development have been applied to cell cultures of mammalian cerebral cortex in order to evaluate possible deleterious effects of the anticonvulsants phenytoin, valproate, diazepam and carbamazepine. Differential effects of these agents have been noted, but the data are incomplete for a complete evaluation of their relative toxicities. A striking long-term down regulation of diazepam receptors by diazepam has been demonstrated which may be related to the marked tolerance developed against this drug when it is used for control of seizures.

Detailed statistical models of the transmitter release process in central neuronal cultures can be tested by physiological means. The anatomical unit through which the probalistic release is regulated would appear to be the individual synaptic bouton.

The central α-adrenergic synaptic response has been analyzed in co-cultures of locus coeruleus and spinal cord. A prolonged depolarization produced by a decrease in membrane conductance is the mechanism of the response and a substantial increase in excitability results. Iontophoretic sensitivity to catecholamine is greatly increased in spinal cord cells by the presence of innervating locus coeruleus neurons, so that long term regulation of receptor may result from this cellular interaction.

We have begun experiments on the mechanism of synaptic competition during development. Excitation of neuroblastoma cells reduces the incidence of polyneuronal innervation in neuroblastoma - muscle cocultures. Thus, synaptic competition is increased by activity in this in vitro system.

A sharply defined period of activity dependent neural development has been defined during the 2nd and 3rd week of culture with mouse spinal cord cultures. Mechanisms involved in this interaction between the environment and the neuronal developmental program are in progress.

The effect of the ACh receptor aggregation factor (RAF), a protein isolated from medium conditioned by NG 108-15 cells, is long lasting. In cultured rat embryonic myotubes, the increased number of receptor aggregates

remained stable even 3 days after removal of the RAF. Laminin increased receptor aggregate formation and the effect of laminin also was long lasting. Several fatty acids were also tested for their ability to aggregate AChR. Only one, linoleic acid produced an increase in receptor aggregates. Other fatty acids tested (myristic acid, steric acid, oleic acid and arachnidonic acid) had no siginificant effect on the number of ACh receptor aggregates per myotube.

Unlike laminin however, there was no synergistic effect of linoleic acid when applied together with RAF. Whether the receptor aggregating activity of linoleic acid is due to increased fluidity of the plasma membrane of the myotubes or some other mechanism remains unclear.

Crosslinking substances such as the lectin Concanavalin A or rabbit antiserum directed against the ACh receptor inhibit the formation of receptor aggregates. A positive correlation between the titer of various antisera tested and the degree of inhibition of the RAF effect was observed. The inhibitory effect is also associated with a decrease in the rate of degradation of the ACh receptor. These antisera and the lectin Con A can themselves produce microaggregates of the receptor, which appear in randon distribution on the surface of the myotubes.

Further characterization of RAF has been performed. This protein has been found to bind to and be specifically eluted from wheat germ and Ricinus communis Type II lectin columns. A weak interaction has also been found with heparin immobilized on agarose. When conditioned medium from NG 108-15 cells was incubated with laminin immobilized on sepharose, the activity was extracted from the conditioned medium. An interaction between laminin and RAF can be postulated. However, all attempts to release RAF in active form from the immobilized laminin have thus far been unsuccessful.

II. Section on Functional Neurochemistry

The section continues the study of peptides and proteins which are involved in intracellular communication (i.e. as neurotranmitters, modulators and trophic agents), and on the cellular mechanisms by which these molecules are synthesized, transported and secreted.

The prohormones for vasopressin (propressopysin) and oxytocin (prooxyphysin) and their associated neurophysins have been identified and characterized. Cyanogen bromide cleavage of these prohormones indicates that the oxytocin is on the N-terminal and neurophysin is near the C-terminal in prooxyphysin; and vasopressin is near the N-terminal, neurophysin is in the middle, and the glycopeptide is near the C-terminal in propressophysin.

A method for preparing a highly purified preparation of neurosecretory vesicles from bovine posterior pituitaries has been developed. The pH of the interior of these secretory vesicles was found to be 5.7, using radioactive method methylamine distribution. Further, the vesicles were shown to have an internal negative membrane potential. ATP in the presence of Mg^{2+} caused a large depolarization of these vesicles, and the presence of permeable Cl^-

ions caused them to lyse osmotically. Work now in progress involves the use of these secretory vesicles to study the proteolytic enzymes involved in the conversion of the prohormone and for experiments on the mechanism of secretion.

The study of the biosynthesis of ACTH, α-MSH and endorphin in the toad, mouse and rat intermediate lobe has continued. These peptides have been shown to be synthesized from a common, glycoprotein precursor (Mol. wt. 32,000). Glycosylation of the prohormone is important for stabilizing the molecule from non-specific proteolysis, and in the processing to correct post-translational cleavage products. Recently, a converting activity for the prohormone has been detected in purified secretory granules fractions from rat intermediate and neural lobes, and bovine neural lobes. The activity appears to be due to an acid arginyl protease. The protease is currently being purified.

Studies on the turnover and regulation of release of the ACTH, α-MSH and endorphin peptides from the toad neurointermediate lobe have led to the discovery of two pools of peptides that differ in their peptide content (one pool contains α-MSH and ACTH but no endorphin while the other contains all three peptides), turnover and regulation of release. Work is now in progress to determine the cellular mechanisms underlying the selective synthesis and storage of the different hormones in the different pools.

Finally, studies on the Ca^{++} activated protease in squid giant axon will continue with Dr. I. Tasaki. These studies will be extended to synaptosomal preparations representative of nerve terminals in the squid brain.

III. Section on Intermediary Metabolism

Investigators in the Section are interested in the biochemical and physiological actions of nerve growth factor and of other growth factors on neural tissue. Our studies are designed to elucidate the role of such factors in the development and maintenance of the nervous system. In both normal sympathetic neurons and the PC12 clone of rat pheochromocytoma, nerve growth factor initiates a chain of biochemical events culminating in the induction of neuron-specific enzymes and the outgrowth of neurites. Among these biochemical alterations is an increase in the phosphorylation of a specific nuclear protein. In recent months we have shown that this protein is a member of the "low mobility group" proteins, is bound loosely to DNA, and is present in the nucleoplasm. We also have obtained reasonable evidence that the phosphorylation of this protein, induced by nerve growth factor, enhances its binding to DNA. This latter observation permits the hypothesis that the nerve growth factor-induced phosphorylation alters either the packing or the transcription of portions of the DNA in responsive cells.

We have purified the nuclear protein using salt extraction and a combination of G-75 Sephadex and Phosphocellulose. Although not homogeneous as yet, there is a reasonable chance that we can purify it completely in the near future. This will permit a complete characterization and the preparation of an antibody for use in quantitative studies on its distribution. Most important, the availability of pure material will allow us to initiate studies on the kinase responsible for the phosphorylation. This kinase should be one

step closer to the molecular target for nerve growth factor.

Current evidence suggests that nerve growth factor acts at two sites in the cell. Rapid cellular responses are elicited by nerve growth factor binding to its surface receptor; longer-term responses results from nerve growth factor actions, either direct or indirect, on the nucleus.

We have recently obtained evidence that the nuclear actions of nerve growth factor can be effected by agents other than nerve growth factor itself. Thus, we now know that phosphorylation of the same non-histone nuclear protein, induction of ornithine decarboxylase, and priming of the cells for subsequent neurite outgrowth can be produced by adenosine analogs as well as by nerve growth factor. This data permits the hypothesis that nerve growth factor exerts, as part if its action, some general activation or stimulation of nuclear events and further, that the more specific action of nerve growth factor is at the membrane. Experiments designed to test these hypotheses are in progress.

IV. Section on Neuroendocrinology

The Section on Neuroendocrinology continues to conduct multidisciplinary investigations of the melatonin rhythm generating system. There are four special areas on which current research is focused. First, the purification of pineal N-acetyltransferase has continued with the ultimate goal of using this gene product to understand how neural signals control gene expression. Computer assisted image analysis of two dimensional gels is now used to study neurally stimulated gene expression. Second, neural control of rapid changes in enzyme activity has been studied. Based on studies with N-acetylatransferase, it appears that peptides, acting by virtue of disulfide bonds, can specifically control the activities of enzymes; the mechanism involved is thiol: disulfide exchange. A third effort is direct towards discovering the mechanisms involved in regulation of pineal hydroxyindole-O-methyltransferase. Current results indicate the enzyme can be stimulated by administration of adrenergic agents for prolonged periods. This indicates that this enzyme provides an integrated indication of prior adrenergic stimulation of the pineal gland, while N-acetyltransferase provides a minute-to-minute indication of ongoing stimulation. The fourth area of study is the control of cyclic GMP. Of special interest is the finding that responsiveness to adrenergic agonists decreases if the tissue is not adrenergically stimulated, and increases when the tissue receives the normal daily pattern of stimulation.

V. Section on Brain and Behavior

Plans to move the progams of this section to NIHAC (Poolesville, MD) have progressed to the point of contract award and physical relocation within calender year 1981.

Research accomplishments in this Section for FY 81 may be summarized as follows:

1) The vocal ethology program has continued to focus on the vocal behavior associated with affiliation in naturally formed (imported intact) social units. Findings which identified the Chuck call as the primary vocal signal of this important aspect of social relationships have been extended to other groups and contexts. Specifically, the affiliative bonds found earlier in the main study group have been shown to endure through the reproductive cycle and birth of young, and some clues as to the development of Chuck usage among the infants have emerged. Present studies are investigating in greater detail correlations between Chuck microstructure and social context, with the hope of both clarifying the nature of the exchanged messages and of increasing the depth and focus of neurophysiological studies. During FY 81 one member of the Section (Dr. Harriet Smith) spent five months in the field as an invited member of a team organized by Dr. John Terborgh of Princeton University which visited the Cocha Casu National Park in Peru, an extremely remote wilderness area. The purpose of this field trip was to validate laboratory findings on vocal behavior and sex differences.

2) The neurophysiology program has been active both in continuing to refine the analysis of auditory cortical neurons which can be characterized as feature detectors and in new initiatives. Significant new findings have emerged in the collaboration with Dr. Paul McLean of the NIMH regarding the effects of carefully placed small lesions on spontaneous vocal behavior of squirrel monkeys. Effects both on the quantity and structure of vocalizations have been documented following tegmental lesions. A new series of experiments was initiated aimed at applying advanced techniques for quantifying vocal behavior developed in the Section in prior years to the problem of stimulation evoked vocal behavior. It is hoped that significant new data will be obtained on the relationships between evoked and spontaneous vocalizations.

3) The behavioral genetics program has continued to study the heritability of vocal traits in laboratory-bred hybrid squirrel monkeys from vocally distinct parental types. A significant advance in this work has been development of a statistical procedure for classifying infant vocalizations as to parental type. Applying this procedure to Isolation Peeps from hybrids indicates that one phenotype ("Roman" type) predominates and my be the wild type.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00047-12 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical studies of neuronal and other cell types

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.A. Neale	Physiologist	LDN NICHD	0.5
	B.K. Schrier	Medical Officer	LDN NICHD	0.6
	P.K. Sher		LDN NICHD	0.5
	P.G. Nelson	Chief	LDN NICHD	0.1
OTHER:	S.C. Fitzgerald	Biologist	LDN NICHD	0.4
	K.F. Swaiman	IPA	LDN NICHD	0.1
	K.W. Cheng	Guest Worker	U. of Mannitoba	0.5
	D. Warren	Bio. Lab. Tech.	LDN NICHD	0.5
	L.M. Bowers	Bio. Lab. Tech.	LDN NICHD	0.5
	S. Neal	Photographer, Lab.	LDN NICHD	0.2
	D. Van Putten	Summer Intern	LDN NICHD	0.2

COOPERATING UNITS (if any)

W.H. Habig, DBP BB; D.W. Gallager, AP M; W.H. Oertel LCS M

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	2.3	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this research are the investigation of the biochemical characteristics of developing central nervous system (CNS) cells in dispersed cell cultures, and the use of these cultures and sensitive biochemical tests to assess the toxicity of nervous system active drugs. We have refined several assay methods so that they can be performed in situ in the culture dishes, thus minimizing variablilty and tissue losses, and resulting in more accurate and efficient assays. The use of these various techniques has provided evidence that the anticonvulsants phenytoin and valproic acid cause toxic effects on developing cerebral cortical neurons, whereas diazepam and carbamazepine appear less toxic.

Project Description:

Objectives: Two principal goals are being pursued: 1) to understand the nature and regulation of development in the nervous system, and 2) to assess the potential toxicity resulting from the use of nervous system active drugs in very young children.

Methods employed: The new methods include *in situ* assays for the activities of choline acetyltransferase (CAT), acetylcholinesterase (AChE), glutamic acid decarboxylase (GAD), and for the receptor-mediated binding of benzodiazepines (BDZ). The innovation involves the enzymatic or binding assays performed directly in the culture vessel, without any need to harvest, homogenize and apportion the cells in advance. In all cases, separation of the unreacted substrate and quantification of the product are accomplished by established procedures.

In addition, cortical neurons in cultures are analyzed, by radioautography and immunohistochemistry, for the coexistence of high affinity ^{3}H-γ-amino-butyric acid (GABA) uptake and GAD immunoreactivity within the same neurons.

We are further able to combine assays for ^{3}H-GABA uptake, ^{125}I-tetanus toxin binding and protein content, so that all three are performed on the same culture. High affinity uptake of ^{3}H-β-alanine is used as an indicator of the glial component of these cultures.

Major findings:

Approximately 12-15% of cortical neurons in culture demonstrate high affinity ^{3}H-GABA uptake. Ninety-five percent of neurons that accumulate ^{3}H-GABA also show immunoreactivity for GAD, thus validating the use of high affinity ^{3}H-GABA uptake as a marker for neurons which synthesize and use GABA as a neurotransmitter.

Studies of the characterists of tetanus toxin binding to neuronal cultures have defined those parameters which maximize receptor-mediated neuronal binding, providing the conditions for the use of this binding as an index related to the number of neurons in a given culture.

BDZ binding studies in cortex and spinal cord (SC) cultures reveal: 1) non-neuronal binding sites are disproportionately represented in the cultures as compared to membrane preparations from adult mouse brain or cortex, 2) Scatchard plots of diazepam (DZP) binding data are consistently nonlinear showing at least two binding sites with K_d values of 5.5 and 303 nM, and 3) the course of development of the neuronal and non-neuronal receptors suggest that they are independently regulated in both cortex and SC cultures.

In adult mouse brain membrane preparations, there is evidence for multiple types of diazepam binding sites. Scatchard plots of flunitrazepam (FNZ) binding are precisely linear, and 100% of this binding is blocked by 10^{-7} M clonazepam. In contrast, Scatchard plots of specific DZP binding are non-linear, becoming more horizontal as the concentration of DZP is increased.

The highest affinity DZP binding sites have a K_d of ca. 3.3 nM, are enhanced by incubation with GABA, and are eliminated when the membranes are pre-incubated with FNZ in the presence of UV light. In such FNZ-blocked preparations, the highest affinity sites have a K_d of 7.2-8.5 nM and the Scatchard plot remains non-linear. Ninety percent of the DZP binding in the FNZ-blocked membranes is blocked by 10^{-7} M clonazepam (100% by 3 X 10^{-7} M clonazepam), but a Hill plot of these date give a slope which is consistent with two sites in common to DZP and clonazepam. Thus, assuming that the FNZ site and the highest affinity DZP site are the same, at least two additional DZP binding sites are present in the adult mouse brain.

Studies of the effects of chronic exposure to anticonvulsants on cerebral cortical cells in culture were extended to include diazepam and carbamazepine in addition to phenytoin and valproic acid, at concentrations twice their respective high therapeutic levels. Results of these studies to date indicate that phenytoin and valproic acid cause depressions in all assays, interpreted as a loss of developing neurons. In contrast, cultures treated with diazepam and carbamazepine show no evidence of neuron loss. Whereas diazepam causes a total block and carbamazepine, a partial block, in neuron-specific BDZ binding, this binding 'recovers' to control levels after removal of the drug.

Significance to Biomedical Research and the Program of the Institute:

These studies incidate that 1) neuronal cell cultures are effective systems for evaluating certain aspects of relative drug toxicity, and that 2) developing neurons are adversely affected by phenobarbital, phenytoin, and valproic acid, compounds which are among neuroactive drugs administered to pregnant women, neonates and children.

Proposed Course:

The anticonvulsant toxicity research will be extended to include: 1) dose-response studies, 2) studies to define those periods of development when neurons are most and least sensitive, and 3) 'recovery' studies to determine the reversibility of toxic effects.

Publications:

Bergey, G.K., Swaiman, K.F., Fitzgerald, S.C., Schrier, B.K., and Nelson, P.G. (198) Adverse effects of phenobarbital on morphological and biochemical development of cultured fetal mouse spinal cord neurons. Ann. Neurol. 9, 548-589, 1981.

Schrier, B.K.: Nervous system cultures as toxicologic test systems. In Mitchell, C.L. (Ed.): Nervous System Toxicology, Raven Press, New York, in press.

Gallager, D.W., Mallorga, P., Swaiman, K.F., Neale, E.A., and Nelson, P.G. 1981. Effects of phenytoin on [^{3}H]-diazepam binding in dissociated primary cortical cell cultures. Brain Research 219, in press.

Schrier, B.K., Neale, E.A., Sher, P.K., Swaiman, K.F., Brenneman, D.B., Cheng, K. -W., Habig, W.H., Oertel, W.H., and Nelson, P.G.: GABA uptake and other cell markers distinguish cell types in drug toxicity studies with dispersed cultures of developing CNS cells. Excerpta Medica, in press.

Sher, P.K., Neale, E.A., and Nelson, P.G.: The effects of anticonvulsants on fetal mouse cerebral cortex in culture. Ann. Neurol. In press.

Study, R.E.: Phenytoin inhibition of cyclic guanosine 3':5'-monophosphate (cGMP) accumulation in neuroblastoma cells by calcium channel blockage. J. Pharm. & Exp. Therap. 215: 575-581, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00048-07 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of transcriptional level control of neurobiologic and developmental phenomena.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.K. Schrier	Medical Officer	LDN NICHD
OTHER:	K.W. Cheng	Guest Worker	Univ. Manitoba,-Can.
	C.H. Letendre		NHLBI
	P.G. Nelson	Chief, LDN, NICHD	LDN, NICHD
	D. Warren	Biological Lab. Tech.	LDN, NICHD
	D. Van Putten	Biological Aide	LDN, NICHD

COOPERATING UNITS (if any)
P. Ross, Department of Biology, University of Chicago

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Molecular hybridizations of polysomal poly A-containing messenger RNAs of undifferentiated (S) and differentiated (P) mouse neuroblastoma cells with their complementary DNAs (cDNAs) had shown that there were many messenger sequences which were unique to each differentiation step. This year has been devoted to preparations for molecular cloning of cDNAs made from messenger RNAs of S and P cells, using recombinant DNA techniques. Differentiation-step-specific clones will be identified by the use of ^{32}P-labelled unique cDNA sequences purified by recycling hybridizations.

Project Description:

Objectives: The immediate objective is to clone the cDNAs which are complementary to the differentiation-step-specific sequences. We plan to use the cloned sequences (1) in a variety of ways to monitor nervous system development and (2) to learn about some of the regulatory steps which control nervous system development. A long-term goal is to understand diseases of development in the nervous system and be able to prevent and/or treat them.

Methods Employed: The methods for RNA and cDNA preparation, hybridization, and recycling of cDNA probes have been described in previous reports. An analysis of most of the factors involved in making cDNAs double-stranded with E. Coli DNA polymerase I allowed us to develop a protocol for accomplishing this step. Conditions were worked out for tailing with deoxyguanosine using terminal deoxynucleotidyl transferase such that the tailing reaction can be used to measure the number of free 3' ends in a double-stranded cDNA preparation. The time course of addition of 3'-poly (dC) tails has also been worked out. The reaction conditions which are best suited to complete cutting of plasmid pBR322 with the restriction endonuclease Pst I are now being determined. In order to obtain larger amounts of messenger RNAs we devised a method to grow as much as 20 liters of suspension culture at once and to convert that number of S cells into P cells without exchanging culture vessels. Dr Schrier attended a 3-day wet workshop in DNA sequencing by both the Sanger dideoxy and the Maxam-Gilbert techniques.

Major Findings:

We found that the rate and extent of double-stranding with DNA polymerase I varied from one cDNA to the next; with some templates, reaction for 30 hrs was necessary to reach a point at which no further nucleotides were added. Since we also found that after 3 hours of incubation both the dNTPs and the enzyme were inactivated, our protocol requires testing (with S_1 nuclease) the progress of the reaction every 3 hours, with the simultaneous addition of fresh enzyme and deoxy-nucleoside triphosphates.

In the tailing reaction with terminal deoxynucleotidyl transferase, the reaction conditions are such that 14 pmol of dG residues are added to each pmol of 3' ends and then the enzyme stops. The same enzyme, in much more rapid fashion, will add dC residues to free 3' ends until it runs out of substrate. Hence, we can use the dG tailing reaction to determine the number of pmol of 3' ends in a small portion of a double-stranded cDNA preparation. A pilot dC tailing reaction time course is then used to determine the best incubation time for adding 15-50 dC residues.

At present we have 500mg of P-cDNA which is double-stranded and has about 45-50 dC residues per 3' terminus and a smaller quantity of S-cDNA with about 100dC residues per terminus. Both of these are ready to use to form recombinant plasmids. The plasmid is being cut with Pst I and will be tailed with dG. We will start with some P-cDNA which is small in size, but relatively abundant, and make our first clones with that material.

In vitro translation of S-cell and P-cell mRNAs, followed by 2-dimensional gel electrophoresis of the translation products, has revealed at least one P-cell specific polypeptide of about M_{12} = 100,000.

Significance to Biomedical Research and the Program of the Institute

We are working toward an understanding of transcriptional controls which are operative during differentiation and development in the nervous system. We hope to map out critical developmental requirements and essential gene products for differentiation in the mammalian nervous system. We believe that results of such studies will contribute significantly to an understanding of normal and abnormal CNS development. In addition, the S-cell specific sequences may aid us in understanding the control of mitotic activity in tumor cells.

Proposed Course

We will continue to work toward completion of the cloning and identification of S-cell and P-cell specific cDNAs. The clones recovered will be used in the following studies: (1) They will be used as probes to identify clones of their genes in the mouse gene library in phage. (2) The genes thus recovered, and restriction fragments of those genes, will be cloned in phage M13 mp7 and sequenced for the purpose of comparing regulatory sequences. (3) In situ hybridization (to mRNAs) to determine when and where they are expressed in nervous system development. (4) Attempts to identify the proteins coded for by these genes using hybrid arrest of translation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00053-13 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Information processing in the central auditory system of mammals and birds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Symmes	Chief, Section on Brain and Behavior	LDN, NICHD
	J.D. Newman	Physiologist	LDN, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Activity in this project during FY 81 has consisted entirely of statistical analysis of data obtained previously, and preparation of a review article based on that analysis and literature search (Dr. Symmes). No new electrophysiological results have been obtained, in part due to space renovation and in part due to greater emphasis on collecting and analyzing data related to other projects within the Section.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: This project is concerned with utilizing neurophysiological and neuroanatomical techniques to study processing of complex sounds in the primate central auditory system. Special attention is being paid to sounds used in intra-specific communication, to determine which acoustic features are used by the brain to encode this input and to classify vocalizations.

Methods Employed and Major Findings: Project inactive during FY 81.

Publications:

1. Mueller-Preuss, P., Newman, J.D. and Juergens, U.: Anatomical and physiological evidence for a relationship between the "cingular" vocalization area and the auditory cortex in the squirrel monkey. Brain Res. 202: 307-315, 1980.

2. Symmes, D.: On the use of natural stimuli in neurophysiological studies of audition. Hearing Research 4: 203-214, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00054-07 LDN

PERIOD COVERED
October 1, 1980 to Septmember 30, 1981

TITLE OF PROJECT (80 characters or less)

Structural and behavioral analysis of vocal communication in squirrel monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN, NICHD
	D. Symmes	Chief, Section on Brain and Behavior	LDN, NICHD
	H.J. Smith	Guest worker	LDN, NICHD
OTHER:	Deborah Bernhards	Bio Lab Technician	LDN, NICHD
	Janet Goodwine	Bio Aide	LDN, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project in concerned with 1) obtaining a detailed description of vocal behavior of squirrel monkeys, including the frequency of occurrence of defined microstructural features in a variety of behavioral contexts; 2) describing the developmental course of vocal behavior by serial recording during growth of laboratory-reared infants; 3) comparing the vocal behavior of captive monkeys of known and unknown social histories with vocal behavior in the natural state (via field studies in the Peruvian Amazon). Primary emphasis in FY 81 has been on studying the effect of the reproductive cycle on affiliative vocalizations in captive social groups. Two call types (one used by females, one by males) have been correlated with the estrous period, representing a significant new finding in the area of functional parcellation of the vocal repertoire of this species. Data collected in a 4-month field trip (Dr. Smith) support findings obtained in a captive group and reported last year.

Methods Employed and Major Findings: A longitudinal study of affiliative vocalizations in captive squirrel monkeys continued throughout FY 1981, with the primary purpose of observing affiliative behavior over a variety of seasonal changes in reproductive activity. It was discovered that the stable affiliative relationships between adult females observed during anestrous periods (documented in last year's report) persist through the time of these same females' estrous period. Affiliative relationships involving males, however, were not stable. Three vocalizations not occurring during anestrous periods were recording during estrous. These were the err and err chuck (both uttered by reproductively active females) and the oink, uttered only by the reproductively active male.

The birth of 2 male infants in this group later in the year provided an oppor tunity to record the vocal concomitants of adult-infant interactions, and to study the development of the infants' behavior within the context of normal interactions with their mothers and other members of the group. Data collected immediately after the birth of the infants showed a significant increase in both chucks and err chucks by all adult females in the group. Instances of infant-carrying and nursing were observed in the non-reproductively active females, each of whom adopted the role of "aunt" to the infant born to their preferred female affiliative partner. Infant chucks occurred primarily in the contexts of searching for the nipple and, when independently mobile, upon returning to their mother following a disturbance in the group.

Analysis by Dr. Smith of data collected by her in the field in Peru is still underway. It is apparent, however, that many of the same social interactions observed by her in our captive group are also readily observable in wild squirrel monkey troops. In particular, these include affiliative groupings consisting only of females, and females being the primary source of chuck vocalizations.

Significance to Biomedical Research and the Program of the Institute: Squirrel monkeys possess one of the most specialized and discrete systems of vocal communication in infra-human primates. Analysis of the semantic aspects of this system would provide valuable insight into the functions of animal languages in general and an excellent model for the study of language disorders in Man. Knowledge of the development of squirrel monkey vocalizations and role of social experience in their incorporation into the adult repertoire can contribute to an understanding of the normal and abnormal development of human language.

Proposed Course: To continue studies of social behavior and vocalizations in captive and free-ranging squirrel monkeys. Transfer of the Section to the NIHAC will provide further opportunities to study captive groups of monkeys under seminatural conditions.

Publications:

Smith, H.J., Newman, J.D., and Symmes, D: Vocal concomitants of affiliative behaviour in squirrel monkeys (Saimiri sciureus). In Brown, C.H., Petersen, M.R. and Snowden, C.T. (Eds.): Primate Communication. New York, Cambridge University Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00056-06 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of ACTH, Endorphin, and MSH synthesis and secretion.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y.P. Loh	Research Chemist	LDN	NICHD
OTHER:	H. Gainer	Chief, Sec. on Functional Neurochemistry	LDN	NICHD
	C. Chang	Visiting Fellow	LDN	NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have demonstrated the synthesis of the 32,000 mw common precursor for ACTH-endorphin in intact toad and mouse intermediate lobes, and have studied its conversion to the final peptide products in vivo and in vitro. These studies have revealed that glycosylation of the prohormone plays a regulatory role in its conversion, and that the toad lobe has two independently regulated and distinct pools of hormones and precursors. A converting-enzyme for the prohormone has been detected in secretory granules and characterized.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To study the regulation of biosynthesis, turnover and secretion of ACTH, αMSH and endorphin peptides in the neurointermediate lobe of the toad (Xenopus laevis) and mouse.

Methods Employed: Acid-urea gel electrophoresis, high performance liquid chromatography (HPLC), radioimmunoassays (RIA) for ACTH, αMSH and βendorphin.

Major Findings: αMSH, ACTH, and βendorphin are synthesized in the toad neurointermediate lobe from a glycosylated prohormone. Pulse-chase studies on the intact lobe using the inhibitor of glycosylation, tunicamycin, and in vitro studies on the cleavage of glycosylated and nonglycosylated forms of the prohormone by trypsin, revealed that the carbohydrate is important for the processing of the prohormone to correct products. Similar results have also been observed for the prohormone in the mouse neurointermediate lobe. Long-term (10-16h) pulse-chase studies on the toad neurointermediate lobe, inhibited from release by dopamine, revealed the existence of two pools of ACTH, αMSH and endorphin related peptides. One pool consisting of ACTH, αMSH and βendorphin, was rapidly degraded, while the other pool consisting of ACTH, αMSH and βLPH (but no βendorphin) was stored when the lobe was inhibited from release. Both pools were inhibited from release by dopamine, but the storage pool was also inhibited from release by L-isoproterenol.

Converting-activity for the above prohormones has been detected in purified secretory granule fractions from rat intermediate and neural lobes, and bovine neural lobes. The activity appears to be due to an acidic, thiol, arginyl protease.

Significance to Biomedical Research and the Program of the Institute:

Peptides such as αMSH and βendorphin have been implicated in higher brain functions (e.g. avoidance learning and analgesia, respectively). While αMSH is clearly important for melanophore control in lower vertebrates, and more recently βendorphin has been found to potentiate this action, the function of these peptides in mammals are less clear. Studies on the biosynthesis and regulation of secretion of these peptides from the pituitary and brain may provide insights into their functional significance. Recently, αMSH has been implicated in fetal development and elevated levels of βendorphin has been detected during labor, suggesting a possible role for this peptide in the relief of the pain during childbirth.

Proposed Course: 1) To further determine the mechanisms (e.g. selective processing of the prohormone or selective degradation of the cleavage products) by which different peptides, presumably synthesized from a similar prohormone, are accumulated in the two hormone pools.

2) To further study and isolate the proteolytic enzymes involved in the processing of the ACTH/αMSH-endorphin prohormone. 3) To examine the roles of the ACTH, αMSH and endorphin peptides in fetal development and neuronal differentiation (using tissue culture models as well).

Publications

1. Loh, Y.P. and Gainer, H.: In vitro evidence that glycosylation of pro-opiocortin and corticotropins influences their proteolysis by trypsin and blood. Molecular and Cellular Endocrinology 20: 35-44, 1980.

2. Ruchel, R., Loh, Y.P. and Gainer, H.: Polyacrylamide gel electrophoresis: Principles, techniques, and micromethods. In Lahue, R. (Ed.): Methods in Neurobiology. New York, Plenum Press, 1980, pp. 245-300.

3. Loh, Y.P.: Processing, turnover and release of corticotropins, endorphins and melanotropin in the frog pituitary intermediate lobe. In Evered, D. and Lawrenson, G. (Eds.): CIBA Symposium No. 81, "The Intermediate Lobe of the Pituitary." Bath, Pitman Press, 1981, pp. 55-78.

4. Loh, Y.P., Jenks, B.G. and Broadwell, R.D.: The role of the carbohydrate in the stabilization, processing and packaging of pro-ACTH/ α MSH-endorphin in frog and mouse pituitary intermediate lobes. In Koch, G. and Richter, D. (Eds.): Biosynthesis, modification and processing of cellular and Viral Polyproteins. New York, Academic Press, 1981, pp. 151-162.

5. O'Donohue, T., Handelmann, G.E., Loh, Y.P., Olton, D.S., Liebowitz, I. and Jacobowitz, D.M.: Comparison of biological and behavioral activities of alpha and gamma melanocyte stimulating hormone. Peptides 2: 101-104, 1981.

6. Loh, Y.P. and Jenks, B.G.: Evidence for two different turnover pools of ACHT, MSH and endorphin related peptides released by the frog pituitary neurointermediate lobe. Endocrinology 109: 54-61, 1981.

7. Gainer, H., Loh, Y.P. and Neale, E.A.: The organization of post-translational precursor processing in peptidergic neurosecretory cells. In Haber B., Blankenship, J. and McAdoo, D. (Eds.): Proteins of the Nervous System: Structure and Function. New York, Alan Liss Inc. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00057-06 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Axonal proteins: Biosynthesis, transport, neuronal cytoskeleton, and secretion.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Gainer Chief, Sec. on Functional Neurochemistry LDN NICHD

Others: J.T. Russell Staff Fellow LDN NICHD
T. Wheler Visiting Fellow LDN NICHD

COOPERATING UNITS (if any)

I. Tasaki and J. Baumgold, Laboratory of Neurophysiology, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our major focus has been on processes occurring in nerve axons and endings (i.e., axonal transport and secretion). These studies have emphasized the role of cytoskeletal proteins in these processes, and have characterized these protein interactions with membranes. Regulation of neurofilaments by calcium-activated proteases, and intra-vesicular pH by membrane-bound ATPases have been demonstrated. Four new techniques have been developed: 1) An HPLC system for analysis of taurine, 2) An iso-osmolar density gradient for secretory vesicle isolation, 3) A covalent-labelling method to study retrograde axonal transport in vivo, and 4) a new histochemical method for thiols and disulfides.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To study the role of axonal cytoskeletal proteins in axonal excitability, transport and secretion.

Methods Employed: Protein biochemical techniques, autoradiography, and intraaxonal and membrane protein labeling techniques, ultracentrifugation, HPLC, and stereotaxic techniques.

Major Findings: The relationship of certain cytoskeletal proteins to the axonal membrane is essential for its excitability. Removal of these proteins from the inner surface of the perfused squid axon membrane reduces its excitability. The intra-axonal cytoskeleton is a complex matrix of proteins, amongst which the neurofilaments play a major role. We have characterized one factor which regulates this cytoskeleton, i.e., a Ca^{+}-activated protease which selectively degrades neurofilament proteins. The axonal transport of neurosecretory vesicles in the hypothalamus-neurohypophysial system is being investigated as a model system to study intracellular organelle-cytoskeleton interaction. These vesicles are transported at a rate of 150 mm/day through the dense cytoskeletal matrix, and we have recently succeeded in isolating stable vesicles (using isoosmolar density gradients) in order to study their interactions with axonal proteins. Studies on the structures of the vesicles are currently underway (i.e., intravesicular and membrane proteins and lipids). Thus far an ATPase on the vesicle membrane has been detected, which appears to behave as an electrogenic-proton pump. This proton-pump appears to generate a pH gradient across the vesicle membrane (i.e., an intravesicular pH of 5.7). We have recently developed a covalent-labelling approach to study retrograde axonal transport in vivo, and have uncovered a slow retrograde transport component which is carrying plasma proteins intra-axonally towards the neuron perikaryon.

Significance of Biomedical Research and the Program of the Institute:
The demonstration that plasma proteins (e.g., serum albumin) are found intra-axonally and are transported retrogradely may indicate a new source of trophic information for neurons from the periphery. Serum constituents through this route may have influence on neurons during development. The Ca^{++}-activated proteases we have studied may play a major role in Wallerian degeneration, and formation of new nerve endings during regeneration and development.

Proposed Course: To study the purified vesicles in experiments with axonal cytoskeletal proteins, so as to evaluate which of these proteins bind to the vesicle membranes. Such binding proteins may be the critical proteins involved in the axonal transport of these vesicles. In addition, we plan to investigate the role of intravesicular organization, the vesicle membrane ATPase and other membrane proteins in the process of secretion in the posterior pituitary. Further investigation of the calcium-activated protease's role in synaptic endings will be made.

Publications:

1. Gainer, H. and Kosower, N.: Histochemical demonstation of thiols and disulfides by the fluorescent labeling agent, monobromobimane: An application to the hypothalamo-neurohypophysial system. Histochemistry 68: 309-315, 1980.

2. Baumgold, J., Teraleawa, S., Iwasa, and Gainer, H.: Membrane associated proteins in squid giant axon. J. Neurochem. 36: 759-764, 1981.

3. Wheler, G.T. and Russell, J.T.: Separation and quantitation of o-phthalaldehyde derovatoves of taurine and related compounds in an HPLC system. J. Liquid Chromatog. In press.

4. Thorn, N.A., Russell, J.J. and Treiman, M.: The neurosecretory granule. In Poisner, A. and Trifaro, J.M. (Eds.): The Secretory Granule Amsterdam, Elsevier. In press.

5. Russell, J.T.: The isolation of purified neurosecretory vesicles from bovine neurohypophysis using iso-osmolar density gradients. Analyt. Biochem. In press.

6. Russell, J.T. and Holz, R.W.: Measurement of pH and membrane potential in isolated neurosecretory vesicles from bovine neurohypophyses. J. Biol. Chem. In press.

7. Fink, D.J., Russell, J.T., Brownstein, M.J., Baumgold, J. and Gainer, H.: Multiple rate components of axonally transported proteins in the hypothalamo-neurohypophysical system of the rat. J. Neurobiol.. In press.

8. Gainer, H. and Fink, D.J.: Covalent Labelling Techniques and Axonal Transport. In Weiss, D. and Gross, G. (Eds.): Axoplasmic Transport. New York, Raven Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00058-06 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biosynthesis and secretion of peptides in the vertebrate nervous system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. Gainer Chief, Sec. on Functional Neurochemistry LDN NICHD

OTHERS: J. T. Russell Staff Fellow LDN NICHD

COOPERATING UNITS (if any)

M. Brownstein, Laboratory of Clinical Science, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Functional Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Using the rat hypothalamo-neurohypophysial system as a model, we have isolated two large molecular weight common precursors, one for oxytocin and its neurophysin (Prooxyphysin), and the other for vasopressin and its neurophysin (Propressophysin). Propressophysin has a mw = 19,500 (pI=6.1) and is a glycopeptide. Prooxyphysin has a mw = 15,000 (pI=5.4) and lacks the glycopeptide component. The glycopeptide from propressophysin has been isolated, its amino acid composition and the first 20 amino acids of its sequence have been determined. We have also demonstrated that the two prohormones are present in secretory granules, and their processing to the respective hormones can occur intragranularly.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: 1) To determine whether the neurohypophysial peptide hormones, vasopressin and oxytocin and their respective neurophysins are synthesized from a common precursor, and to further characterize these precursors. 2) To study the cellular mechanisms involved in the biosynthesis and secretion (by exocytosis) of these peptides. This vertebate neurosecretory system is ideal as a model for the development of necessary techniques and approaches for the study of peptidergic neurons and systems in general. The latter represents the major long term objective of this section.

Methods Employed: Gel electrophoresis, high performance liquid chromatography, thin-layer chromatography, radioisotope technology, stereotoxic intracerebral micro-injection, and isolation of brain nuclei for analysis, affinity chromatography, radioimmunoassay of vasopressin, oxytocin and neurophysin, sub-cellular fractionation techniques, spectrophotometric and spectrofluorometric techniques for measuring membrane potential.

Major Findings: The prohormones for vasopressin (propressophysin) and oxytocin (pro-oxyphysin) and their associated neurophysins have been identified and characterized. Cyanogen bromide cleavage of these prohormones indicates the oxytocin is on the N-terminal, and the neurophysin is near the C-terminal in prooxyphysin; and vasopressin is near the N-terminal, neurophysin is in the middle, and the glycopeptide is near the C-terminal in propressophysin. We have demonstrated that vasopressin and its neurophysin and oxytocin and its neurophysin are each synthesized from a common prohormone. Subcellular fractionation studies revealed the presence of the two prohormones in secretory granules and their processing can occur intragranularly. We have also shown that the vasopressin prohormone is a glycoprotein, and have isolated two small glycopeptide products that were presumably cleaved from the vasopressin precursor, since these peptides were absent in rats with hereditary diabetis insipidus which lack vasopressin. One of these glycopeptides was isolated from rat posterior pituitaries in a pure form and its amino acid composition and partial sequence were determined.

A method for preparing a highly purified preparation of neurosecretory vesicles from bovine posterior pituitaries was developed. The pH of the interior of these secretory vesicles was determined to be 5.7 using radioactive methylamine distribution. Further, the vesicles were shown to have an internal negative membrane potential. ATP in the presence of Mg^{2+} caused a large depolarization of these vesicles, and the presence of permeable Cl^- ions caused them to lyse osmotically.

Significance to biomedical research and the program of the institute:
The identificaton of another peptide product derived from the vasopressin prohormone raises the possibility that the hypothalamo-neurohypophysial neurons may synthesize and release other biologically active peptides besides oxytocin and vasopressin. Studies directed at the understanding of the cellular mechanisms underlying the prohormone processing, packaging, axonal transport and secretion of the peptides products synthesized by these neurons, in adult rats, are prerequisites for the subsequent analysis of the regulatory and integrative roles these peptidergic neurons may play during development.

Proposed Course: To study the proteolytic enzymes, involved in the conversion of the oxytocin and vasopressin prohormones to the hormones. To examine the development of the oxytocin and vasopressin synthesizing neurons in the hypothalamus both in vivo and in vitro (using tissue culture methodology), and their interaction with neurons in other central nervous system regions.

Publications:

1. Russell, J.T., Brownstein, M.J. and Gainer, H.: Biosynthesis of vasopressin, oxytocin and neurophysins; Isolation and characterization of the two common precursors (Propressophysin and prooxyphysin). Endocrinology 107: 1880-1890, 1980.

2. Russell, J.T., Brownstein, M.J. and Gainer, H.: ^{35}S-Cysteine labelled peptides transported to the neurohypophyses of adrenalectomized, lactating, and Brattleboro rats. Brain Res. 201: 227-234, 1980.

3. Gainer, H., Loh, Y.P. and Russell, J.T.: Biosynthesis of neuronal peptides: implications for neurobiology. Progs. Biochem. Pharmacol. 16, 60-68, 1980.

4. Russell, J.T., Brownstein, M.J. and Gainer, H.: Biosynthesis of common precussors of vsopressin, oxytocin, and their respective neurophysins. In J.L. Barker and T.G. Smith (Eds.): The Roles of Peptides in Neuronal Function. New York, Marcell Dekker, 1980, pp. 86-108.

5. Russell, J.T, Bennett, C., Gainer, H. and Brownstein, M.J.: Biosynthesis of posterior pituitary hormones. Adv. Physiol. Sci. 13: 103-110, 1980.

6. Russell, J.T., Brownstein, M.J. and Gainer, H.: Time course of appearance and release of ^{35}S-Cysteine labelled neurophysins and peptides on the neurohypophysis. Brain Res. 205: 299-311, 1981.

7. Gainer, H.: The biology of neurosecretion. In Martin, J.B., Reichlin, S. and Bick, K.L. (Eds.): Neurosecretion and Brain Peptides: Implications for Brain Function and Neurological Diseases, New York, Raven Press, 1981, pp. 5-20.

8. Gainer, H. and Brownstein, M.J.: Neuropeptides, In: Albers, R.W. and Siegel, G. (Eds.): Basic Neurochemistry, Amer. Soc. Neurochem., in press.

9. Schwartz, W.J. and Gainer, H.: The localization of the "biological clock" in the brain. JAMA, in press.

10. Brownstein, M.J. and Gainer, H.: Biosynthesis of posterior pituitary hormones. In Martini, L. and Ganong, W.F. (Eds.): Frontiers in Neuroendocrinology. Vol 6, in press.

11. Brownstein, M.J. and Gainer, H.: Neuropeptides: An overview. In Lajtha, A. (Ed.): Handbook of Neurochemistry, New York, Plenum Press, in press.

12. Russell, J.T., Brownstein, M.J. and Gainer, H.: Synthesis and processing of posterior pituitary prohormones. In Motta, M. (Ed.): Pituitary Hormones and Related Peptides: From Cell Biology to Clinical Applications. In press.

13. Gainer, H., Russell, J.T. and Brownstein, M.J.: Axonal transport, neurosecretory vesicles, and the endocrine neuron. In Weiss, D. and Gross, G. (Eds.): Axoplasmic Transport. New York, Raven Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00062-05 LDN

PERIOD COVERED
Oct. 1, 1980 to Sept. 30, 1981

TITLE OF PROJECT (80 characters or less)

Brain mechanisms of vocal production in squirrel monkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Newman	Physiologist	LDN NICHD
	P.D. MacLean	Chief	LBEB NIMH
OTHER:	D. Symmes	Head, Section on Brain and Behavior	LDN NICHD
	M. Murphy	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)

P.D. MacLean and M. Murphy, LBEB, NIMH

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The current phase of this project involves continuing studies on the effects of effects of selective brain lesions on production of the Isolation Peep. Since lesions in the thalamus and midbrain that disrupt production of this vocalization also may encompass fibers arising from more rostral structures, it is important to determine the effects on the Isolation Peep of damage to these rostral structures, particularly the anterior limbic cortex. We have attempted to deactivate the anterior limbic cortex in one subject by surgically occluding the anterior cerebral artery. Postoperatively, this animal failed to utter Isolation Peeps until the third postoperative week, although other vocalizations were recorded during the intervening period. A new study assessed the role of opiates and opiate antagonists on Isolation Peep production. Preliminary results suggest that an opiate-dependent mechanism underlies production of the Isolation Peep. Another new study involved electrical stimulation of the cingulate gyrus in squirrel monkeys, attempting to locate brain structures that reliably produce vocalizations.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: This project is concerned with the brain sites and neural mechanisms by which vocalizations are initiated and controlled. A major objective is to form a link with our ongoing studies of the perceptual and behavioral aspects of vocal communication, leading to an overall understanding of the communicative process. A further goal is to correlate the brain mechanisms of vocalization with those controlling other forms of emotional behavior, to enlarge our understanding of the neural control of social behavior in general.

Methods Employed and Major Findings: Work on the effects of tegmental lesions on squirrel monkey vocalizations was submitted for publication during FY 81. Preoperative vocal data on 6 new animals was collected, subsequent to a series of lesions scheduled for Fall, 1981. In addition to the Isolation Peep, recordings were made of typical vocalizations uttered during the trump display (a species-typical display involving thigh-spreading, penile erection, and characteristic vocalizations). Investigation of the role of the anterior limbic cortex on the production of the Isolation Peep and other vocalizations was initiated. A pilot study of the feasibility of surgically occluding the anterior artery was attempted in one animal. It was found that surgical intervention under visual control permits access to the anterior cerebral artery at the level of the genu of the corpus callosum without risking damage to other nearby neural structures. Occlusion of the artery at this level failed to completely eliminate supply of blood to the anterior limbic cortex. Nevertheless, postoperative testing of this animal showed a failure to utter Isolation Peeps up to the third postoperative week, at which time the subject showed a level of Isolation Peep performance comparable to preoperative values. During the time when Isolation Peep production was absent, this subject produced other call types typical of normal animals. This work is also described in this year's report of Project No. Z01 MH 000787-02 LBEB. A new series of experiments begun this year by Dr. Symmes is also related to the role of the anterior limbic cortex in vocal behavior. Electrical stimulation of the brain is carried out and elicited vocalizations are acoustically analyzed with the goals of locating structures that reliably produce vocalizations used in contact and affiliation, and of establishing the subspecific conformity of elicited vocalizations to those occurring under natural conditions. This study has so far concentrated on the cingulate gyrus, because of its implication from other work in production of contact vocalizations.

A new study on the effects of exogenous opiates and opiate antagonists on Isolation Peeps was started with Dr. Michael Murphy (LBEB). Two monkeys treated with 2 mg/kg naltrexone increased the rate of their Isolation Peeps over their normal average rates during the 30 minute period following injection. Administration of a 5mg/kg dose of the opiate, morphine, resulted in a dramatic drop in Isolation Peep calling rate, as well as in altered structure of the calls. These results implicate an opiate-dependent mechanism in the control of Isolation Peep production.

Significance to Biomedical Research and the Program of the Institute: This project combines methods for the production and anatomical analysis of experimental brain damage, procedures for testing subjects in naturalistic contexts, and quantitative methods for evaluating changes in vocal behavior. This significance of this combined effort is the promise it offers for discovering the specific brain structures controlling different aspects of vocal communication, including articulation, contextual analysis and emotional expression. This work is therefore relevant both to the pathology of social communication as well as to the neural mechanisms controlling emotional expression.

Proposed Course: Both lesion and electrical stimulation studies will focus primarily on the rostral limbic cortex. Additional studies on the effects of tegmental damage on vocalization will be performed, in an attempt to link areas relevant to Isolation Peep production to those involved in expression of the "trump display." Further work with opiates and opiate antagonists will be done, to determine dosage parameters for Isolation Peep performance, and to evaluate the relative effectiveness of additional pharmacological agents.

Publications:

1. Newman, J.D. and MacLean, P.D. Effects of tegmental lesions on the isolation call of squirrel monkeys. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00064-05 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neurobiologic studies of neurons and glia from the mammalian central nervous system in cell cultures.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	P. G. Nelson	Chief, Lab. of Dev. Neurobiol.	LDN NICHD
	E.A. Neale	Research Physiologist	LDN NICHD
OTHERS:	K. Marshall	Guest Worker	U. of Ottawa
	R. Pun	Visiting Fellow	LDN NICHD
	W. Habig		DBP BB
	W. Hendelman		U. of Ottawa
	W. Oertel		LCS M
	A. Schaffner	Guest Worker	LDN NICHD
	S. Fitzgerald	Biologist	LDN NICHD
	L. Bowers	Bio. Lab. Tech	LDN NICHD
	S. Neal	Photographer	LDN NICHD

COOPERATING UNITS (if any)

Departments of Anatomy and Physiology, University of Ottawa

LAB/BRANCH
Laboratory of Deelopmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.2	5.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Release of transmitter from spinal cord (SC) and dorsal root ganglion (DRG) cells in cell culture is better described by binomial than by Poisson statistics, indicating that the probability of transmitter release is relatively high for each release element. The statistically defined release element corresponds to the individual synaptic bouton. Catecholaminergic connections between locus coeruleus explants and dissociated spinal cord neuron are predominantly depolarizing, alpha receptor mediated, and are due to a decrease in neuronal membrane conductance, which results in a large increase in excitability. Polyneuronal innervation of muscle by neuroblastoma x glioma hybrid cells is decreased by veratridine activation of the cells. Specific developmental periods have been found during which spinal cord cells are sensitive to tetrodotoxin (TTX) induced inactivity. The degree and age dependency of this effect varies with the neuronal marker used. In addition, our data suggest the existence of a trophic factor which prevents TTX toxicity. Combined radioautography and immunohistochemistry of cerebral cortical neurons in cell culture have shown that ^{3}H-GABA accumulating neurons are those which contain immunoreactive glutamate decarboxylase (GAD), the GABA-synthesizing enzyme.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To understand the mechanisms underlying mammalian central synaptic action and to study the factors affecting formation and development of central neurons and their synapses. Catecholaminergic and peptidergic mechanisms are particular areas of interest as is the role of neuronal activity in regulating neuronal development.

Method Employed: As in previous reports, we prepare cell cultures of mouse or rat cerebral cortex or mouse spinal cord (SC) plus dorsal root ganglia (DRG). Intracellular recordings are done in combination with a variety of methods for applying pharmacological agents and media with altered ionic composition. Tissue from the brain stem (including the locus coeruleus) is explanted in conjunction with SC cell cultures. Fluorescent microscopic techniques visualize catecholamine containing fibers. Light and electron microscopy are utilized to monitor morphological development, and to characterize cell type specificity and synaptic interrelationships of neurons in cultures prepared from a variety of central nervous system structures.

Light and electron microscopy are utilized to monitor morphological development, and to characterize cell type specificity and synaptic interrelationships of neurons in cultures prepared from a variety of central nervous system structures. Light microscope radioautography and immunohistochemistry are used to distinguish neurons from non-neuronal cells and to identify those neurons which contain a specific neuropeptide, or neurotransmitter-related enzyme or high affinity uptake system. Biochemical methods for quantitating development of neuronal cultures are as described in Project Z01 HD 00048-07.

Major Findings:

1. Statistical analysis of electrophysiological measures of transmitter release from SC and DRG neurons indicate that the commonly used Poisson model is inadequate and binomial statistics are more appropriate. The number of release elements corresponds well to the number of synaptic terminals connecting neurons, and the probability of release from a terminal may be relatively high.

2. Pharmacological properties of physiological interactions between locus coeruleus (LC) explants and dissociated SC neurons have been characterized. Both LC stimulation and noradreneline iontophoresis elicit slow depolarization of SC cells which is associated with decreased neuronal membrane conductance. Both responses are blocked by piperoxan (an alpha adrenergic receptor blocker) and enhanced by des-methyl imipramine, which blocks re-uptake of noradrenaline by neurons. The occurrence of neuronal responsiveness to iontophoretically noradrenaline is highly correlated with catecholaminergic synaptic input.

3. When neuroblastoma x glioma hybrid neuronal cells are co-cultured with skeletal muscle they form cholinergic synapses, with a substantial degree of multiple innervation of the muscle. Chronic activation of the hybrid cells with veratridine results in a reduction of multiple innervation without a decrease in

the incidence of innervated muscle cells. This shows that activation of nerve (and/or muscle) can accelerate the process by which muscle normally progresses to the singly innervated state, a process involving both elimination of some synapses and stabilization of others.

4. A method was developed in which cerebral cortical cell cultures are incubated for high affinity ^{3}H-GABA uptake, stained immunocytochemically for GAD, and processed for radioautography. In mature cultures, of those neurons showing high silver grain densities and/or GAD-immunoreactivity, approximately 90% show the coexistence of both labels. These results demonstrate that ^{3}H-GABA accumulating neurons contain the enzyme that synthesizes GABA. In developmental studies, the high affinity ^{3}H-GABA uptake system is demonstrated 1-2 week prior to GAD immunoreactivity.

5. Developmental stage-dependency of TTX-induced toxicity was evaluated with tetanus toxin binding, GABA uptake, and CAT activity in the SC-DRG cultures

A. Specific tetanus toxin binding was reduced to 50-55% of control values if TTX was applied during the 2nd or 3rd week after plating. Application of TTX during the 1st or 4th week resulted in no changes from control binding. Chronic exposure (4 weeks) did not result in further decreases in tetanus toxin binding from that observed during the 2nd or 3rd week.

B. High affinity GABA uptake was not affected by TTX exposure throughout the testing period of 4 weeks. Neither DABA (primarily neuronal) or B-alanine (glial) displaced uptake was significantly altered by the TTX.

C. CAT activity was not affected by TTX applied 2-4 days after plating. However, TTX exposure from day 2-7 reduced CAT activity to 60% of control values. Similar decreases were observed during acute exposure during the 2nd, 3rd, and 4th week in culture. Chronic application of TTX produced a progressively greater loss of activity (38% of control) through 21 days.

D. These data indicate that there is a differential response of various neuronal populations to blockade of electrical activity with TTX.

Significance to Biomedical Research and the Program of the Institute:
Establishing the appropriate mathematical models for synaptic transmission between central neurons is a basic goal of neurobiological research, and is fundamental to a number of pharmacological and developmental studies. The mechanism of action of catecholamines and the regulation of receptors for the amines is important from both functional and developmental standpoints. Synapse formation, elimination, and stabilization are critical in the development of synaptic circuitry and the mechanisms underlying these phenomena not known. The use of appropriate models is essential for progress in this area.

The role of neural activity in modulating nervous system development during critical periods may have important implications for experimental effects on development in intact animals.

Proposed Course: Further work on the pharmacology and biophysics of catecholaminergic and other synaptic interactions is underway. Attempts to identify the critical factors mediating the influence of neural activity on neuronal survival and maturation have begun using conditioned media from active cultures and some neural transmitters as additive to inactive cultures.

Radioautography of ^{3}H-GABA accumulation will be combined with immunohistochemical localization of neuropeptides to determine the coexistence or relationship of GABAergic systems with particular peptides.

On-going studies, involving light and electron microscopic radioautography, are concerned with localizing ^{125}I-tetanus toxin bound to neurons, and following the movement, with time, of the toxin within neurons. These results will be correlated with electrophysiologic analysis of the onset and time course of tetanus effects.

Publications:

1. Bergey, G.K., Fitzgerald, S.C., Schrier, B.K. and Nelson, P.G.: Neuronal maturation in mammalian cell culture is dependent on spontaneous electrical activity. Brain Res. 207: 49-58, 1981.

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6. Neale, E.A., Macdonald, R.L., and Nelson, P.G.: Neurons in culture are developmental models. JAMA, in press.

7. Nelson, P.G. Neuronal cell culture as toxicologic test systems. Env. Health Perspectives 26: 125-133, 1978.

8. Nelson, P.G. and Bergey, G.K.: Pharmacological and developmental studies on mammalian central neurons in cell culture. In Giacobini et al., (Eds.): Tissue Culture in Neurobiology. New York, Raven Press, 1980, pp. 221-227.

9. Nelson, P.G., Neale, E.A., Matthew, E., and Zimmerman, E.A.: A presynaptic locus of action for the opiates. In: Barker, J.L. and Smith, T.O. (Eds.): The Role of Peptides in Neuronal Function. New York, Marcel Dekker Inc., 1980, pp. 727-739.

10. Nelson, P.G., Neale, E.A. and Macdonald, R.L.: Electrophysiological and structural studies of neurons in dissociated cell cultures of the central nervous system. In Nelson, P.G. and Lieberman, L. (Eds.): Excitable Cells in Tissue Culture. New York, Plenum Press, 1981, pp. 39-80.

11. Nelson, P.G. and Lieberman, M. (Eds.): Excitable Cells in Tissue Culture. New York, Plenum Press, 1981, 422 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00089-07 LDN

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pineal-pituitary interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD

COOPERATING UNITS (if any)
J. Martin, Ph.D., Dept. of Pharmacology, Washington Univ. Med. School; K. Kirk, LC, NIAMMD; Kevin Catt, Reproductive Biology Branch, NICHD; L. Tarmarkin, NICHD

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many life processes are regulated by the pituitary gland. This project studies how the pineal gland, influences pituitary function. The primary control of the pituitary gland is via the release of brain hormones. One of these hormones, luteinizing hormone releasing factor, regulates the release of a pituitary hormone necessary for normal reproduction, luteinizing hormone. A pineal secretion, melatonin, prevents the brain hormone from stimulating the release of luteinizing hormone. Similar effects are found using new synthetic derivatives of melatonin. The function of the pineal gland is drastically altered by environmental light and by drugs; pineal function also changes markedly during development. Thus it seems possible that as a result of these changes, and subsequent pineal-pituitary interactions, there will be significant changes in pituitary gland function resulting in turn in alterations in general health. Such an interaction may explain why the pineal gland can stop reproduction in certain animals when they are deprived of light. A second interaction of the pineal gland and the pituitary is through the pituitary adrenal axis. Studies have been performed to determine whether or not such a relationship exists in the rhesus monkey.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To chemically and physiologically characterize pineal compounds which influence function of the pituitary gland, including those aspects concerned with reproduction and adrenal function. To describe the mechanism through which pineal compounds act and to design drugs which are more potent and long lived as compared to the parent compound, or block the effects of the pineal compounds.

Methods Employed: A pituitary organ culture system and a pituitary cell culture system using neonatal and adult pituitary tissue are the model systems studied. This approach provides a highly sensitive and precise means of measuring the effects of pineal hormones on the pituitary gland. In vivo systems are also used to further explore the effects of compounds of interest and of alterations in pineal function. Immunoassay procedures are used to measure the amount of pituitary hormones released following treatment with test compounds, or under physiological conditions. The number of receptors present on the pituitary gland and on pituitary target tissues is determined using radioreceptor assays.

Major Findings: (1) The effects of changes in photoperiod on the number of gonadotrophin receptors in the testis have been examined. It has been found that during a 24 hour period there is no change in the apparent number of receptors present in this tissue. (2) It has been discovered that shifts in the environmental lighting changes in the numbers of FSH, LH, and prolactin receptors in the testes, which raises the possibility that the pineal gland influences reproduction through mechanisms which alter the number of receptors for pituitary hormones that are present in the testis.

Significance to Biomedical Research and the Program of the Institute: These studies are consistent with the mission of the Institute to support research in reproductive biology. The results of these studies may lead to an increased understanding of the role of the pineal gland in reproduction and to the development of new drugs to alter reproduction by either mimicking the effects of pineal compounds or blocking their effect. One consequence of these studies is that abnormalities in reproductive physiology may be explained on the basis of pineal function. Finally, these studies may uncover a interaction between pineal and the adrenal gland.

Proposed Course of Project: (1) The relationship of pineal secretion of melatonin and the adrenal secretion of steroids will be studied under a variety of environmental conditions and as a function of shifts and photoperiods to determine if these two parameters are closely linked. (2) The changes in testis receptors for pituitary hormones will be analyzed in depth, with one goal being the determination of the relative rate of change in receptor number seen during testicular regression and recrudesence. (3) The effects of melatonin on prolactin secretion will be studied.

Publications: none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00093-07 LDN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Mechanism of Action of Nerve Growth Factor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

G. Guroff	Chief, Section on Intermediary Metabolism	LDN NICHD
L. Pevzner	Visiting Scientist	LDN NICHD
S. Vinores	Staff Fellow	LDN NICHD
S. Hashimoto	Visiting Fellow	LDN NICHD
G. Dickens	Bio Lab Tech	LDN NICHD
N. Tolson	Biologist	LDN NICHD
D. End	Guest Worker	LDN NICHD

COOPERATING UNITS (if any)

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C. Londos, LNE A	
P. Marangos, LCS M	

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Intermediary Metabolism

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this research is to provide information on the mode of action of nerve growth factor. Nerve growth factor is required for the development and maintenance of the sympathetic and sensory nervous systems and may be a prototype of the many factors now known to be involved in the growth and maturation of various cell types. The mechanism of action of nerve growth factor is of interest because it should lead to an understanding of the way in which gene expression is regulated in neurons and how such factors induce the synthesis of specific enzymes for the biosynthesis of the neurotransmitter, norepinephrine, or for the morphological changes involved in the outgrowth of neurites. In turn, such information may lead to an understanding of the development and differentiation of the non-dividing, non-replaceable neurons of the central nervous system, and to information about the tumors which arise from them.

Objectives: (1) To elucidate the mechanism of action of nerve growth factor on the differentiation and maintenance of the sympathetic and sensory nervous system; (2) to delineate the intracellular events mediating the action of nerve growth factor in responsive cells; (3) to detect and characterize other factors which direct the development of other types of neurons; (4) to study the interactions of nerve growth factor with other factors impinging on the cell, especially those which stimulate cell division or prevent cell differentiation; (5) to investigate the effects of nerve growth factor and its antiserum on the growth rate and characteristics of other cells of neural crest origin and of the tumors which arise from such cells; (6) to explore the enzymology of neurite formation and the effect of nerve growth factor on these enzymes; (7) to characterize the action of nerve growth factor and of other growth factors on the central nervous system; (8) to measure the levels of nerve growth factor and of nerve growth factor receptors in clinically relevant conditions, such as those involving tumors of the sympathetic nervous system or of other neural crest-derived tissues.

Methods employed: The methods include standard techniques of cell culture and *in vivo* investigations of the peripheral and central nervous system. Enzyme assay, cell fractionation, and surgery on small animals and embryos are used, as well as biological and immunological methods by which nerve growth factor and its receptor are localized. Radioisotope techniques are employed with heavy emphasis on the use of protein labeling with ^{125}I and phosphorylation studies with ^{32}P. Slab gel electrophoresis provides an integral tool for the separation of nuclear proteins, and isoelectric focussing methods are used. Standard immunological techniques are employed for the preparation and use of the relevant antibodies.

Major findings: (1) Interaction of nerve growth factor with PC12 cells leads to the phosphorylation of a specific nuclear protein and the subsequent binding of that protein to the DNA of the cell; (2) phosphorylation of the same specific nuclear protein can be stimulated by adenosine analogs and these adenosine analogs can prime PC12 cells for neurite outgrowth; (3) nerve growth factor treatment leads to an increase in the neuron-specific protein, NSE; (4) receptors for nerve growth factor and for epidermal growth factor can be detected by immunofluorescence techniques and both are present on single PC12 cells; (5) cells originating from the neural crest of chick embryos, and which are precur-sors of the neurons of the sympathetic nervous system, respond to nerve growth factor and have nerve growth factor receptors.

Significance to Biomedical Research and the Program of the Institute: Nerve growth factor is the best characterized and, indeed, the first known growth factor. There are now more than 30 growth and maturation factors known, and they act on a wide variety of cell types. Nerve growth factor is required for the growth and maintenance of the sympathetic and sensory nervous systems. Its mechanism of action, therefore, is of extreme interest and only the outlines are presently apparent. A full understanding of the mechanism of action of nerve growth factor might lead to a better understanding of the mode of regulation of the growth of neurons and of their differentiation. Further, understanding of the mechanism by which nerve growth factor affects cell development may give clues as to the normal regulation of the growth of mammalian cells, and, specifically, of neurons, and to the process of genetic expression. Then,

studies on nerve growth factor may indicate ways in which the selectivity of neural connectivity can be understood and, indeed, altered. A molecular understanding of nerve growth factor action may lead to a common thread for the action on growth and maturation factors in general on their target cells. Finally, an understanding of genetic expression and growth regulation in normal cells and in the tumors which arise from them may give some new insights into tumor cell biology.

Proposed course of the project: (1) Purification of the nerve growth factor-responsive protein from the nuclei of PC12 cells; exploration of its properties and sites of phosphorylation; (2) characterization of the binding of the non-phosphorylated and the phosphorylated forms to PC12 DNA; elucidation of the site of binding on the DNA; exploration of the effects of the binding on the structure and the stability of the DNA; effects of the binding on transcription; (3) production of an antibody to the sensitive protein and development of quantitative immunochemical methods for studying the distribution of the protein; (4) search for the kinase responsible for catalyzing the nerve growth factor-dependent phosphorylation; (5) exploration of the other agents, e.g. epidermal growth factor, adenosine analogs, tumor promoters, which also promote the phosphorylation of the nerve growth factor-sensitive protein; elucidation of the similarity of their effects on transcription and on neurite outgrowth; (6) elucidation of the effects of nerve growth factor on neuroblast precursors of the sympathetic neurons; identification of the influences which direct the development and differentiation of these cells in culture; (7) study of the effects of nerve growth factor and other factors on the enzymes which are involved in the formation of the matrix of neurite outgrowth.

Publications:

1. Guroff, G., Montgomery, P., Tolson, N., Lewis, M.E., and End, D.: The induction of ornithine decarboxylase by renin-free nerve growth factor. Proc. Nat'l. Acad. Sci. U.S.A. 77: 4607-4609, 1980.

2. Yu, M.W., Tolson, N.. and Guroff, G.: Increased phosphorylation of specific nuclear proteins in superior cervical ganglia and PC12 cells in response to nerve growth factor. J. Biol. Chem. 255: 10481-10492, 1980.

3. Guroff, G.: Inborn errors of amino acid metabolism in relation to diet. Nutrition in Health and Disease, Franklin Institute Press, in press.

4. Huff, K., End, D., and Guroff, G.: Nerve growth factor-induced alteration in the response of PC12 pheochromocytoma cells to epidermal growth factor. J. Cell Biol. 88: 189-198, 1981.

5. End, D. and Guroff, G.: Nerve growth factor and epidermal growth factor responsiveness of pheochromocytoma cells. Maturation Factors and Cancer, Raven Press, in press.

6. Ikeno, T., Dickens, G., Lloyd, T., and Guroff, G.: The receptor-mediated activation of tyrosine hydroxylation in the superior cervical ganglion of the rat. J. Neurochem. 36: 1632-1640, 1981.

7. Guroff, G., Dickens, G., and End, D.: The induction of ornithine decarboxylase by nerve growth factor and epidermal growth factor in PC12 cells. J. Neurochem. 37: 342-349, 1981.

8. Vinores, S. A., Marangos, P. J., Parma, A. M., and Guroff, G.: Increased levels of neuron-specific enolase in PC12 pheochromocytoma cells as a result of nerve growth factor treatment. J. Neurochem., in press.

9. Guroff, G., Dickens, G., End, D., and Londos, C.: The action of adenosine analogs on PC12 cells. J. Neurochem., in press.

10. End, D., Tolson, N., Yu, M., and Guroff, G.: Effects of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on rat pheochromocytoma (PC12) cells: Interactions with epidermal growth factor and nerve growth factor. J. Cell. Physiol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00094-11 LDN

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Circadian, Stress, Light and Drug Influences (rat, hamster, rhesus monkey)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	J.L. Weller	Chemist	LDN	NICHD

COOPERATING UNITS (if any)
R.Y. Moore, Dept. of Neurosciences, Univ. of CA, School of Medicine; R. Kvetnansky, LCS, NIMH; H. Hoffman, EBR, NICHD: M. Perlow and M. Mishkind, NIMH; S. Reppert, Harvard Medical School

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to describe the factors which regulate the metabolism of a neuroendocrine organ, the pineal gland. These investigations are conducted within a heirarchy of levels of biological organization, including the interactions of the pineal gland with environmental lighting, stress, drugs, endocrine organs, and the central nervous system. Studies related to the central nervous system also probe the fundamental nature of the biological clock governing biological rhythms.

PHS-6040
(Rev. 2-81)

Project Description;

Objectives: To determine how stress, central neural structures, environmental and pharmacological factors influence horomonal secretion by the pineal gland, and to study the fundamental nature of circadian rhythms.

Methods Employed: (1) To determine the central neural, environmental and pharmacological factors influencing the pineal gland, pineal glands are removed from treated animals and the activity of metabolites and enzymes are measured. In addition, the amount of meltatonin in blood, CSF and the pineal gland is measured. The animals are treated with drugs, hormones, or experimental environmental regimes, including stress and constant darkness, and in some cases are surgically prepared by the precise destruction of small areas of the brain and neural structures thought to be part of the neuronal circuit regulating the pineal gland.

In addition, small areas of the brain known to be involved in the regulation of the pineal gland are removed and the amounts of neurotransmitters they contain are determined after experimental treatment.

(2) Circadian rhythms are studied by measuring the effects of destruction of brain areas on the circadian rhythm in pineal N-acetyltransferase activity, and in melatonin in the circulation.

Major Findings: (1) It has been found that the rhythm in melatonin in the rhesus monkey can be shifted by shifting environmental lighting. Interestingly, we have found that there is entrainment within a one-day period when the rhythm is shifted 12 hours by extending the normal day period twelve hours, providing the animal with a continuous period 24 hours of constant light. In contrast when an animal is provided with an extended night period, providing 24 hours of constant darkness, the period required for the animal to entrain to the new lighting cycle is three days. (2) The effects of alterations in environmental lighting on the daily rhythm in CSF melatonin were studied in the rhesus monkey. It was found that acute esposure to darkness during the day did not increase normally low daytime CSF melatonin levels, that light suppressed the normally high CSF melatonin values at night, and that 12 hr phase shifts in the diurnal lighting cycle caused 12 hr phase shifts in the rhythm. The daily rhythm persisted for 6 and 1/2 d of study in constant darkness and the phase of the rhythm was not affected in constant darkness by a 12 h phase shift in the daily delivery of food and daily care of the animals.

These results support the conclusion that the melatonin rhythm in this primate species is endogenous in nature, and that light can act to both coordinate the rhythm to the 24-hr day and to acutely suppress melatonin production.

(3) In the Syrian hamster, a sharp peak in pineal melatonin occurs toward the end of the dark period. In the present communication, we describe characteristics of this rhythm. First, the time of the initial increase in pineal melatonin is not altered by daily melatonin injections that induce gonadal regression. Second, the rapid decrease of pineal melatonin in the

morning is not prevented by acutely extending the dark period. Third, a consistent nocturnal increase in pineal melatonin is not observed in a 20-h light, 4-h darkness lighting schedule. However, acute exposure to a longer dark period, at the appropriate time of day, allows expression of the pineal melatonin rhythm in these animals. Finally, the rhythm in pineal melatonin appears to be truly circadian and is tightly coupled to the circadian rhythm in running activity.

Significance to Biomedical Research and the Program of the Institute: A growing body of knowledge points to the pineal gland as the source of a compound that inhibits reproduction, and that this compound is melatonin. Large changes in melatonin production are regulated by N-acetyltransferase. Thus, an increase in understanding of how melatonin production is regulated provides a more complete description of the interaction of the pineal gland in reproductive physiology.

With knowledge in hand regarding the factors which regulate melatonin production, including drugs, environment, developmental and stress, it may be possible to regulate the production of this compound, and to predict when production would be enhanced.

Our studies on circadian rhythms in animals may have direct influence on the modern day problem of "jet lag." If we can alter the rate at which new circadian patterns are established in rats and hamsters after a shift in lighting schedules, it seems possible that this might be applied to humans to prevent "jet lag."

Proposed Course of Project: (1) Studies on the neural structures involved in the regulation of the pineal melatonin rhythm in the rhesus monkey will continue. (2) Experiments in the rhesus monkey will be performed of a pharmacological nature, designed to shed light on the transmitters involved in regulating pineal melatonin rhythm. These studies will include analysis of animals in which the superior cervical ganglia, which probably innervates the pineal gland, has been removed. This will allow the evaluation of the effects of administration of drugs on the pineal gland in this situation in which neural elements innervating the pineal gland are absent.

Publications:

1. Reppert, S., Perlow, M., and Klein, D.C.: CSF Melatonin. In J.H. Wood, ed.: Neurobiology of Cerebrospinal Fluid, Plenum Press, New York, 579-589.

2. Reppert, S., and Klein, D.C.: The mammalian pineal gland: basic and clinical aspects. In M. motta, Ed: Endocrine Functions of the Brain; Comprehensive Endocrinology IV, Raven Press, New York, 327-372.

3. Tamarkin, L., Reppert, S.M., Klein, D.C., Pratt, B. and Goldman, B.P. 1980: Studies on the daily pattern of pineal melatonin in the Syrian Hamster. Endocrinology 107, 1525-1529.

4. Reppert, S.M., Perlow, M.J., Tamarkin, L., Orloff, D., and Klein, D.C. (1980): The effects of environmental lighting on the diurnal melatonin rhythm in primate CSF fluid. Brain Research (in press).

5. Reppert, S.M., Perlow, M.J., Ungerleider, L., Mishkind, M., Tamarkin, L., Orloff, D.G., Hoffman, H., and Klein, D.C. (1981): Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the Rhesus monkey. J. of Neurosciences (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00095-11 LDN

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Transsynaptic Mechanisms in the Pineal Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	J.L. Weller	Biologist	LDN	NICHD

COOPERATING UNITS (if any)
G. Aurbach, K.Kirk, C.L. Creveling, MDB, NIAMMD; L. Tamarkin, NICHD; D. Auerbach, Duke University

LAB/BRANCH
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SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the transsynaptic mechanisms which are important in regulating metabolism of a neuroendocrine tissue, the pineal gland. The specific topics of interest include the interaction during differentiation of the pineal gland and innervating structures, the role of nerves in both releasing and taking up transmitters, the interaction of transmitters in transsynaptic regulation and the characterization of postsynaptic adrenergic receptors.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To describe the transsynaptic mechanisms through which neuronal activity regulates biochemical activity in the pineal gland.

Methods Employed: (1) Cultures of pineal tissue are used to study the molecular mechanisms through which neuronal activity regulates biochemical activity. These preparations are treated with neurotransmitters, drugs, and hormones to determine their effects on pineal cell metabolism. (2) To study development, pineal glands are removed at various ages and the activity of metabolites and enzymes are measured. The animals are also treated with drugs known to block specific neural processes. (3) Beta-adrenergic receptors are characterized using a radioligand binding assay.

Major Findings: (1) We have found that the efflux of taurine from the pineal gland is regulated by adrenergic-cyclic AMP mechanism. The treatment of glands with norepinephrine results in a 100-fold increase in the rate of efflux of taurine. This release appears to be related to changes in membrane potential and the driving force behind the release is not the taurine concentration gradient. (2) Techniques have been developed which make it possible to study β-adrenergic receptors on intact neuroendocrine cells. Receptors were characterized on neonatal pinealocytes using the radioligand [^{125}I]iodohydroxybenzylpindolol ([^{125}I]IHYP). Specific binding of [^{125}I]IHYP, which is 4-fold greater than nonspecific binding, is concentration and temperature dependent, reversible and saturable. [^{125}]IHYP binds noncooperatively (K_d = 35 pm), and Scatchard analysis indicates that only a single class of receptor sites for [^{125}]IHYP is present. Under the conditions used, it appears that there are about 12,000 $\pm$ 1,100 sites/cell. Inhibition of specific [^{125}I]IHYP binding by β-adrenergic agonists and antagonists is stereospecific, and the relative potency of agonists is characteristic of binding to β-adrenergic receptors. Analysis of adrenergic stimulation of intracellular cAMP accumulation indicates that similar half-maximal concentrations of antagonists inhibit [^{125}I]IHYP binding and adrenergically stimulated cAMP accumulation. In contrast, β-adrenergic agonists are considerably more potent in stimulating cAMP than in inhibiting [^{125}I]IHYP binding. Unexpected differences, not previously reported, were found in the shapes of the cAMP accumulation doses-response curves of norepinephrine and isoproterenol. The relative potencies of these two agonists appear to be partially concentration dependent. This raises the possibility that there may be distinct differences in the intrinsic effects of these compounds on the regulation of intracellular cAMP accumulation in pinealocytes. (3) Alterations in β-adrenergic agonist activity resulting from fluorine substitution on the 2-, 5- or 6-position of the aromatic ring of norepinephrine were investigated, using dispersed neonatal rat pinealocytes. Increases in cyclic AMP in these cells are known to be governed entirely by β-adrenergically coupled adenylate cyclase. Norepinephrine (NE), 2-fluoronorepinephrine (2F-NE), 5-fluoronorepinephrine (5F-NE), and 6-fluoronorepinephrine (6F-NE) were tested for their effects on cyclic AMP. In parallel studies, the inhibition of [^{125}I]iodohydroxybenzylpindolol (IHYP) binding to pineal β-adrenergic receptors by these compounds was studied. All of the agonists inhibited IHYP binding and elevated intracellular cyclic AMP in a dose-dependent fashion. The relative potencies of norepinephrine and its fluorine derivatives in both studies were

found to be 5F-NE>NE>2F-NE>6F-NE. Increases in cyclic AMP were stereospecifically blocked by (-)-propanolol. From these studies it appears that fluorine substitution on NE at the 5-position enhances, and at the 2- and 6-position reduces, the ability to elevate cyclic AMP, as compared to that of norepinephrine, by altering agonist affinity for the β receptor. Additionally, subtle differences in the effects of the different fluorinated analogs on pinealocytes in the presence of weak antagonists were detected. In studies of antagonist action on agonist-induced cyclic AMP accumulation, substitution at the 5- or 6-position resulted in derivatives, which, when compared with NE and 2F-NE, appeared less affected by the weak β-adrenergic antagonist (+)-propanolol and more affected by the α-adrenergic antagonist phentolamine. (4) Studies of the adrenergic regulation of cyclic GMP in the pineal gland show that (-)-norepinephrine stimulates cyclic GMP primarily in pineal cells, rather than in nerve endings as previously thought. The response exhibits the interesting and unusual characteristic of homologous sensitization: It is maintained by neural stimulation and disappears gradually as a consequence of depressed neural stimulation, due to denervation or decentralization of the superior cervical ganglia or to constant light. The response is restored in intact animals that had been in a constant-light environment when they are returned to a normal light cycle and in ganglionectomized animals by norepinephrine treatment. These findings are especially interesting because the pineal adrenergic-cyclic AMP stimulus-response system exhibits homologous desensitization. The occurrence of homologous sensitization of a cyclic AMP response, which we term seesaw signal processing, in the same tissue or cell has intriguing implications. It provides a mechanism through which the qualitative nature of a multicomponent response can be modified. Such a mechanism could play a role in signal processing by neural or neuroendocrine tissues that release two or more extracellular messages.

Significance to Biomedical Research and the Program of the Institute: The pineal gland represents one of the most attractive models of adrenergic control mechanisms presently available. Our understanding of the transsynaptic mechanisms involved in regulating this tissue will lead to a fuller understanding of neural functions in humans and especially in the development of neural control mechanisms. It may be possible, with an increased understanding of the specific structures and proteins involved in neural transmission and neural biochemical transduction, to identify a set of specific proteins required for normal adrenergic functioning. With these markers in hand it may be possible to evaluate retardation of mental development against these standards and thus to fully diagnose these neural problems on a molecular basis. This should provide a thorough understanding of the nature of alterations in neural development and mental retardation and also help provide a means of designing strategies to solve problems associated with these issues.

Proposed Course of Project: (1) The effect of taurine on transsynaptic control of adrenergic function will be studied. (2) The role of cyclic GMP in pineal function will be studied. (3) The regulation of taurine efflux will be investigated further. (4) The regulation of cyclic AMP will be studied.

Publications:

1. Wheler, G.H.T. and Klein, D.C.: Function and regulation of taurine in the pineal gland. in: S.I. Baskin, J.J. Kocsis, and S.W. Shaffer, eds.: Actions of Taurine on Excitable Tissues, Spectrum Press, New York.

2. Klein, D.C., Auerbach, D., Namboodiri, M.A.A., Wheler, G.H.T.: Indole metabolism in the mammalian pineal gland. In Pineal Gland: Anatomy and Biochemistry, Ed. R. Reiter, CRC Press, 1980, 199-227.

3. Auerbach, D.A., Woodard, Auerbach, G.D., and Klein, D.C.: Neonatal rat pinealocytes: Characteristis of typical and atypical ^{125}I-iodohydroxy-benzylpindolol binding and cyclic AMP accumulation. Endocrinology, 1980, 108, 559-567.

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5. Auerbach, D.A., Weller, J., and Klein, D.C.: Perinatal development of the pineal β-adrenergic adenylate cyclase control system. Journal of Neurochemistry, 1980 (in press).

6. Klein, D.C., Auerbach, D., and Weller, J.L. (1981): Seesaw signal processing in pineal cells: Homologous sensitization of adrenergic stimulation of cyclic GMP accompanies homologous desenitization of β-adrenergic stimulation of cyclic AMP. Proc. Natl. Acad. Sci. (in press).

7. Klein, D.C. (1981): Pineal Gland: Adrenergic regulation of function. In (Weiner, N., and Trendelenberg, U) Calecholamines II, Springer Press, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00096-11 LDN

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Neuroendocrine Metabolism: Intracellular Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.C. Klein	Physiologist	LDN	NICHD
OTHER:	A. Namboodiri	Visiting Associate	LDN	NICHD
	J.L. Weller	Biologist	LDN	NICHD
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COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations have centered on understanding on a molecular level the intracellular regulation of metabolism in a neuroendocrine organ, the pineal gland. The topics of specific interest include the factors regulating cyclic AMP production, the function of cyclic AMP, the control of synthesis of pineal N-acetyltransferase activity, the control of activation and inactivation of this enzyme and related enzymes, the role of mRNA synthesis in the regulation of the activity of N-acetyltransferase activity, the regulation of pineal biopterin and control of guanosine cyclohydrolase.

Project Description:

Objectives: To describe the intracellular mechanisms through which neuronal activity regulates biochemical activity in the pineal gland.

Methods Employed: (1) The experimental systems used to study the intracellular mechanisms through which neuronal activity regulates biochemical activity are cultures of pineal glands and broken cell preparations. The intact preparations are treated with neurotransmitters, drugs, and hormones to determine their effects of pineal cell metabolism. Other measures of cell function are studied, including the generation of cyclic nucleotides, the production of new protein, the production of new RNA, the activity of specific enzymes, and the production of metabolic products, including several tryptophan derivatives. (2) Broken cell preparations are used to study the regulation of pineal N-acetyltransferase activity and other parameters. In addition this enzyme is purified.

Major Findings: (1) Substantial progress has been made in purifying N-acetyltransferase from the sheep. The major advances have been the incorporation of techniques based on disulfide exchange. This coupled with affinity and hydrophobic chromatography yields a purified preparation of the enzyme. (2) Pineal indoleamine N-acetyltransferase activity in homogenates is rapidly reduced at pH 6.8 by treatment with cystamine or arginine vasotocin. Other disulfides including glutathione disulfide, penicillamine disulfide, and N,N'-diacetylcystami are either ineffective or less effective. The diamine analogous to cystamine, diaminohexane, is also ineffective. Inactivation by cystamine is accelerated at higher pH and is temperature-and time-dependent. It was also found that cystamine treatment inactivated N-acetylatransferase in intact pineal cells. Treatment with dithiothreitol reactivated the cystamine- or arginine vasotocin-inactivated enzyme formed in broken cell preparations and the cystamine-inactivated enzyme formed in intact cells. These observations indicate that pineal N-acetyltransferase can be inactivated by protein thiol-disulfide exchange; further research is required to ascertain whether this mechanism is of physiological significance. (3) Using intact pinealocytes in suspended cell culture it has been determined that acetyl CoA hydrolase activity can be rapidly increased by treatment with cystamine. Similar results are seen with diacetylcystamine, but not with GSSG, penicillamine disulfide, nor with oxidized DTT. The activation of acetyl CoA hydrolase by cystamine is reversible: after cystamine treatment is terminated, enzyme activity decreases slowly in cell culture. It is also possible to reverse the activation by treating homogenates of cystamine-treated cells with dithiothreitol. These observations are consistent with previous findings indicating that pineal acetyl CoA hydrolase activity can be regulated *via* protein thiol:disulfide exchange. The observations presented in this report also indicate that conditions within the cell allow this type of reaction to take place, and raise the possibility that disulfide exchange mechanisms may be physiologically involved in the intracellular regulation of the activity of this and perhaps other enzymes. (4) Pineal N-acetylatransferase can be inactivated through a mechanism of disulfide exchange. It has now been found that a number of disulfide containing peptides can inactivate this enzyme, and that the most potent is insulin. The findings point to the interesting possibility that disulfide containing peptides may be involved in the regulation of pineal

N-acetyltransferase activity. (5) Pineal glands in organ culture synthesize and release biopterin and are able to maintain in vivo concentrations of biopterin for up to 54 hours in vitro The intracellular biopterin content is reduced 50% by treatment with (1)-norepinephrine or cyclic AMP derivatives, but not by (d)-norepinephrine, indicating that biopterin levels are regulated by an adrenergic-cyclic AMP mechanism. The decline in tissue biopterin is produced mainly by an inhibition of biosynthesis, is maximal at 6 hours, and is not associated with either an increase in biopterin relase or a shift in the reduction state of biopterin. This is the first indication that the biosynthesis of biopterin within reural target tissue is under adrenergic control.

Significance to Biomedical Research and the Program of the Institute: The pineal gland offers an attractive model system for the study of the neural control of gene expression. Although other neural systems are available, none is capable of exhibiting such large and rapid changes in the expression of single gene as is the pineal gland. In addition, with the knowledge and confidence that the increase in pineal N-acetyltransferase depends upon the synthesis of new messenger RNA, it is now appropriate to determine whether or not the neural regulation of this gene, which must be considered to be a high information-low abundance gene, is similar to gene regulation seen in other tissue, as studied through the analysis of major gene products. Our understanding of the factors which control rapid changes in enzyme activity as a function of neural stimulation will be of great importance in understanding the neural control of metabolism in general. It is highly possible that transmitters control many enzymes in a manner similar to that in which norepinephrine controls N-acetyltransferase. Knowing what we know now about the regulation of pineal N-acetyltransferase, we may be able to use these lessons to study and analyze the regulation of enzyme activity in other neurally-controlled tissues, including neurons from the central nervous system.

Proposed Course of Project: (1) The isolation and characterization of pineal N-acetyltransferase will continue to be pursued. (2) Efforts to generate antibodies against pineal N-acetyltransferase will be continued. (3) The role of disulfide exchange and the regulation of pineal N-acetyltransferase and acetyl CoA hydrolase will be investigated. (4) The molecular mechanism involved in the regulation of both enzymes will be investigated. (5) The possible role of peptides in the regulation of this enzyme will be investigated. (6) The regulation of pineal biopterin will be investigated.

Publications:

1. Namboodiri, M.A.A., Klein, D.C.: Evidence of inactivation of pineal N-acetyltransferase by protein thiol-disulfide exchange. Journal of Biological Chemistry, 1980, 253, 6032-6035.

2. Namboodiri, M.A.A., Weller, J.L., and Klein, D.C.: Rapid and reversible activation of pineal acetyl CoA hydrolase in intact pineal cells by disulfide exchange. Biochemical and Biophysical Research Communications, 1980, 96, 188-195.

3. Klein, D.C., Weller, J.L., Auerbach, D., and Namboodiri, M.A.A.; Regulation of pineal N-acetyltransferase: Focus on "turn-off". In Enzymes and Transmitters in Mental Disease (ed. M. Youdim and E. Usdin), 1980, 603-627.

4. Kapatos, G., S. Kaufman, Weller, J.L., and Klein, D.C. (1981): Biopterin: Adrenergic regulation in the pineal gland. Science (in press).

5. Namboodiri, M.A.A., Favilla, J.T., and Kelin, D.C. (1981): S-S peptides intactivate pineal N-acetyltransferase: Insulin is the best. Science (in press).

6. Klein, D.C. and Namboodiri, M.A.A. (1981): Inactivation of pineal N-acetyltransferase by disulfide exchange: A possible role of S-S peptides. in (Weiner, N., Usdin, E., and Youdim, M., eds.) Monoamine Enzymes (in press).

7. Namboodiri, M.A.A. and Klein, D.C. (1981): Purification of sheep pineal N-acetyltransferase. In (Weiner, N., Usdin, E., and Youdim, M., eds.) Monoamine Enzymes (in press).

8. Kapatos, G., Kaufman, S., Weller, J.L., and Klein, D.C.; Regulation of pineal biopterine in (Weiner N., Usdin, E., and Youdim, M., eds.) Monoamine Enzymes (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00097-11 LDN

PERIOD COVERED
October 1, 1980 through September 31, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of Neuroendocrine Metabolism: Melatonin Physiology (rat, hamster, rhesus monkey)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	D. Sugden	Visiting Fellow	LDN	NICHD

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LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Efforts are directed at fully describing the physiology of a putative neuroendocrine hormone, melatonin. Current interest is centered on describing the amount of this coumpound present in body fluids throughout life, and understanding the factors controlling circulating melatonin.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To study the factors regulating the amount of circulating melatonin, a putative pineal hormone during life.

Methods Employed: (1) Melatonin metabolism, fate, and distribution is studied using [^{3}H] melatonin. Thin layer chromatography is used to isolate [^{3}H] melatonin and its metabolites. (2) Melatonin is measured by radioimmunoassay.

Major Findings: (1) The development of the rhythm in pineal melatonin has been studied in several species including the hamster and the rat. In both animals the rhythm in melatonin is absent at birth and develops at about the end of the second week of life. The development of this rhythm closely parallels the development of enzymatic machinery required for the last step in melatonin production, the enzyme involved being hydroxyndole-O-methyltransferase. In addition, it has been found that the photic regulation of the melatonin rhythm in the rat is apparent as soon as the animal is capable of synthesizing melatonin on a rhythmic basis. This would indicate that the neural connections between the eye and the pineal gland are complete by the end of the second week of life. (2) Cerebrospinal fluid was continuously collected from the cisternal-cervical subarachnoid space of chair-restrained rhesus monkeys. The concentrations of melatonin and cortisol were measured in the cerebrospinal fluid. Under diurnal lighting (light: dark, 12:12h) melatonin concentrations were elevated during darkness and low during illumination. The melatonin rhythm persisted in constant darkness but was suppressed in constant illumination. Under diurnal lighting, cortisol concentrations were elevated in the early portion of the light period. This daily rhythmicity of cortisol secretion was not altered by constant illumination or constant darkness. The differential response of the two hormones to constant light suggests that the daily fluctuation of melatonin secretion was not responsible for the daily rhythmicity of cortisol secretion in the rhesus monkey. (3) A Perifusion system for studying rat pineal cells was developed. Data show that the effect of adrenergic stimulation of melatonin secretion agree with those obtained in static systems. This system makes it now possible to study pineal cells under a dynamic situation with noninvasive techniques which allows for the monitoring of membrane potential. (4) The effects of alterations in environmental lighting on the daily rhythm in CSF melatonin were studied in the rhesus monkey. It was found that acute exposure to darkness during the day did not increase normally low daytime CSF melatonin levels, that light suppressed the normally high CSF melatonin values at night, and that 12 hr phase shifts in the diurnal lighting cycle caused 12 hr phase shift in the rhythm. The daily rhythm persisted for 6 and 1/2 d of study in constant darkness and the phase of the rhythm was not affected in constant darkness by a 12 h phase shift in the daily delivery of food and daily care of the animals. These results support the conclusion that the melatonin rhythm in this primate species is endogenous in nature, and that light can act to both entrain the rhythm and to acutely suppress melatonin production.

Significance to Biomedical Research and the Program of the Institute: A growing body of knowledge points to the pineal gland as the source of a compound that inhibits reproduction, and that this compound is melatonin. An

increase in our understanding of how melatonin production is regulated, when production is first regulated, when production is initiated, the relative importance of melatonin from mother's cow milk, the fate of melatonin, and the biological effects of melatonin analogs and metabolites is in accord with the Institute's mission to study reproduction.

Proposed Course of the Project: To determine if there are genetically distinct varients available among the genetic strains of mice in which melatonin synthesis is altered. To evaluate the possible use of the melatonin rhythm in testing neurotoxicity during development.

Publications:

1. Tamarkin, L., Reppert, S.M., Orloff, D.J., Klein, D.C., Yellon, S.M., and Goldman, B.D.: Ontogeny of the pineal melatonin rhythm in the Syrian (Mesocricetus auratus) and Siberian (Phodopus sungorus) hamsters and in the rat. Endocrinology, 1980 107, 1061-1064.

2. Perlow, M.J., Reppert, S.M., Boyar, R.M., and Klein, D.C. (1981): Daily rhythms in cortisol and melatonin in primate cerebrospinal fluid: Effect of constant light and dark. Neuroendocrinology 32, 193-196.

3. Klein, D.C., Namboodiri, M.A.A., and Auerbach, D.A. (1981): The melatonin rhythm generating system: Developmental aspects. Life Sciences 28 1975-1986.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00700-04 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cell interactions in synaptogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C.N. Christian	Staff Fellow	LDN NICHD
	P. Sonderegger	Guest Worker	LDN NICHD
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	C.E. Morris	Guest Worker	LB NINCDS
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	Z. Vogel	Visiting scientist	Weizman Inst.

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project continues the study of the interaction between nerve and muscle during synaptogenesis. A receptor aggregation factor (RAF), produced specifically by neurons, aggregates the acetylcholine receptors (AChR) of cultured embryonic myotubes. The nature of the RAF and its effects on muscle are relevant to the development of the neuromuscular junction.

The mechanism of RAF action was studied. Substances known to bind to and to crosslink the AChR produce small AChR microaggregates but prevent the formation of large aggregates by RAF. When the lipid composition of the myotube membrane was changed by the addition of fatty acids, only linoleic acid increased the AChR aggregation. Various components of basement membrane alone or with RAF, had no effect on AChR distribution, except laminin, which acted synergistically with RAF to produce AChR aggregation.

Further purification of RAF has shown that it is a glycoprotein. Factors derived from neurons also produce a general trophic effect on myotube cultures and decrease the rate of AChR degradation. These effects co-purify with the RAF.

PHS-6040
(Rev. 2-81)

Objectives:

To purify, isolate and characterize the RAF, define the biological processes to which it is involved, and to study the mechanisms of its activity. To relate its molecular properties to the cellular interactions in synaptogenesis.

Methods Employed:

As described in a previous annual report. Cultured embryonic rat myotubes are used as the bioassay system for the various effects of factors produced by neurons.

The distribution of surface AChR is assessed by labelling muscle cultures with fluorescent alpha-bungarotoxin and observing myotubes with a fluorescence microscope. The metabolism of the AChR is determined by turnover studies using radioactive I^{125} alpha-bungarotoxin.

The pharmacological responsiveness of muscle cells and the AChR channel kinetics are determined by standard electrophysiological methods and patch clamp techniques.

Binding studies involve measuring the on-rate of alpha-bungarotoxin binding in the presence of various nicotinic agonists and antagonists.

Purification of RAF has employed general analytical and preparative biochemical techniques. Serum-free cell culture is used to increase the specific activity of RAF. For large-scale purification, RAF is extracted from embryonic brains or continuous neuronal cell lines grown as tumors in vivo.

Major Findings:

The role of direct cross-linkage of the AChR in aggregation was explored. The lectin Concanavalin-A or a rabbit antiserum to the AChR were incubated with myotube cultures. Both substances inhibited the formation of receptor aggregates. A positive correlation between the titer of various anti-AChR antisera and the degree of inhibition of the RAF effect was observed. Some anti-AChR antiserum decreased the rate of degradation of the surface AChR. These antisera and the lectin Con A can themselves produce microaggregates of the receptor, which appear in random distribution on the surface of the myotubes.

The addition of various fatty acids to myotube cultures was tested for their ability to aggregate AChR. Linoleic acid alone produced an increase in receptor aggregates but did not act synergistically with RAF. Other fatty acids tested (myristic acid, steric acid, oleic acid and arachidonic acid) had no significant effect on the number of ACh receptor aggregates per myotube. The effect of linoleic acid will be related to changes in the fluidity of the myotube plasma membrane or to changes in the lipid composition of the membrane.

The role of the basement membrane in AChR aggregation was assessed by adding purified basement membrane components to myotube cultures. Although the proteo-

glycams and collagens tested had little effect, laminin increased AChR aggregation and acted synergistically with RAF. It is proposed that RAF interacts directly with laminin to promote basement membrane formation, which directs the local accumulation of muscle surface AChR.

The effect of RAF or laminin in vitro is relatively long lasting. After treatments of as short as 12 hours, an increased number of AChR aggregates is seen 3 days after removing these compounds.

Medium conditioned by neuroblastoma x glioma hybrid cells NG108-15 is a rich source of RAF. This medium also increases the leucine incorporation into myotube cultures. This trophic activity co-purifies with the RAF. Medium conditioned by hybrid cells which lack detectable RAF also lacks trophic activity. Medium conditioned by NG108-15 cells also decreases the rate of degradation of the myotube surface AChR. This activity also co-purifies with the RAF.

Another developmental parameter of striated muscle is its responsiveness to d-tubocurarine. Whereas d-tubocurarine is an antagonist in adult muscle, it is an agonist in embryonic muscle. Cultured myotubes exhibited an embryonic response to d-tubocurarine and it was demonstrated that it briefly opens AChR channels. Binding studies indicate that with respect to d-tubocurarine binding, the AChR of cultured myotubes exhibit either multiple states or negative cooperativity.

Further purification of RAF has been achieved. This protein has been found to bind to and be specifically eluted from wheat germ and Ricinus communis Type II lectin columns. A weak interaction has also been found with heparin immobilized on agarose. The RAF activity in medium conditioned by NG108-15 cells is lost when incubated with laminin immobilized on sepharose. An interaction between laminin and RAF can be postulated. However, attempts to release biologically active RAF from immobilized laminin have thus far been unsuccessful.

Although the yield was small, the specific RAF activity of medium conditioned by NG108-15 cells was markedly increased by adapting these cells to growth in serum free medium. To scale up the purification of RAF, we are currently extracting it from embryonic pig brain or from neuroblastoma tumors grown in mice.

Proposed Course: To continue the purification of RAF and to produce antibodies against it. We will then study the distribution and developmental regulation of RAF in neuronal tissues. We will also characterize the interaction of RAF with the muscle membrane and basement membrane.

Publications:

Axelrod, D., Bauer, H.C., Stya, M. and Christian, C.N.: A factor from neurons induces partial immobilization of nonclustered acetylcholine receptors receptors on cultured muscle cells. J. Cell Biol. 88: 459-462, 1981.

Bauer, H.C., Daniels, M.P., Pudimat, P., Jacques, L., Sugiyama, H. and Christian, C.N.: Characterization and partial purification of a neuronal factor which increases acetylcholine receptor aggregation on cultured muscle cells. Brain Res. 209: 395-404, 1981.

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Christian, C.N., Bergey, G.K., Daniels, M.D. and Nelson, P.G. Synapse formation and stabilization in neuronal developments. J. Exp. Biol. 89: 85-101, 1980.

Christian, C.N. and Nelson, P.G.: Synapse formation of continuous cell lines. Pontifical Acad. Scientiarum Scripta Varia 45: 135-162, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00702-01 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Genetics of Primate Vocal Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.D. Newman Physiologist LDN NICHD

COOPERATING UNITS (if any)

N.F. Ma, New England Regional Primate Research Center

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Brain and Behavior

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project will focus on studying the inheritance patterns of well-defined vocalizations in selected primates, particularly the squirrel monkey. Cross-breeding of closely related species or subspecies with distinctly different vocalizations will provide the opportunity for analysis of the parental contributions of hybrid vocal traits. Collaboration with other units expert in cellular genetic methods is expected to provide information regarding the transmission of genetic material to hybrid offspring as well as the genetic distances between members of the parental populations. Additional primate species will be examined for presence of distinctive vocal traits and interspecific hybridization, to evaluate their usefulness in this area of genetic research.

PHS-6040
(Rev. 2-81)

Objectives: To understand the genetic mechanisms underlying the transmission of vocal traits in primates.

Methods Employed: Work on the inheritance patterns of squirrel monkey vocalizations has so far concentrated on one vocalization, the Isolation Peep (IP). Our prior work has shown that this vocalization is highly stereotyped, that distinctive differences in its structure in 2 different subspecies of squirrel monkey are present at birth, and that these differences remain throughout the life of the individual, regardless of subsequent experience. Quantitative procedures have been developed that permit measuring selected parameters of a large number of IPs, and statistically classifying individual IPs as to one or the other of these 2 types. This statistical procedure is highly reliable, correctly classifying IPs as to species origin with 100% accuracy. In as much as these 2 subspecies of squirrel monkey have been shown to produce viable offspring when cross-bred, the analysis of the transmission of parental IP characteristics to their hybrid offspring in these animals was initiated.

Major Findings: Isolation Peeps of 12 F_1 hybrid squirrel monkeys have been subjected to discriminant analysis for the purpose of classifying each call as to parental type ("Roman" or "Gothic"). Isolation Peeps were collected at intervals from 1 week to 1 year of age. Calls from hybrids with "Roman" mothers were classified as Roman in 98% of the cases. However, IPs from hybrids with "Gothic" mothers were classified as "Gothic" in only 46% of the cases. Discriminant analysis was also used to classify the IPs of backcross offspring (hybrid mother x Roman or Gothic father). Backcrosses from Roman-type hybrid mothers and Gothic fathers produced IPs that were classified as Roman in 90% of the cases. A backcross from a Gothic-type hybrid mother and Roman father produced IPs that were classified as Roman in 87% of the cases.

Taken together, these results suggest that the "Roman" phenotype is more effectively expressed than is the "Gothic" phenotype. Inspection of individual discriminant scores of the IPs from purebred Roman and Gothic individuals fit this suggestion, in that Roman scores are tightly clustered around the average score for the Roman population, whereas the Gothic scores are widely dispersed, some nearly overlapping with Roman scores. Our collaborations with Dr. Nancy Ma may yield data at the cytogenetic level that correlate with differences in IP phenotype in different squirrel monkey populations.

In an effort to extend the work on inheritance of primate vocalizations, Dr. Newman visited other facilities with hybrids of other primate species. A visit to the Field Station of the Yerkes Primate Research Center was made to record vocalizations from hybrids between 2 species of macaques (Macaca nemestrina and M. nigra). A two-day trip to the Primate Center at Duke University resulted in tape recordings from infants of 3 species of lemurs, Lemur macaco, L. fulvus, and L. catta. Calls of L. macaco and fulvus infants separated from their mother were similar in structure. The only hybrids currently available involved these 2 species, and the similarity of their calls rendered evaluation of hybrid vocalizations problematic. The calls of

isolated infant L. catta were distinctive, being tonal in quality (like the IP of squirrel monkeys but much lower in frequency). Discussions with the manager of the Center concerning future crossbreeding of L. catta and one of the other 2 species were initiated.

Significance to Biomedical Research and the Program of the Institute:

The importance of genetic mechanisms for development of normal communication and their involvement in communicative disorders is widely recognized but poorly understood. The availability of a vocalization which differs in quantifiable dimensions between 2 species producing viable hybrid offspring provides a unique opportunity to study the genetic basis of communicative behavior in primates. Correlating the inheritance patterns of communicative behavior with transmission mechanisms of genetic information to offspring will provide knowledge essential to an understanding of the genetic programming of normal behavior.

Proposed Course: Current plans include production of additional hybrids by enlarging our breeding colony. Investigations of fertility in male hybrids is in progress, and it should soon be clear whether F_2 hybrids can be produced in the future. Possible collaborative efforts with investigators using isoenzyme analytical methods will be explored, to attempt to identify transmission characteristics and genetic distance measures between vocally distinct primate species at the cellular level.

Publications:

Newman, J.D. and Symmes, D.: Inheritance and experience in the acquisition of primate acoustic behavior. In Brown, C.H., Petersen, M.R. and Snowdon, C.T. (Eds.): Primate Communication. New York, Cambridge University Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00703-01 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of long chain fatty acids on developing neurons in cell culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. E. Brenneman	PRAT Fellow	LDN NICHD
Others:	R. McGee		Georgetown Univ.

COOPERATING UNITS (if any)

Dept. of Pharmacology, Georgetown University

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The electrophysiological effects of long chain free fatty acids (FFA) have been studied on large multi-polar spinal cord cells in tissue culture. In vitro enrichment with FFA produced alterations in action potential characteristics which were dependent on the structure of the FFA supplement and the duration of FFA exposure. Resting membrane potential was not affected by the FFA treatments. Phospholipid fatty acyl composition of spinal cord cultures revealed small but significant changes with FFA supplementation. These studies indicate that lipids can alter the functional properties of developing neurons in vitro.

PHS-6040
(Rev. 2-81)

Objectives: Two goals are being pursued: 1) to investigate lipid nutritional factors which could influence developing neurons in tissue culture and, (2) to develop an understanding of the relationship between neuronal functions and the lipid phases of neuronal membranes.

Methods Employed: Cell cultures of fetal mouse spinal cord were prepared as described in previous reports from this laboratory. Intracellular recordings were made with 4 M KAc electrodes using a standard bridge configuration. Free fatty acid-bovine serum albumin complexes were prepared by a soap method. The concentration of FFA in the supplement was 100 M at a molar ratio of 4 (FFA/BSA). The fatty acyl analysis of cell phospholipids was performed by standard techniques employing column and gas chromatography.

Major Findings: The relationship between action potential (AP) characteristics and nutrient fatty acid structure was investigated in spinal cord neurons maintained in culture for 3 weeks. Fatty acid supplementation had no effect on resting membrane potential even after 1 month of incubation with the lipids. These data indicate that there was no gross toxicity associated with this supplement paradigm.

Short term incubation (18-24 hrs) with various fatty acid structures produced effects on electrical activity with only 2 of the 5 lipids tested. Myristic acid (14:0) produced a 60% increase in AP rate of rise as compared to control cells. In addition, cells given 14:0 had a 43% increase in AP rate of fall and a significant increase ($p<0.025$) in spike height (11 mV). Addition of linolenic acid (18:3) increased the AP rate of rise by 23% but had no effect on the rate of fall or spike height. Supplementation with oleic acid (18:1), linoleic acid (18:2) or arachidonic acid (20:4) had no effect on AP characteristics after 18-24 hrs exposure.

Neurons which had been incubated 4 days with 14:0, 20:4, 18:2, or 18:3 exhibited a 23-50% increase ($p<0.025$) in AP rate of rise as compared to control cells. In contrast, cells given 18:1 had 22% decrease ($p<0.025$) from control values. Similar relationships were observed between rate of fall and FA structure. AP height was significantly increased with 20:4, 18:2, or 14:0 but not with 18:3 or 18:1.

Significance to Biomedical Research and the Program of the Institute: These studies indicate that fatty acids can alter the electrical properties of developing neurons in tissue culture. These findings are related to the overall interest of the Institute in evaluating nutritional factors in developing organisms.

Proposed Course: The mechanism(s) by which certain fatty acid structures produce alterations in the electrophysiology of large spinal cord neurons will be the focus of future experiments. Membrane capacitance measurements and neurotoxin binding studies may contribute to our understanding of these effects. In addition, we want to investigate membrane-related neurotransmitter functions after lipd supplementation. Such functions include: GABA uptake, QNB binding, and diazepam binding.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00704-01 LDN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Physiologic Effects of Tetanus Toxin on Nerve Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.K. Bergey	Medical Officer	LDN, NICHD
	H. Bigalke	Visiting Scientist	LDN, NICHD
Others:	P.G. Nelson	Chief, Lab. of Dev. Neurobiol.	LDN, NICHD
	S. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS (if any)

W. Habig, Bureau of Biologics, FDA

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0		

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dissociated mouse spinal cord neurons in culture were used to investigate the effects of tetanus toxin at the synaptic level. After a dose-dependent lag period tetanus toxin produced convulsant activity in the cultures as characterized by depolarizing shifts and burst of action potentials. This lag period suggests some membrane and/or cellular interactions with the toxin are necessary for convulsant action. The convulsant activity was synchronous in adjacent neurons and synaptically mediated. No effects of tetanus toxin on postsynaptic membrane properties or responses to iontophoretically applied amino acids could be demonstrated. After several hours in tetanus toxin the toxin produced blockade of both excitatory and inhibitory synaptic activity (spontaneous and evoked). This tetanus produced synaptic blockade was long lasting, persisting for weeks after removal of exogenous toxin. No effects of the toxin on the calcium component of the spinal cord action potential could be demonstrated, suggesting that the toxin produces blockade of synaptic transmission by presynaptic mechanisms different from those mediating calcium flux.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Utilizing spinal cord neurons in culture the effects of direct application of tetanus toxin were investigated. The acute and chronic effects of the toxin on individual neurons and synaptic networks were characterized to further elucidate the synaptic mechanisms of action of the toxin.

Methods Employed: The dissociated fetal mouse spinal cord system previously characterized in this laboratory was utilized. Purified, crystallized tetanus toxin was prepared by the Bureau of Biologics, FDA. Intracellular recordings were obtained using potassium acetate-filled electrodes and a conventional bridge circuit.

Major Findings:

Tetanus toxin when applied to the spinal cord neurons in culture resulted in convulsant effects characterized by abrupt paroxysmal depolarizations and triggered action potentials. The onset of convulsant action occurred in a dose-dependent manner that was roughly exponential. However, even at the highest toxin concentrations used (10^{-5} g/ml, equivalent to 10^5 mouse lethal doses per ml) an obligatory lag period of about 20 minutes was observed prior to the onset of convulsant activity. This is in contrast to the abrupt onset of action of convulsants such as strychnine and picrotoxin that act a post-synaptic antagonists of inhibitory amino acids. Presumably the lag required for tetanus toxin action results from binding of the toxin and subsequent membrane and/or cytosol interactions. That the lag period is not simply due to binding is demonstrated by the marked temperature dependence of the convulsant action (Q_{10} of between 3 and 10 from 25° to 35° C), whereas binding of the toxin is actually enhanced at lower temperatures.

The convulsant action of tetanus toxin was synaptically mediated. All cells in a given network fired synchronously and convulsant action was abolished by high magnesium.

No effects of tetanus toxin on postsynaptic responses to the putative amino acid neurotransmitters, GABA, glycine and glutamate could be demonstrated. No postsynaptic effects of tetanus toxin on membrane parameters was seen.

With more chronic exposure to the toxin, the frequency of the paroxysmal events diminished and ultimately synaptic silence occurred after two to six hours, depending upon toxin concentration (10^{-5} to 10^{-8} g/ml). No spontaneous or evoked synaptic connections could be demonstrated after such chronic exposure to tetanus toxin. This is in distinct contrast to control situations where over 85% of spinal cord neurons sampled electrophysiologically demonstrate spontaneous synaptic activity and evoked monosynaptic pairs can be rountinely identifed.

While the acute convulsant action of tetanus toxin on the spinal cord neurons appears to result from functional disinhibition, chronic exposure to the toxin results in blockade of excitatory as well as inhibitory synaptic transmission. Whether inhibitory transmission is indeed preferentially affected by tetanus toxin remains to be determined. The tetanus toxin produced syaptic blockade

is long lasting; even three weeks after removal of exogenous toxin mature cultures demonstrated continued synaptic silence. Cells exposed chronically to tetanus toxin appeared morphologically normal and had normal membrane properties.

To determine whether the synaptic blockade produced by tetanus might be a result of reduction of calcium flux, the effects of tetanus toxin on the tetrodotoxin resistant calcium component of the action potential of spinal cord neurons were measured. No significant effect on the rate of rise or maximum duration of the calcium component could be demonstrated. This negative result is consistent with data from other laboratories regarding measured calcium flux in synaptosomes and suggests that a mechanism distinct from calcium is responsible for the presynaptic effects of tetanus toxin on synaptic transmission.

Significance to Biomedical Research and the Program of the Institute:

An in vitro system for the study of effects of tetanus toxin upon spinal neurons is appealing as an adjunct to intact systems because it eliminates the requirements of axonal transport and permits direct access of known toxin concentrations to all the neurons in the preparation. The ability to study selected neurons and pairs at various times after toxin application provides a direct approach to the synaptic action of the toxin. Chronic actions of the toxin can be investigated using toxin concentrations that would be lethal in intact animals.

Tetanus toxin is distinct from other convulsants in acting at a presynaptic locus. The evolution of convulsant activity seen with tetanus in contrast to other convulsants may provide insights into early network mechanisms underlying paroxysmal activity.

The ability of tetanus toxin to provide chronic blockade of synaptic transmission even after removal of exogenous toxin makes it an interesting probe for the study of certain neurobiologic phenomena, specifically how chronic blockade of synaptic activity affects neuronal development and synaptogenesis.

Proposed Course

The effects of tetanus toxin on identified monosynaptic inhibitory and excitatory cell pairs will be studied to determine whether the toxin preferentially affects inhibitory synaptic transmission versus excitatory. This will hopefully elucidate whether the convulsant action of the toxin is due to initial blockade of inhibitory synaptic events or whether perhaps functional disinhibition occurs via other mechanisms whereby all synaptic transmission is affected. All evidence to date indicates that tetanus toxin acts at a presynaptic site, however, this evidence is indirect. Quantal analysis of monosynaptic connections affected by tetanus should provide more direct evidence for a presynaptic mechanism.

Experments have shown that tetanus toxin can produce chronic synaptic blockade lasting weeks. Using I^{125} labeled toxin with subsequent removal of exogenous toxin cultures will be examined autoradiographically (light and EM) and on gel columns. Correlative physiology will also be performed. Hopefully the composition of the toxin (i.e. whether whole or in subunits) as well as cellular and subcellular associations can be determined.

This laboratory has been interested in the affects of chronic blockade of synaptic activity on subsequent neuronal development. Tetrodotoxin has been shown to have profound affects on spinal cord neuron survival when applied early in development. Tetanus toxin with its ability to provide chronic blockade of synaptic transmission now provides us with another tool for investigating these phenomena via a different mechanism.

Publications

None

Laboratory of Molecular Genetics

Project Number	Title	Principal Investigator
Z01 HD 00066	Control of Mechanisms in Temperate Baceriophage λ	Robert Weisberg
Z01 HD 00067	Integrative Control of Macromolecular Synthesis	Michael Cashel
Z01 HD 00068	Factors Influencing Genetic Transcription- Initiation and Termination	Robert J. Crouch
Z01 HD 00069	Molecular Aspects of the Replication of Enveloped Animal RNA Viruses	Judith G. Levin
Z01 HD 00070	Morphogenesis of Animal Viruses During Infection of Mammalian Cells	Jacob V. Maizel, Jr.
Z01 HD 00071	Study of Adenovirus Gene Functions	Heiner Westphal
Z01 HD 00072	Crystallin Synthesis in Development and Disease	Joram Piatigorsky
Z01 HD 00073	Regulation of Immune Systems at the Cellular Level	Edgar E. Hanna
Z01 HD 00074	Regulated Gene Expression During Cell Growth and Differentiation	Philip Leder
Z01 HD 01000	The Molecular Genetics of the Major Histocompatibility Complex	Jonathan G. Seidman

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981

Laboratory of Molecular Genetics

The Laboratory of Molecular Genetics conducts research directed towards understanding the molecular processes involved in the transmission of genetic information and its regulated expression during growth and differentiation. A variety of model systems are under investigation, ranging from bacterial and animal viruses to complex vertebrate regulatory responses involving the synthesis of specific antibodies and the development of specific organ systems. Taken together, the Laboratory joins active workers in the areas of genetics, biochemistry, electron microscopy, immunology, virology and cellular biology to bring their skills to bear on problems of mutual interest. At present the Laboratory is comprised of ten research groups distributed among six sections.

I. Section on Gene Organization and Control

A. Studies have been directed towards understanding the regulated expression and organization of two sets of mammalian genes, those involved with globin and those involved with immunoglobulin production. In earlier reporting periods we developed strategies for cloning a number of genes derived from each system. During the past year we have progressed considerably with both a structural and functional analysis of these genes. In terms of the globin system we have completed physical maps of the murine alpha and beta loci and determined the location and complete structural sequence of an embryonic beta-like gene. We have also discovered that the previously noted alpha globin pseudogenes are dispersed; that is, they reside on chromosomes that differ from the major locus. This observation suggests the operation of a very dynamic mechanism that can amplify and convey gene sequences during evolution. In terms of the human immunoglobulin system we have cloned genes covering the light and heavy chain loci and identified and, in many cases, sequenced genes corresponding to the kappa, lambda, mu, delta, gamma, epsilon and alpha chains. We have also identified sequences within these loci that are associated with V/J, V/D/J and switch region recombination. All of the switches are involved with the somatic rearrangement of DNA. The last mentioned signals occur in multiple copies outside this locus.
(Dr. Philip Leder -- Principal Investigator)

B. Studies have been directed towards understanding the regulatory pathways controlling immune systems at the cellular level. The integrated roles of regulatory T-lymphocytes, macrophages and responder B-lymphocytes are being delineated in vitro. We use monoclonal and perpetual cell lines constructed within fractionated cell populations obtained from NFR/N nude (nu/nu) and +/nu mice by the hybridoma technology. Microbial and plant agents are known to variously affect the immune system in animals and humans. This natural circumstance is being exploited in order to understand pathways toward the regulation of the immune system. A streptococcal protein (SPE, an exotoxin) was shown to profoundly cancel the activity of normal suppressor T-lymphocytes in experimental antibody responses to haptenic immunogens. In recent experiments we have been able to distinguish and separate two populations of T-lymphocytes. One resonds to SPE while the other does not. This has allowed us to formulate two alternative models of the development of immune regulation by T-cells. One is that suppression and help are different functions associated with the same T-cells but at different stages of their development. The other is that suppression and help are different functions of different T-cell lineages. In the former, SPE could drive the development selectively along the helper course. In the latter, SPE could inactivate the suppressor population causing an unbalanced helper activity. A helper T-cell hybridoma has been constructed and cloned. Other T-cell hybridomas have been identified which possess graded activity profiles from suppression to elevated help. It is now possible to ask the questions about dynamics and stability of these phenotypically different cell lines. Thus this approach should allow us to map the various pathways of cellular regulation of immune responses and immunocyte circuitry within immune systems. (Dr. Edgar E. Hanna -- Principal Investigator)

C. Studies have been directed towards understanding the molecular events involved in the replication and assembly of RNA tumor viruses. Stability of viral messages and utilization of intracellular viral RNA species for encapsidation or messenger function are being investigated under conditions which block ongoing synthesis of viral RNA (actinomycin D system). Kinetic studies involving liquid hybridization experiments as well as Northern blot analysis have demonstrated that (i) viral 35S and 21S mRNA species have a slow rate of turnover; and (ii) the rate of turnover of viral messages is slower than that of 35S viral RNA which is ultimately packaged into virions. These results indicate that two functionally independent pools of genome-size (35S) viral RNA exist within the infected cell. The data also suggest that there may be an intrinsic difference in the metabolic stabilities of individual viral mRNAs. Work has continued on a system in which metaphase chromosomes are used to transfer viral genetic information to uninfected cells. Both mouse cells and heterologous mink cells can be productively infected in an apparently stable manner. Restriction enzyme analysis of genomic DNA from transfected mouse cells indicates that murine leukemia virus genetic information is integrated into high molecular weight DNA of the recipient. In addition, chromosomes isolated from a murine sarcoma virus-infected nonproducer cell line have been shown to induce characteristic foci in previously uninfected, nontransformed recipients. Finally, efforts are being directed towards identifying the genetic lesion in a nonconditional reverse transcriptase mutant, using recombinant DNA technology. (Dr. Judith G. Levin -- Principal Investigator)

D. Studies have been directed towards understanding the organization of the genes encoding the major transplantation antigens (murine major histocompatibility antigens). We expect that recombinant DNA techniques will eventually allow much better understanding of this multigene complex. As an initial step we have obtained cDNA clones corresponding to beta-2 microglobulin, and an H-2-like mRNA. We are attempting to clone murine I-A cDNA. The single beta-2 microglobulin gene and the multiple H-2-like genes have been cloned with the aid of these cDNA clones. The organization and the expression of these cloned genes are being studied.
(Dr. Jonathan G. Seidman--Principal Investigator)

II. Section on Cellular Differentiation

Studies are being directed towards understanding the molecular events related to crystallin gene expression and protein synthesis in the vertebrate lens during development and disease (cataract). cDNAs for the main structural protein of the verbetrate lens (crystallins) have been cloned and are being sequenced. These cDNAs are being used as probes for analysis of their respective genes and for analysis of crystallin gene expression in the lens. Temperature-sensitive viruses (Rous sarcoma virus) have been used in order to examine the shut-off of crystallin transcription in cultured lens epithelial cells. Further studies are being directed towards understanding the inhibitory role of ions for crystallin synthesis during cataractogenesis, a phenomenon discovered in this section several years ago.
(Dr. Joram Piatigorsky -- Principal Investigator)

III. Section on Molecular Structure

Studies have been directed towards understanding the structure of animal virus genomes and the way viruses gain control of the host cell's molecular processes. The poliovirus genome has been examined for features that allow modulation of its expression through interaction with enzymes or regulating proteins and that can alter the direction of the information flow toward replication, translation or morphogenesis. DNA clones from reverse transcripts of the RNA have been made. Computer analysis of the published sequence has begun. Comparative studies with rhinoviruses and hepatitis A virus are underway. Liposomes have been used to introduce anti-RNP antibodies, into adenovirus cells where they alter the expression of fiber protein selectively. Specific separated complementary nucleic acids are being introduced into cells to observe transformation, transient expression or antagonistic effects using biochemical and electron microscopic procedures. Computer analysis of poliovirus, adenovirus, globin, and immunoglobulin sequences has continued to be employed in the study of molecular genetics. Recently developed programs to predict secondary structure in single-stranded polynucleotides have been correlated with other observations that suggest a subtle and considerable role for these structures in gene expression. (Dr. Jacob V. Maizel, Jr.--Principal Investigator)

IV. Section on Animal Viruses

Studies have been directed towards an understanding of early adenovirus gene control. Pathways of viral gene regulation were analyzed by injecting viral DNA fragments into nuclei, or mRNAs into the cytoplasm of suitable host cells. Expression of the injected macromolecules was recorded by gel analysis of DNA or proteins. We found that one early adenovirus function, needed to promote growth of a defective adeno-associated virus, is expressed after no less than three separate early gene blocks have interacted in an ordered sequence of regulatory events.

Growth properties of adeno-SV40 hybrid viruses in simian and human cells is the topic of a joint study with Dr. A.M. Lewis, NIAID. The host was found to determine the structure of the SV40 DNA segment in the hybrid virus genome. Mechanisms causing these structural changes are under current investigation. (Dr. Heiner Westphal -- Principal Investigator)

V. Section on Molecular Regulation

A. Work is concerned with mechanisms coordinating cellular gene expression with growth rate transitions. Focus is on the physiological behavior of mutants displaying altered patterns of guanosine 3',5'-pyrophosphate (ppGpp) regulation. Ribosomal RNA gene regulation is studied as a model gene sensitive to nutritional and growth rate control. The regulatory characteristics are localized to the promoter region, which is fused to galactose operon genes both in plasmid and in lambda vectors. Dissection of structural features determining regulatory characteristics of this region is by genetic deletion analysis, mutation and by chemical synthesis. In vitro assay systems for gene expression in crude extracts are being improved as a result of removal of nuclease activities accompanying the introduction of mutant alleles in the source strain. (Dr. C. Michael Cashel -- Principal Investigator)

B. Studies have been directed toward understanding the role of certain ribonucleases in processing RNA. Studies of the processing of ribosomal RNA from chick embryos by RNase DII, an enzyme first isolated in this laboratory from chick embryos, has centered on the primary and secondary structure of the sequences involved in processing. Restriction enzyme analysis and sequence determination suggest that at least one of the cleavage sites is formed by the 3' terminus of 5.8S rRNA forming a stable duplex with the 5' terminus of 28S rRNA and cleavage occurs at--or near--the mature ends. 58S rRNA can exist in the precursor form in two alternate forms. One form occurs if the 5.8S rRNA hydrogen bonds with itself while a second interation occurs when 5.8S rRNA interacts with the region of the precursor containing the sequences for 28S rRNA. Either form could easily be a site for recognition of RNase DII. (Dr. Robert J. Crouch -- Principal Investigator)

VI. Section on Microbial Genetics

Studies have been directed towards understanding the mechanisms by which a virus, the bacteriophage λ, inserts its chromosome into and excises it from the chromosome of its host. Both insertion and excision are examples of a general process called site-specific recombination that involves interactions between specific recombination proteins and "attachment" sites on the DNA substrate. An attachment site mutant that changes the specificity of site-specific recombination has been isolated. The properties of this mutation strongly suggest that it alters the region of the attachment site whose normal role is "homologous pairing"; that is, two recombining attachment sites may interact directly and specifically with each other over this region. A novel method has been devised that should make it possible to saturate this segment of the attachment site with mutations. The nucleotide sequences of several recombinants that arose by crossing a wild type attachment site with a naturally occurring mutant have been determined. These sequences confirm the location of the two permissible crossover points used for site-specific recombination and suggest an explanation of the high recombinogenicity of the mutant site. An endonuclease that specifically cleaves branched DNA has been characterized. One substrate for this enzyme is an intermediate in recombination, and cleavage occurs in such a way as to give a molecule that is on the pathway to recombinant DNA. (Dr. Robert A. Weisberg - Principal Investigator)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00066-11 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Control of Mechanisms in Temperate Bacteriophage λ

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Weisberg	Microbiologist	LMG NICHD
OTHERS:	Eric Flamm	Staff Fellow	LMG NICHD
	Edward Appelbaum	Staff Fellow	LMG NICHD
	Christine Gritzmacher	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any) Dr. Howard Nash, LNC, NIMH; L. Enquist, LMV, NCI; A. Landy, Brown University, Providence, R.I.; B. Kemper, University of Cologne, Cologne, W. Germany

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Microbial Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long term goal of this project is to determine the mechanism of recombination of virus DNA with the host chromosome. Recombination between virus and host involves specialized regions in each DNA called attachment (att) sites. We have established that recombination between att sites is promoted by a direct interaction between identical nucleotides in a specific sub-region of each site. We have devised a novel way to isolate many different mutations of this sub-region, and we are also using standard techniques to isolate mutants outside of it. We have determined the sequence of a new secondary attachment site and can account for its recombinogenicity. Finally, we have characterized an enzyme that specifically cleaves branched DNA; such an enzyme is thought to have a key role in recombination.

PHS-6040
(Rev. 2-81)

Project Description:

Objective: To understand the mechanism of virus-host recombination as exemplified by the insertion of the bacteriophage λ genome into the Escherichia coli chromosome.

Major Findings:

1. Role of homology in site-specific recombination. We have isolated and characterized a mutation of the "overlap" region of the λ attachment site. The nucleotide sequences of the wild-type and mutant overlap regions are shown below (the changes are in italics):

   TTTATAC -- Wild Type
   TTT*TC*A*A* -- safG mutant

   We call the mutation saf (for site affinity) because it alters the specificity of insertion of λ DNA into the host chromosome. We have found that when one of the parents in a cross is mutant and the other is wild-type the frequency of recombination is depressed 5 to 100-fold relative to a fully wild type cross. However, when both of the parents are mutant, the frequency of recombination is restored to near wild type levels. We conclude that the nucleotides altered by this mutation participate in a DNA-DNA interaction that requires match of the DNA chains to each other rather than (or perhaps in addition to) match to the wild type sequence. In this way site-specific recombination resembles ordinary or homologous genetic recombnation.

2. Isolation of saf mutants. We have shown that mutations of the overlap region can be isolated in the following way. After λ infection, the phage DNA is sometimes inserted into the bacterial chromosome at secondary att sites, which differ in nucleotide sequence from the wild type site. Last year, we showed that all known insertions of λ DNA into secondary sites can be accounted for by assuming that the crossover point (i.e., the point at which the polynucleotide chain was broken and rejoined) could occur at either of two positions: immediately to the left or immediately to the right of the overlap region. The limited evidence we had suggested that excision of the prophage from a secondary site also uses one or the other of these two points, but not necessarily the same point used for insertion. Therefore, a cycle of insertion into and excision from an appropriate secondary site can generate phage with mutations in the overlap region. Indeed, the safG mutation described above was produced in just such a way. Preliminary evidence indicates that we have isolated two new saf mutants by excision from two different secondary attachment sites.

3. Deletion mutants formed by int protein. We have described a λ mutation called xin that increases the activity of the int protein so that att site recombinations that ordinarily occur at low frequency now occur at high frequency. Contrary to previous indications, the mutation appears to alter the structure rather than the level of int protein. One of the

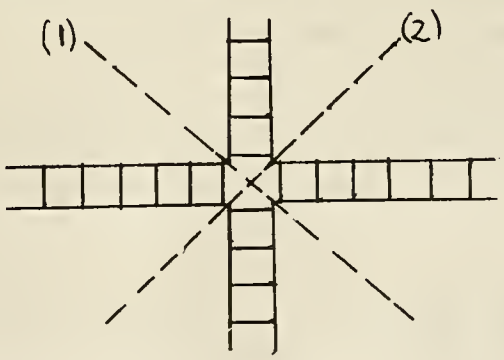
recombinations promoted by xin occurs between the phage attachment site and a particular secondary attachment site. We have sequenced several recombinants with the following results:

C A G C T T T T T A T A C T A A G T T G	Phage site
C A G C T T T↓T↓G C C C G A G A A G A T G	Recombinant I
C A G C T T T T T T A T A C↓G A A G A T G	Recombinant II
C G A C T T A T G C C C G A G A A G A T G	Secondary site

Two types of recombinants have been found, and the possible crossover points are indicated by arrows. Recombinant I could have been produced by crossing over at either of two points. Of these, the left hand point is at the predicted location immediately to the left of the overlap region. What is distinctive about the sequence of this secondary att site? Recently, W. Ross and A. Landy (personal communication) have found that int protein binds there. Comparison of this int binding site to others suggests that CPuPuCTT (or its complement) may be part of a sequence recognized by int protein; these nucleotides are underlined. Note that each putative int recognition sequence (or five of six bases in one case) is present in identical orientation and spacing with respect to each crossover point in the two sites, suggesting a functional relationship between them.

4. Isolation of mutations of the host attachment site. We are isolating, characterizing and sequencing mutants in the host attachment site in order to determine which bases are important for function. We started with a 44 basepair fragment that contains within it the minimum number of bases necessary for fully functional recombination. We have so far isolated five ultraviolet-induced mutants in which recombination is defective, and are in the process of sequencing them.

5. An endonuclease that specifically cleaves branched DNA. In collaboration with Dr. B. Kemper and K. Mizuuchi we have shown that an endonuclease produced by bacteriophage T4 cleaves branched DNA at the branch point. The sites of cleavage are shown in the figure below.



Cleavage occurs along line (1) or line (2), but not both simultaneously.

This is an important finding because branched DNA is thought to be an intermediate in recombination, and cleavage of the type shown is required to convert the intermediate product to recombinant DNA.

Proposed Course of Project:

We plan to saturate the overlap region with mutations in order to determine the role of each nucleotide. We have isolated a transducing phage carrying hip, a host gene required for site-specific recombination. We are using it to identify the protein encoded by hip. In order to analyze the function of the bacterial attachment site, we are sequencing mutants and secondary attachment sites. We are initiating a new project designed to analyze the control mechanisms for nitrogen fixation in the bacterium K. Pneumoniae. We shall determine the nucleotide sequence of a promoter of nitrogen fixation genes and compare it to the sequences of mutant promoters.

Significance to Biomedical Research and the Program of the Institute: The insertion and excision of viral (and other episomal) DNA is known to change the physiology of the host organism. In addition, these viruses (and other episomal elements) provide an important mechanism of genetic transfer and exchange in the microbial world. Viruses promote genetic transfer because of their ability to convert host genes to an infectious form by packaging them inside a shell of viral structural proteins. It is highly likely that such a successful symbiotic relationship has an analogy in the relationship between certain animal viruses and the cells of higher organisms. An understanding of the control mechanisms operative in these easily studied microorganisms has obvious relevance to an understanding of regulatory elements in higher organisms. The ability to direct the insertion of phage DNA to predetermined locations on the host chromosome is an extremely useful and widely applicable method for isolating gene products and studying the control of bacterial gene expression.

Publications:

1. Mizuuchi, K., Gellert, M., Weisberg, R. and Nash, H.: Catenation and supercoiling in the products of bacteriophage λ integrative recombination in vitro. J. Mol. Biol. 141, 485-494, 1980.

2. Nash, H., Mizuuchi, K., Enquist, L. and Weisberg, R.: Strand exchange in λ integrative recombination: Genetics, biochemistry and models. Cold Spring Harbor Symp. Quant. Biol. XLV, 417-428, 1980.

3. Mizuuchi, K., Weisberg, R., Enquist, L., Mizuuchi, M., Buraczynska, M., Foeller, C., Hsu, P., Ross, W. and Landy, A.: Structure and function of the phage λ attachment site: Size, int-protein binding, and location of the crossover point. Cold Spring Harbor Symp. Quant. Biol. XLV, 429-438, 1980.

4. Pinkham, J., Platt, T., Enquist, L. and Weisberg, R.: The secondary attachment site for bacteriophage λ in the proA/B gene of Escherichia coli. J. Mol. Biol. 144, 587-592, 1980.

5. Enquist, L. and Weisberg, R.: Lambda site specific recombination. Microbiology-81, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00067-13 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Integrative Control of Macromolecular Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Cashel	Medical Research Officer	LMG NICHD
OTHERS:	Sara Contente	Staff Fellow	LMG NICHD
	Paolo Sarmientos	Visiting Fellow	LMG NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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3.0	3.0	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this work is to understand how a cell coordinates the expression of its genetic repertoire during normal growth and during nutritional deficiency. One component of this process is a regulatory nucleotide, guanosine 3',5'-bispyrophosphate (ppGpp) whose discovery and characterization have been described in previous reports. This report is concerned with cellular regulation of ppGpp concentrations and regulatory effects of ppGpp. On the basis of mutant studies it appears that the concentration of ppGpp is thought to participate in the coordination of expression of about half the E. coli genes during nutritional imbalance but has virtually no noticeable regulatory effects during normal growth. Lethal consequences of the relS mutation abolishing ppGpp accumulation during energy source starvation are described here. Work continues towards determining the structural basis of gene promoter activity by engineering ribosomal RNA gene alterations. Two assays of these alterations are being developed involving gene fusions and sensitization of in vitro systems by genetic modification to eliminate nuclease activities.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The goal of this project continues to be a molecular understanding of how diverse cellular genetic activities are coordinately regulated during balanced growth and nutritional impoverishment. The project is focussed on regulation mediated by ppGpp, how it is formed and how it acts.

Major Findings:

1. Biosynthesis of ppGpp. Two mutational pathways alter the ability of a cell to synthesize ppGpp. The relA gene product functions on ribosomes and requires mRNA codon binding to uncharged cognate tRNA accumulated as a result of amino acid deficiency. The relS mutation is a recent mutant isolated in our laboratory that when present in a relA host abolishes ppGpp accumulation during carbon-energy source deficiency but has no effect on steady state growth.

   We have observed that the relA relS double mutant is normal with respect to its ability to recover from nutritional impoverishment in rich media. This implies that activation of expression of genes required for growth occurs normally in the absence of ppGpp as does repression of the genetic domain normally repressed by nutritional abundance. Conversely the double mutant seems markedly defective in its ability to curtail cellular functions necessary for adapting from luxuriant growth to nutritional impoverishment. This is partially true during carbon-energy source starvation and strikingly evident during starvations which restrict protein synthesis. Phenotypically, cells from the double mutant display a prolonged lag time before resumption of growth at a new slower rate as compared to a relA relS+ single mutant. In extreme cases, cell death occurs that is independent of the particular amino acid missing as well as the number of amino acids withdrawn. Comparisons to relA+ relS single mutants are impossible due to incompatibility problems (see #2).

   Although the details of the mutant lesion are unknown, these results imply that ppGpp is required only in a regulatory role in the cell. The absence of ppGpp mediated turn off of genes expressed during normal growth can have physiologically diastrous consequences. Preliminary studies have ruled out translational coding errors as the source of impairments described.

2. relS-relA+ Incompatibility. Previously, abnormal frequencies of certain recombinants at the relA locus in bacterial crosses involving relS parents led to deducing that the wild type allele of the relA gene is lethal when present in the same cell with a relS mutation. The unraveling of this clue to relS gene function requires finding means by which the incompatibility phenomenon can be provoked uniformly throughout a cell population.

   This has been achieved by combining a relS mutation together with an amber mutation in relA in a temperature sensitive suppressor (supD) background. Suppression of the relA amber mutation does result in rapid onset of cellular death. Survivors growing at otherwise restrictive temperatures should provide a source of second site mutations and therefore clues to alleles mediating this puzzling phenomenon.

3. Regulation of Gene Expression by ppGpp. We continue to develop both in vitro and in vivo systems to measure regulation of gene expression by ppGpp. The model genes responding to growth rate and nutritional imbalance are ribosomal RNA genes which are present in seven copies in E. coli. Previous reports have described localization of growth rate control and ppGpp regulation to the initial part of the gene which encodes two tandem promoters (P_1,P_2) as well as ribosomal RNA processing sites. These genes are among the most active of all cellular genes during balanced growth. Since ribosomal RNA availability determines the extent of free ribosomal structural proteins available for autogenous regulation of operons encoding protein synthesis components, the activity of these genes may be of primary importance in regulation.

   Attempts to clone P_1P_2 promoters fused to galactokinase have been successful but plagued with the instability of the plasmid vector. Partial stabilization of such fusions has been associated with growth conditions that restrict P_1P_2 activity; these observations have led us to postulate that transcriptional read-through results in reduced plasmid replication. Attempts to stabilize the isolates by introducing transcriptional terminators from other genes (in our laboratory and elsewhere) have resulted in neither stabilization nor measurable termination. Current ideas being tested range from models based on lambda phage antitermination mediated by the lambda N gene and the host NusA gene product to functional matching of promoters and terminators within a given gene. We have isolated the P_1 region separate from the P_2 promoter and found P_1 to exist in stable plasmids. The sequence of the P_1 region is known and highly conserved among ribosomal cistrons. Since much is known of the determinants of promoter activity, we are embarking on chemical synthesis of altered forms of the P_1 promoter region. Fusion of these segments to a galactokinase detector element should enable identifying regulatory determinants of the P_1 promoter region which are currently unknown.

4. Modification of in vitro Assay Systems of Gene Expression. Work from several laboratories has determined that pure component assay of gene expression does not yet reflect cellular regulation. Appropriate regulatory features are displayed with crude S30 systems which must therefore be employed. We have been systemmatically eliminating nuclease activity of such crude extracts by the introduction of well known mutations in the strain used as the source of these extracts. Removal of most of the DNA exonuclease activity was accomplished by introduction of the recB21 allele. This resulted in a significant potentiation of the activity of the S30 extract of over a hundred fold in some cases. More significantly for our purposes, small linear DNA fragments can be assayed. Using transposon linked markers to RNase I, II and III we are in the process of constructing and testing strains deficient in these activities for use in assessing rRNA gene activity.

Significance to Biomedical Research and the Program of the Institute: How cells regulate their activities during normal growth and nutritional impoverishment is a recurrent problem in developing as well as adult stages. A molecular understanding of these processes in any biological system is expected to be of basic significance.

Proposed Course of Project: We shall continue to pursue both biochemical and genetic approaches to understanding how cells respond to their environment by adjusting their growth rate. A concerted effort will be made to identify the basis for cell death during amino acid starvation as well as relA+ incompatibility of relS strains by measuring macromolecular synthesis parameters. Promoter stabilization and read-through studies will be performed with classical NusA mutants and ribosomal RNA gene terminators will be inserted downstream of homologous and heterologous rRNA genes to examine the promoter specificity of termination. Variant segments of the P_1 and P_1P_2 promoter region will be synthesized both for site-specific mutagenesis and for use in chemically synthesized variants of the gene. Finally the in vitro assay system will be further developed to yield a simplified yet precise assay of promoter activity and regulation in a crude system known to display ppGpp mediated regulation.

Publications:

1. Yang, H.L., Ivaskiv, L., Chen, H.-Z., Zubay, G. and Cashel, M. Cell-free coupled transcription-translation system for investigation of linear DNA segments. Proc. Natl. Acad. Sci. U.S.A. 77: 7029-7033, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00068-10 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Factors Influencing Genetic Transcription-Initiation and Termination

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Crouch	Research Chemist	LMG NICHD
OTHERS:	Shigenori Kanaya	Visiting Fellow	LMG NICHD
	Stephanie Seidman	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any)

Jacob V. Maizel, Jr., LMG, NICHD; Robert P. Lenk, LMG, NICHD; Richard J. Feldmann, CCB, DCRT

LAB/BRANCH
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SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

RNA plays an important role in cellular regulation--either by its presence in active form or by its total absence. A number of proteins have been shown to be involved in regulating RNA synthesis and RNA processing in Escherichia coli--among these are RNA polymerase, rho and RNase III. We have described an enzyme from chick embryos (RNase DII) which is an analogue of RNase III. These proteins are involved in the synthesis or inhibition of synthesis of specific species of RNA and in the processing of RNA. Another enzyme which may be involved in regulation of RNA synthesis is RNase H. The objective of this research project is to determine the method by which several of these enzymes act to regulate RNA synthesis in E. coli and chick embryos.

PHS-6040
(Rev. 2-81)

Objectives: our goal has been to understand the control of synthesis and utilization of RNA transcripts at the enzymological level. Primary transcripts generally are cleaved to smaller products during the course of maturation. Such processing events have recently been shown to play a significant role in the formation of both messenger RNA and ribosomal RNA metabolism and has captured the interest of a number of scientists. We are studying an enzyme from chick embryos that was isolated in this laboratory and implicated in processing ribosomal RNA by studies on isolated nucleoli (the organelle in which synthesis and processing of ribosomal RNA is localized) and by the ability of this ribonuclease, RNase DII, to degrade double-stranded RNA to acid soluble-products--a property shared by one of the E. coli enzymes involved in ribosomal RNA processing, RNase III. Our attention is now focused on the sites of cleavage of ribosomal RNA precursors at the level of the precise nucleotide sequence that is cleaved.

Methods Employed:

Recombinant DNA techniques have been utilized in our experiments. These include restriction enzyme analysis of DNA, sequencing of both DNA and RNA, cloning and a variety of techniques for analysis of clones. In vitro transcription requires synthesis of RNA by E. coli RNA polymerase, analysis of the products by "denaturing" gel techniques and electron microscopy. Computer analysis has been on the DEC10 system using a program developed by Richard Feldmann with modifications specially generated for studying chicken ribosomal RNA sequences. Examination of the effect of introduction of antibodies into living cells has required isolation of IgG on StaphA-sepharose columns. Antigen determination was carried out using purified IgG to bind labeled RNA of U1RNP, isolation of the complex by StaphA-sepharose column chromatography and analysis of the RNA on polyacrylamide gels.

Major Findings:

Construction of clones linking E. coli ribosomal promoters (P_1P_2) to chicken ribosomal genes was carried out to generate in vitro transcripts for challenge with various "processing ribonucleases". In vitro transcripts from such clones have the expected size (as determined by agarose gel electrophoresis) and secondary structure (as determined by electron microscopy). More importantly, E. coli RNaseIII cleaves the in vitro transcripts at specific sites whose exact location is under investigation. Various deletions have been constructed to examine the impact of the deleted sequences on in vitro processing. The availability of these transcripts is of central importance in our work to understand the role of structure, sequence and enzymes involved in processing eukaryotic ribosomal RNA.

Ribosomal promoters of E. coli are amongst the most active promoters in E. coli and for many cloning experiments should be very important. However, as initially isolated, plasmids containing P_1P_2 promoters are unstable. Insertion of chick ribosomal genes together with a 1.6 kb fragment of the b2 region of phage lambda into a plasmid containing P_1P_2 leads to a stable P_1P_2 containing plasmid. Deletions of either the lambda sequences or portions of the chicken ribosomal genes is possible without altering the stability of the plasmids but does have an effect on the response of the plasmids to chloramphenicol treatment. Pre-

sumably, as a result of competition between transcription and replication of the plasmid, deletion of certain DNA sequences is accompanied by a decrease in yields of plasmid DNA following the normal treatment of plasmid bearing cells with chloramphenicol.

Secondary structure of ribosomal RNA precursors may play a significant role in processing. To aid our understanding of the relationship between the primary sequence of RNA and its secondary structure, we have used computer analysis to build hypothetical structures. One such structure resembles that observed by electron microscopy of ribosomal RNA precursors. The ability to use computer analysis to predict secondary structure together with the possibilities of construction of in vitro deletions and/or insertions opens up a new area of research.

Small ribonucleoprotein (RNP) complexes have been implicated in messenger RNA processing. Most such evidence has been circumstantial. We have gathered more data supporting the involvement of U1 RNP in splicing mRNA precursors by transfusing anti-U1 RNP antibodies into HeLa cells infected with adenovirus type 2 and observing changes in the amounts of certain late viral proteins. Most late viral proteins are made in normal amounts while fiber protein is greatly diminished and hexon protein is decreased two-fold. In light of the current thinking on the role of U1-RNA in splicing messenger RNAs, we have interpreted our results to reflect the involvement of U1 RNA in splicing fiber mRNA precursor and possibly a subset of hexon mRNA precursor.

Proposed Course of the Project:

Our efforts in the coming year will be a continuation of this year's efforts. In the past year we have developed a system for in vitro synthesis of RNA for processing studies and for construction of deletions or other alterations which should be extremely important for studying (1) the enzymology of processing, (2) the sequence requirements for processing and (3) the effects of changes in sequence on RNA secondary structure. Our work suggesting the involvement of U3 RNPs in ribosomal RNA processing will proceed by utilizing U3 clones for localization of U3 RNA under various conditions and by examination of the effect of transfusion of anti-U3 RNP on ribosomal RNA processing. Other antibodies directed against the nucleolus (the site of ribosomal RNA synthesis and processing) will be used in transfusion experiments.

Publications:

1. McClain, K., Stewart, M., Crouch, R.J., Sullivan, M. and Maizel, Jr, J.V. Ribosomal binding to poliovirus RNA. In Fields, B.N., Naenisch, R. and Fox, C.F. (Eds.): Animal Virus Genetics, New York, Academic Press, 1980, pp. 95-104.

2. Carl, P.L., Bloom, L. and Crouch, R.J. Isolation and mapping of a mutation in Escherichia coli with altered levels of ribonuclease H. J. Bacteriol. 144, 28-35, 1980.

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4. Dirksen, M.-L. and Crouch, R.J. Selective inhibition of RNaseH by dextran. J. Biol. Chem., in press.

5. Crouch, R.J. Analysis of nucleic acid structure by RNaseH. In Chirikjian, J.G. and Papas, T.S. (Eds.): Gene Amplification and Analysis, New York, Elsevier-North Holland, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00069-09 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Molecular Aspects of the Replication of Enveloped Animal RNA Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith G. Levin	Research Biochemist	LMG NICHD
OTHERS:	Lesley I. Messer	Postdoctoral Fellow	LMG NICHD

COOPERATING UNITS (if any) Brenda Gerwin, Laboratory of Tumor Virus Genetics, NCI; Sisir Chattopadhyay, Pediatric Oncology Branch, NCI; Anil Mukherjee, Pregnancy Research Branch, NICHD

LAB/BRANCH
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SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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. The possible role of the viral RNA genome in directing virus assembly as well as certain aspects of viral RNA metabolism are being investigated in a system which uncouples murine leukemia virus RNA synthesis from terminal steps in virus maturation. Results of a recent kinetic study indicate that viral messages have a very slow rate of turnover, whereas viral RNA which is ultimately encapsidated into virions has a short half-life. This finding suggests that genome-size viral RNA is present in two independent metabolic pools in the infected cell. Another interest concerns the process of reverse transcription, and in this connection recombinant DNA technology is being used to map the genetic lesion in a nonconditional reverse transcriptase mutant. Work has also been carried out on chromosome-mediated transfer of viral genetic information.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To study factors which influence expression of viral genetic information; to define in molecular terms the specificity requirements of the virus assembly process; to understand the virus-host relationship in chronic infection.

Methods Employed: Mouse cells chronically infected with murine leukemia virus are maintained as continuous lines in culture. Leukemia virus production is measured by assay of the extracellular fluids for reverse transcriptase activity in response to synthetic templates. Infectivity is determined by the XC plaque assay. The RNA species present in virions and in cells are analyzed by polyacrylamide or agarose gel electrophoresis. Conditions have been devised which yield high resolution of a wide range of RNA molecules, from the high molecular weight 70S viral RNA genome to the low molecular weight 8S and 4S RNA species. Viral DNA is analyzed by restriction enzyme digestion followed by agarose gel electrophoresis and Southern transfer. Virus-specific RNA and DNA sequences are detected by hybridization to "nick-translated" cloned viral DNAs or in some cases to a viral cDNA probe. Viral proteins are analyzed on polyacrylamide slab gels. Electron microscopy is used to follow viral morphogenesis and to delineate the ultrastructural features of the virions.

Major Findings:

1. In earlier work on this project, a system was developed in which synthesis of murine leukemia virus RNA is uncoupled from terminal steps in virus maturation by treating chronically infected cells with actinomycin D. Despite the absence of ongoing viral RNA synthesis, virus particles continue to be assembled for at least 8-12 hours. Most of the virions produced are defective, however, and form a unique and previously undescribed class of RNA tumor virus particles which lack 70S genomic RNA and appear to contain only low molecular weight RNA species. We refer to these defective particles as Act D Virions.

2. Studies on viral RNA metabolism. The actinomycin D system has been exploited to study viral mRNA stability and pathways involved in utilization of viral RNA species for encapsidation or messenger function. In previous kinetic studies we showed that viral RNA which is destined to be packaged into virions has a half-life of approximately 3-4 hours, whereas other intracellular viral RNA molecules are quite stable and have a half-life of 12 hours. Northern blot analysis has now demonstrated that the intracellular virus-specific RNA sequences previously detected by hybridization to a viral cDNA probe are represented in 35S and 21S viral mRNA species. The fact that these mRNAs can be isolated at a time when virion precursor RNA is depleted indicates that (i) viral messages have a slow rate of turnover; and (ii) the rate of turnover for viral messages is slower than that for virion precursor RNA. Thus, it appears that two functionally independent pools of genome-size (35S) viral RNA exist within the infected cell: one serving as message, and the other as precursor to virion RNA. In addition, we have found that in the presence of actinomycin D, 35S mRNA declines more rapidly than 21S subgenomic mRNA. This observation could reflect some drug-related effect or alternatively, an intrinsic difference in the

metabolic stabilities of individual viral mRNAs which becomes apparent in the absence of RNA synthesis.

3. Interest in in vitro methods of gene transfer has led to the development of a system in which metaphase chromosomes from murine leukemia virus-infected cells can be used to transfect uninfected mouse cells as well as heterologous mink cells. The ability of recipient cells to produce virus appears to be stable over many passages. Moreover, restriction enzyme analysis of genomic DNA from transfected mouse cells indicates that the viral genetic information is integrated into high molecular weight DNA of the recipient. Chromosomes have also been isolated from a murine sarcoma virus-infected nonproducer cell line and have been shown to induce characteristic foci with an efficiency of approximately 1 focus formed per 1-2 μg of DNA in the chromosome preparation. The foci can be rescued by superinfection of transformed recipient cells with a murine leukemia virus helper, as evidenced by the fact that 7-10 days after addition of the helper, the supernatant fluids of transformed cells gain focus-inducing activity. This demonstrates that the original transformation was mediated by the sarcoma virus genome and was not the result of a spontaneous transformation event.

4. Another major interest concerns the process of reverse transcription. Our approach involves the study of murine leukemia virus polymerase (pol) mutants to correlate structure and function. The availability of the non-conditional pol mutant, clone 23, which was previously shown to produce an abnormally small enzyme, has stimulated an interest in mapping the genetic lesion in this virus. Recombinant DNA technology will be used. In order to perform the mapping in the absence of other murine viruses, the clone 23 genome has been transferred biologically from mouse to mink cells. Two mink cell clones which produce defective virions lacking reverse transcriptase activity have been isolated and are now being characterized. Further work on this project is in progress.

Significance to Biomedical Research and the Program of the Institute: The advantages of viral systems as models for studying the regulation and expression of genetic information are well recognized. Studies on the RNA tumor viruses are of special interest since these viruses extablish a chronic infection in the host cell by integrating viral genetic information into the host chromosome. An understanding of the various steps involved should aid in efforts to limit the oncogenic potential of these agents. Finally, information on the degree to which host material can be assembled into virus particles could have some application in the field of gene therapy, where the use of pseudotype virions has been contemplated as a means of introducing "foreign" nucleic acid into an individual possessing an inherited biochemical defect.

Proposed Course of the Project:

1. Studies directed towards understanding the process of reverse transcription will continue.

   A) Our plan is to map the genetic lesion in the clone 23 pol mutant. Characterization of the two mink clones containing the clone 23

genome is in progress. Virion RNA, viral mRNA species, and viral proteins will be analyzed and the virions examined by electron microscopy. Preliminary results from restriction enzyme analysis of genomic DNA from the mink cell clones, using a probe which is specific for mouse ectropic viral sequences, indicate the feasibility of molecularly cloning the viral genome. It is anticipated that with a cloned viral DNA, we will be able to construct a detailed genetic map of the pol mutation.

B) Studies of in vitro viral DNA synthesis will also be performed. These studies involve isolation of transcription complexes of wild type and mutant virions, characterization of the protein composition of the complexes with a view towards elucidating interactions between polymerase and other viral proteins, analysis of the DNA products formed and examination of the ultrastructural configuration of the complexes.

2. During the coming year, we will attempt to set up a transcription system to study synthesis and processing of viral RNA. Although the HeLa cell S100 system may prove useful for some experiments, our primary approach will be to utilize the oocyte system. By injecting DNA into the oocyte nucleus, it should be possible to detect spliced RNA products as well as synthesis of viral proteins. We will begin our study with cloned wild type murine leukemia virus DNA, but we eventually hope to use mutant DNAs as well. S1 nuclease mapping will be employed to characterize the RNA products.

Publications:

1. Bassin, R.H., Gerwin, B.I., Levin, J.G., Duran-Troise, G., Benjers, B.M. and Rein, A. Macromolecular requirements for abrogation of Fv-1 restriction by murine leukemia viruses. J. Virol. 35: 287-297, 1980.

2. Levin, J.G. and Seidman, J.G. Effect of polymerase mutations on packaging of primer $tRNA^{Pro}$ during murine leukemia virus assembly. J. Virol. 38: 403-408, 1981.

3. Messer, L.I., Levin, J.G. and Chattopadhyay, S.K. Metabolism of viral RNA in murine leukemia virus-infected cells: evidence for differential stability of viral message and virion precursor RNA. J. Virol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00070-21 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Morphogenesis of Animal Viruses During Infection of Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Barbara Norman	Chemist	LMG NICHD

COOPERATING UNITS (if any) Dr. N. Salzman, LBV, NIAID; Dr. L. Lipkin, DCBC, NCI; Dr. G. vande Woude, VB, NCI; Dr. B. DeCrombrugghe, NCI; Dr. M. Girar, Pasteur Institute, Paris, Dr. J. Leis, Case Western Reserve University

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Structure

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mammalian virus systems have been studied by combined electron microscopic and biochemical methods. Oligonucleotides and ribosome binding sites of the poliovirus genome have been mapped. Polio RNA fragments have been translated in vitro, and cloned in plasmids. Adenovirus early proteins have been localized in the cellular ultrastructure. Newly devised computer programs have been used to analyze nucleics for homologies, secondary structures, splice sites, promoter sites and recombination sites.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Our objective is to describe in molecular detail the components of animal viruses and infected human cells with the aim of thereby pinpointing the molecules that cause genetic and non-genetic changes in transformation and infection. The animal virus systems used as models have been poliovirus, adenovirus and murine oncornaviruses.

Methods Employed: Each virus system has been examined with the techniques of isotopic tracer analysis in vivo and SDS gel electrophoresis to detect all of the viral proteins and nucleic acids as they are newly synthesized and modified and to detect changes in cellular proteins. In combination with subcellular fractionation techniques individual proteins have been followed through their synthesis and assembly into multimeric forms, and localized in subcellular fractions. Such fractionation gives powerful clues as to the structural and functional roles of the components. Electron microscopy is used to characterize individual and complex macromolecules and to localize biologically active molecules through the use of radioautography and immuno-electron microscopy. Computer analysis is used to reveal important structural features in nucleic acid sequences.

Major Findings:

1. Poliovirus. Based on the poliovirus RNA genome and protein maps a correlation has been observed between sites of cleavage by RNase III and the termini of the mature polypeptides cleaved by proteolysis from polyprotein. Though based on different types of biological systems the data suggest models for the differential regulation of the poliovirus genome to permit, at various stages, preferential replication, translation or morphogenesis. Protein p19 from avian myeloblastosis virus binds to specific sites on oncornavirus RNA and seems to alter processing, including altered susceptibility to RNase III. Initial studies on p19 binding sites in polio RNA have shown a pattern by biotin-avidin-ferritin mapping on spread p19-RNA complexes that is consistent with the RNase III sites. The working hypothesis is that both proteins recognize similar kinds of secondary structures in RNA. Computer analysis of the published poliovirus sequence by the graphic matrix procedure finds sequences that could fold into stable structures analogous to the well-known sites in phage T7 RNA.

   In order to pursue further the functional properties of subgenomic RNA fragments work has continued on isolation of RNA fragments which in preliminary studies are translated into polypeptides corresponding in size to the polymerase peptides NC4 (56K) and a 10K polypeptide. Work has also continued on cloning of genome-length RNA/DNA hybrids in pBR322. Full-length clones have never been reported for poliovirus. The inhibitor RNAsin has yielded larger hybrids than previously available. For comparative studies in an effort to understand the common feature of picornaviruses, work began on several strains of rhinoviruses to isolate and prepare RNA, clone them and do selected sequence analysis.

2. Adenovirus. The use of lipid-encapsulated vessicles (liposomes) to introduce macromolecules into cells is a major development in the field of cell and virus biology. A method has been used that simplifies the preparation of liposomes, and can rapidly (within seconds) introduce EM visible labels such as ferritin into cells without destroying the viability of cells. This technique has been used with antibodies to small-nuclear ribonucleoprotein particles (anti-snRNP). When anti-snRNP is introduced into adenovirus infected cells, adenovirus fiber polypeptide synthesis is decreased with little effect on other proteins. This antibody selectively precipitates U1-containing snRNP, which has been implicated in nuclear RNA splicing mechanisms. The adenovirus fiber gene is encoded in a large polymessage that is spliced in a variety of ways to give different messages. Fiber message splicing is known to be unique from the other late messages in the nature of its leader sequences and in its differential expression in cells of different species.

   The liposome technique is currently being used to introduce additional kinds of macromolecules. Nucleic acids are being introduced to see if increased efficiency of gene transformation can be obtained, if transient expression of genes can be observed and if selected nucleic acids may be antagonistic. For the latter experiments labeled, separated strands of adenovirus DNA restriction fragments have been prepared and separated, and will be introduced to adenovirus infected cells to look for perturbations in the expression of proteins, virus and cellular ultrastructure.

   Several new methods for localization of antigens are under investigation to look for antigens in previously fixed and embedded thin sections.

3. Methodology. Computer analysis of nucleic acid sequences has continued with a wider variety of sequences. The graphic matrix procedure has proven to be of great utility in ordering partial sequences. Fragments are concatenated with demarcations between them and then compared directly and to their reverse complements. They can also be compared to a related gene that has already been fully sequenced. By taking advantage of the ability of the GM technique to compare imperfect matches it is possible to recognize the likely order of fragments. The GM and other programs run on the HP9845 microcomputer can be used as an inexpensive guide to direct more sophisticated alignment programs. One such program is the RNA folding program by Zuker employing the Nussinov algorithm. This program has been implemented for sequences up to 230 bases on the NIH central computer. As a guest on a VAX780 mini-computer it has been possible to use sequences of up to 800 bases. A series of mutants in the 0.3 gene of T7 were examined with success in correlating predicted structural changes with known effects on expression. A single base change from wild type eliminates translation by changing the initiator AUG to ACG. It is reverted by a mutation of U to G some 73 bases into the coding region which "reverts" it by causing an AUG six bases to the 3' side of the mutant ACG to function. The computer predicted structure is highly consistent in that the alternate initiation codon is in a more accessible environment following the "reverting" initiation. Work is continuing to test the validity of such programs and to continue to use the graphic matrix programs as a guide to selection of relevant portions of sequence.

4. Collaborations. An important activity of this Section has continued to be collaboration with other units of the Laboratory and elsewhere. With a small Section it has been possible to apply the techniques to a larger number of important problems than otherwise possible.

Proposed Course of Project:

We will continue to develop electron microscope, biochemical and computer techniques to characterize molecules and relate them to the structure and workings of cells. Efforts will be continued to devise similar EM tags for molecules and to use them in mapping molecules and cells. Work will continue on ways to reveal information contained in nucleic acid sequences. We will explore ways to use information from nucleic acid sequences to alter the replication of viruses within cells.

Significance to Biomedical Research and the Program of the Institute:
Biochemical characterization of the early events in viral infections has more completely defined the critical events in cellular and viral regulatory mechanisms. New techniques using electron microscopy are now permitting visualization of molecules in the process of carrying out their regulatory roles. Observations of the specific interactions between cellular and viral genomes and their transcriptional and translational products strikingly verify and extend biochemical findings. Information thus derived will find application in control of reproduction, growth, differentiation and viral diseases in higher organisms. Beginnings to the design of "magic bullets" to selectively affect cellular or viral proteins at the level of genomic expression are being realized.

Publications:

1. M.K. Oskarsson, W.L. McClements, D.G. Blair, J.V. Maizel and G.F. Vande Woude. Properties of a normal mouse cell DNA sequence (Sarc) homologous to the Src sequence of Moloney sarcoma virus. ICN-UCLA Symposium, 1980.

2. M. Oskarsson, W.L. McClements, D.G. Blair, J.V. Maizel and G.F. Vande Woude. Properties of a normal mouse cell DNA sequence (sarc) homologous to the src sequence of Moloney sarcoma virus. Science 207: 1222-1224, 1980.

3. C.J. Sherr, L.A. Fedeles, M. Oskarsson, J.V. Maizel, Jr. and G.F. Vande Wounde. Molecular cloning of Snyder-Theilen Feline leukemia and sarcoma viruses: comparative studies of feline sarcoma virus with its natural helper and with Moloney sarcoma. J. Virol. 241: 200-212, 1980.

4. T.G. Storch and J.V. Maizel, Jr. The early proteins of the nondefective Ad2-SV40 hybrid viruses: the 19K glycoprotein is coded by Ad2 early region 3. Virology 103: 54-67, 1980.

5. M.L. Stewart, R.J. Crouch and J.V. Maizel, Jr. A high resolution oligonucleotide map generated by restriction of poliovirus type 1 genomic RNA by ribonuclease III. Virology 104: 375-397, 1980.

6. R. Lenk, T. Storch and J.V. Maizel, Jr. Cell architecture during adenovirus infection. Virology 105: 19-34, 1980.

7. K. McClain, M. Stewart, R.J. Crouch, M. Sullivan and J.V. Maizel, Jr. Ribosome binding to poliovirus RNA, in Animal Virus Genetics, ICN-UCLA Symposium on Molecular and Cellular Biology, Vol. VIII, eds. B. Fields, R. Jaenisch and C.F. Fox, Academic Press, New York, 1980.

8. V.E. Avvedimento, G. Vogeli, Y. Yamada, J.V. Maizel, Jr., I. Pastan and B. de Crumbrugghe. Correlation between splicing sites within an intron and their sequence complementarity with U1 RNA. Cell 21: 689-696, 1980.

9. P.A. Hieter, E.E. Max, J.G. Seidman, J.V. Maizel, Jr. and P. Leder. Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. Cell 22: 197-207, 1980.

10. B.A. Shapiro, J.V. Maizel, Jr., and L.E. Lipkin. Computer analysis and synthesis of nucleic acid secondary structure. Proceedings IXth Annual World Association for Medical Informatics Meeting, April 27, 1981, pp. 93-98.

11. E.E. Max, J.V. Maizel, Jr. and P. Leder. The nucleotide sequence of a 5.5 kb DNA segment containing the mouse kappa immunoglobulin J and C region genes. J. Biol. Chem. 256: 5116-5120, 1981.

12. M.L. Stewart and R.J. Crouch. Sensitive and rapid analysis of the T1 ribonuclease-resistant oligonucleotides in two-dimensional fingerprinting gels of poliovirus type 1 genomic RNA. Anal. Biochem. 111: 203-211, 1981.

13. K. McClain, M. Stewart, M. Sullivan and J.V. Maizel, Jr. Ribosomal binding sites on poliovirus RNA. Virology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00071-09 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Study of Adenovirus Gene Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Westphal	Research Geneticist	LMG NICHD
OTHERS:	W. Richardson	Visiting Fellow	LMG NICHD
	S.-P. Lai	Chemist	LMG NICHD

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Animal Viruses

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our group is interested in eukaryotic gene control. Presently, we are analyzing pathways of gene regulation in human adenovirus and adeno-SV40 hybrid virus. One early function of adenovirus genes is to promote growth of the defective adeno-associated virus (AAV). Using this helper effect as a test system, we have detected intricate pathways of early adenovirus gene regulation and are now analyzing details of these gene controls. Our work with adeno-SV40 hybrid viruses is focussed on the host cell dependence of viral gene composition and expression. Both studies are expected to contribute to the general understanding of eukaryotic gene control. The techniques used in our work include standard procedures of molecular genetics and biochemistry, as well as electron microscopy, gene cloning in prokaryotic vectors, and micro-injection of animal cells.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Our goal is to analyze the elements of eukaryotic gene control in an animal virus system.

Methods Employed: See Summary.

Major Findings:

A. The Regulation of Early Adenovirus Gene Expression. Examining one biological activity, namely the ability of early adenovirus genes to promote the growth of the defective adeno-associated virus (AAV), we have detected an intricate pathway of regulations, involving the sequential activation of separate early adenovirus gene clusters. AAV expression was monitored in cells infected with the defective virus and injected with purified adenovirus DNA fragments or mRNAs.

B. Growth Properties of Adeno-SV40 Hybrid Vriuses in Simian and Human Cells. In collaboration with Dr. A.M. Lewis (NIAID), we note a profound host cell dependence of SV40 gene expression when we propagate the hybrid viruses in various cell lines. The host determines the structure of the viral DNA as well as the nature of SV40-specific mRNAs and proteins.

Significance to Biomedical Research and the Program of the Institute: Our studies are designed to contribute to an understanding of the molecular events which accompany cell infection by a virus that is known as a human pathogen in childhood diseases, as well as a tumor virus in rodents. As shown above, our experiments offer insights into basic mechanisms of genetic control and regulation in eukaryotic cells.

Proposed Course of the Project: Our study of early adenovirus gene controls will continue with the analysis of the functions that are involved in gene regulation, and with a close examination of the functions responsible for the AAV helper effect. The elements active at the various steps of gene regulation will be identified first at the level of mRNA, then at the level of protein, using the cell microinjection as the assay system of biological activity. From this we hope to gain an insight into the molecular details of the elements involved in eukaryotic gene control. Dr. A.M. Lewis will continue to collaborate with us in our studies on gene regulation of adeno-SV40 hybrid viruses. Deletions in SV40 genes which occur when the viruses are switched from one host cell to the other, will be closely examined, and we will try to define at the molecular level the reason for these host-mediated deletions. This study is expected to open the way for a closer look at host factors directly involved in virus gene expression.

Publications:

1. Westphal, H. The genomic arrangement of an adeno-SV40 hybrid virus, $Ad2^{+}ND_{4del}$. J. Virol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00072-14 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Crystallin Synthesis in Development and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joram Piatigorsky	Biologist	LMG NICHD
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	Harry Ostrer	Clinical Associate	LMG NICHD
	George Inana	Research Associate	LMG NICHD
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	J. Fielding Hejtmancik	Medical Research Officer	LMG NICHD
	Charles R. King	Guest Worker	LMG NICHD
	Jacques Treton	Fogarty Fellow	LMG NICHD

COOPERATING UNITS (if any) Toshimichi Shinohara, LVR, NEI; Jin H. Kinoshita, LVR, NEI; Deborah Carper, LVR, NEI; Leah A. Williams, University of W. Virginia; David C. Beebe, Uniformed University of the Health Sciences

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Cellular Differentiation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.3	6.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Emphasis has been placed on characterizing crystallin gene sequences in the chicken and the mouse. α-, β- and γ-crystallin cDNAs of the mouse have been cloned. Four different γ-crystallin clones were obtained, suggesting a complex gene family for this protein. Sequencing studies showed that the α- and β-crystallins are evolutionarily related proteins and that the principal β-crystallin polypeptide of the mouse is internally duplicated. Restriction enzyme analysis indicated that the chicken δ-crystallin gene is hypomethylated at three sites when the gene is expressed in the lens; these sites are hypermethylated in cells that do not transcribe this gene. Studies with chickens and ducks showed a relationship between the structure of δ-crystallin mRNA and the ionic control of δ-crystallin synthesis observed earlier. Further evidence was obtained with galactosemic rat lenses that Na+ and K+ impair translation of cyrstallin mRNAs during the onset of osmotic cataracts. Finally, evidence was obtained for translational control of δ-crystallin mRNA during the maturation of embryonic chicken lens epithelial cells.

Major Findings:

1. Significant advances have been made toward characterizing crystallin DNA sequences in the mouse. α-, β- and γ-crystallin cDNA clones have been obtained. The α- and β-clones have been almost entirely sequenced. The sequencing data have provided the primary structure of these mouse crystallins. The data have also shown that the principal chain of the β-crystallins has an internal duplication and is about 25% homologous to γ-crystallin, suggesting an evolutionary relationship between these two families of protein. Four γ-crystallin cDNA clones have been isolated which have different restriction maps. This suggests that the multiple polypeptides (at least seven exist) of the mouse γ-crystallin are encoded by separate genes.

2. Hypomethylation at three specific -CCGG- sites has been found to be associated with δ-crystallin transcription in the embryonic lens. These three sites were found to be highly methylated in DNA of nonocular embryonic tissues and in DNA of lens epithelial cells that have shut-off δ-crystallin transcription after infection with Rous sarcoma virus.

3. Hybridization with gene specific, intervening sequence probes have established that both δ-crystallin genes that have been cloned are transcribed in the lens. This suggests that the two similar δ-crystallin chains (50K and 48K) are products of the two similar δ-crystallin genes.

4. Restriction enzyme analysis of cloned δ-crystallin cDNAs from the chicken suggests that there are at least two similar but distinct δ-crystallin mRNAs. One cDNA of 1200 nucleotides has been almost completely sequenced. δ-Crystallin mRNA is approximately 2000 nucleotides long.

5. Cell-free translation tests have indicated that there are at least four δ-crystallin mRNAs in the duck. All four are translated in the lens, producing minor amounts of 50K and 49K polypeptides and major amounts of 48K and 47K polypeptides. Synthesis of the two smaller δ-crystallin polypeptides is differentially reduced in cultured lenses treated with ouabain. This is similar to the differential reduction of synthesis of the lower molecular weight δ-crystallin polypeptides promoted by increased Na+ and decreased K+ in the chicken lens that we have demonstrated previously. Hybridization experiments with cloned chicken DNA sequences showed that duck and chicken δ-crystallin mRNAs have very similar sequences. This suggests a possible relationship between the structure of δ-crystallin mRNA and the ionically-controlled differential reduction in synthesis of the lower molecular weight δ-crystallin polypeptides. Recent experiments with the duck have resulted in the cloning of a large segment, if not a complete copy, of a δ-crystallin gene from this organism. Electron microscopic examination has revealed the presence of at least a dozen intervening sequences in this cloned gene.

6. Cell-free translation experiments on galactosemic rat lenses have shown that crystallin mRNAs persist in these cataractous lenses although their translation is severely reduced. The translational arrest correlates well

with an increase in Na+ and a decrease in K+ concentration during onset of the cataract. Following the impaired translation of crystallin mRNAs in the galactosemic lenses, there is a degradation of the crystallin mRNAs. These data support our idea that reduced utilization of crystallin mRNAs occurs in osmotic cataracts as a consequence of altered electrolyte concentrations within the lens.

7. A combination of hybridization, cell-free translation and in vivo protein synthesis experiments have provided evidence that efficiency of translation of δ-crystallin mRNA is reduced during maturation of the lens epithelial cells in the chicken embryo. Culture experiments also showed that the rate of translation of δ-crystallin mRNA may be modulated in the lens epithelial cells. These experiments indicate the total amount of δ-crystallin mRNA in the cytoplasm is not the only criterion affecting the rate of δ-crystallin synthesis in the lens epithelial cells.

Significance to Biomedical Research and the Program of the Institute: These studies contribute to our knowledge of the mechanisms of cellular differentiation and disease, especially with regard to the mechanisms of specific protein synthesis in developing and cataractous lenses.

Proposed Course of the Project: Sequencing of the crystallin cDNAs will be continued. α- and β-crystallin cDNAs of the chicken will be cloned and used as probes for analysis of lens development in that organism. Crystallin genes will be cloned from DNA libraries and studied with respect to organization and structure in the mouse and the chicken. Transcription of the chicken δ-crystallin gene will be examined in the lens and, possibly, in vitro. Further studies on duck δ-crystallin DNA sequences will be conducted.

Publications:

1. Piatigorsky, J.: Intracellular ions, protein metabolism and cataract formation. In: Current Topics in Eye Research (ed. J. Zadunaisky and H. Davson), Vol. 3, pp. 1-39, Academic Press, New York, 1980.

2. Piatigorsky, J., Shinohara, T., Bhat, S.P., Reszelbach, R., Jones, R.E., and Sullivan, M.A.: Correlated changes in δ-crystallin synthesis and ion concentrations in the embryonic chick lens: Summary, current experiments and speculations. Ann. N.Y. Acad. Sci. 339, 265-279, 1980.

3. Jones, R.E., DeFeo, D. and Piatigorsky, J.: Initial studies on cultured embryonic chick lens epithelial cells infected with a temperature-sensitive Rous sarcoma virus. Vision Res. 21, 5-9, 1981.

4. Horwitz, J. and Piatigorsky, J.: Evolutionary and developmental differences in δ-crystallin from bird and reptile lenses: circular dichroism and flourescence studies. Biochem. Biophys. Acta 624, 21-29, 1980.

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6. Ostrer, H. and Piatigorsky, J.: β-Crystallins of the adult chicken lens: relatedness of the polypeptides and their aggregates. Exp. Eye Res. 30, 679-689, 1980.

7. Shinohara, T. and Piatigorsky, J.: Persistence of crystallin mRNAs with reduced translation in hereditary cataracts in mice. Science 210, 914-916, 1980.

8. Jones, R.E., Bhat, S.P., Sullivan, M.A. and Piatigorsky, J.: Comparison between two δ-crystallin genes in the chicken. Proc. Natl. Acad. Sci. U.S.A. 77, 5879-5883, 1980.

9. Beebe, D.C. and Piatigorsky, J.: Translational regulation of δ-crystallin synthesis during lens development in the chicken embryo. Develop. Biol. 84, 96-101, 1981.

10. Jones, R.E., Bhat, S.P., Shinohara, T. and Piatigorsky, J.: New directions for cataract research: use of recombinant DNA technology. In: New Directions in Ophthalmic Research (ed. M. Sears) Yale University Press, in press.

11. Shinohara, T., Piatigorsky, J., Carper, D.A. and Kinoshita, J.H.: Crystallin synthesis and crystallin mRNAs in galactosemic rat lenses. Exp. Eye Res., in press.

12. Piatigorsky, J.: Lens differentiation in vertebrates. A review of cellular and molecular features. Differentiation, in press.

13. Jones, R.E., DeFeo, D. and Piatigorsky, J.: Transcription and site-specific hypomethylation of the δ-crystallin genes in the embryonic chicken lens. J. Biol. Chem., in press.

14. Ostrer, H., Beebe, D.C. and Piatigorsky, J.: β-Crystallin mRNAs: Differential distribution in the developing chicken lens. Develop. Biol., in press.

15. Piatigorsky, J.: Structural and functional similarities of δ-crystallin mRNAs from duck and chicken lenses. Biochemistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00073-10 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of Immune Systems at the Cellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edgar E. Hanna	Microbiologist	LMG NICHD
OTHER:	Glenda C. Webb	Staff Fellow	LMG NICHD
	Michael L. Misfeldt	Staff Fellow	LMG NICHD

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

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.85	2.75	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experiments are directed toward understanding processes by which regulation of the immune system is controlled at the cellular level. The integrated pathways of regulatory cells such as thymus-derived helper and suppressor T-cells are being mapped. We are seeking to understand T-cell circuitry from the precursor stage through the various phenotypic stages between suppressor and helper functional stages. Our results involving the preferential inactivation of suppressor function of T-cells by natural microbial agents such as the streptococcal exotoxin, SPE, have delineated a novel approach towards understanding regulatory pathways and T-cell circuitry in the immune system. We have recently achieved stabilization of the helper T-cell phenotype as a functional T-cell hybridoma. This approach is facilitating the ascertainment of definitive regulatory roles of T-cells. These monoclonal and perpetual T-cell clones also provide stable cellular targets for understanding the mechanisms of interference of microbes and their products in the immune system.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To understand the processes by which regulation of immune systems are controlled at the cellular level. Specifically, we seek to understand the mechanisms by which thymus-derived T-cells regulate antibody formation and secretion by interaction with bone marrow derived precursors (B-cells) of antibody-forming cells. We seek to understand the interrelationships between the various T-cell phenotypes, from the precursor stage progressively through those having suppressive activity, to those expressing helper activity. We also seek to understand the origins and mechanisms of those T-cells which have a native effector function. We hope to derive information on the relationship of such effector T-cells and the regulatory T-cells. It remains unclear whether helper cells and suppressor cells are distinct cell types or the same cell type expressing different phenotypes in response to different stimuli, or whether such different phenotypes may reflect different stages of development and maturation. We are establishing a library of cloned and perpetual immunocytes so as to determine the molecular nature of the T-cell receptor(s). Monoclonal lines of cells will also allow a better understanding of the receptors involved in binding of microbial and plant agents such as SPE and other mitogens, thus supporting modulation of the immune system. Our progress in constructing monoclonal lines of functional T-cells will support the isolation of the genes coding the various T-cell receptors.

Methods Employed: Major advantage is being taken of a library of NFR/N mouse T-cell hybridomas which we are constructing. The cellular reconstruction system (cellular complementation in vitro) which has been a major advantage to the progress of our experiments will facilitate the testing of our T-cell hybridomas for function. We anticipate construction of monoclonal cell lines that represent other major classes of immunocytes. We will continue to use nude mice as our source of T-cell deficient immunocytes. Nude mouse immunocytes will also be the cell source for the construction of B-cell and macrophage monoclonal lines. Type II, nude mice were the source of presumptive T-cell precursors from which we have also contructed hybridomas. NFR-nu/nu mice are now highly inbred to greater than 99.9% homogeneity. Standard immunological methodology has been detailed in previous reports.

Major Findings:

We have succeeded in constructing several hybridoma cell lines from fractionated T-cells from normal and antigen-primed NFR/N mice. We have also constructed several hybridomas within fractionated nylon wool excluded NFR/N nude mouse splenocytes. These are a source presumptively of T-cell precursors. Two of these clones are observed to be low Thy1.2+, Lyt1+, Lyt2+ and presumably Lyt3+. One of these clones derived from antigen-primed Type II nudes is capable of complementing the PFC response of Type I nude splenocytes. These were all constructed by the fusion of the respective fractionated splenocytes with malignant cells of the AKR mouse thymus lymphoma line BW5147. We have established a large library reserve of such hybridoma cell lines. We have cloned a small number of these lines so far representing Pool I (helper) and Pool II (suppressor) cells of normal and primed NFR/N mice. We have tested representative Pool I and Pool II hybridoma lines for activity and for T-cell surface markers.

All are hyperploid lines. All of them tested so far express the Thy 1.2 T-cell marker of their NFR/N parent cells in various amounts. These hybrids express the Lyt-1 marker characteristic of helper T-cells like their Pool I T-cell parents. Some hybrids express the Fc and VH T-cell surface markers. One of the two hybridomas has a high capacity to express a helper T-cell function and others express no helper function as assessed in our cell complementation assay in vitro. Other hybrids appear to represent a gradation of T-cell phenotypes and we have obtained good representatives of both helper and suppressor phenotypes.

Proposed Course of Project:

We will continue the screening of our library of hybridomas for activities representing the major activities which have been ascribed to regulatory T-cells. We will begin construction of effector cell lines, e.g. cytotoxic T-cells. Those expressing interesting activity profiles will be cloned and characterized on the basis of the battery of T-cell surface marker tests. We observe that surface marker expression is a dynamic and changing expression, even on monoclonal cells. We hope to understand this process with the aid of a cell sorter and single cell cloning apparatus. We have begun to prepare alloantisera against the hybrid lines by immunization of NFR/N mice and AKR/N mice with selected hybrid clones. These antisera are expected to provide standard antisera which should facilitate screening of subsequent monoclonally produced antisera in a related project designed to classify the NFR/N mouse and our hybrid lines for allotype and idiotype. We expect as our work progresses to map major pathways of cellular regulation and control in immune systems by using our accumulating library of monoclonal and perpetual cell lines.

Significance to Biomedical Research and the Program of the Institute: Understanding the natural mechanisms of the regulated expressions of the immune system may reveal pathways by which defective immune systems may be amelioriated. The potential ability to deregulate and to re-organize the immune system is highly relevant to amelioration of disease. Our demonstration that a representative microbial agent such as SPE can deregulate the immune system is relevant to an understanding of mechanisms of disease processes. Moreover, results of our study should complement scientific thought on cellular control mechanisms in general.

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4. Hanna, E.E. and Misfeldt, M.L. ANTIBODY-Monoclonal Antibodies (Lymphocytic Hybridoma), Method and Application. In The 1981 McGraw-Hill Yearbook of Science and Technology, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00074-10 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulated Gene Expression During Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Leder	Chief/Guest Worker	LMG NICHD
OTHERS:	N. Hansen	Guest Worker	LMG NICHD
	P. Hieter	Postdoctoral Fellow	LMG NICHD
	K. Kelly	Staff Fellow	LMG NICHD
	I. Kirsch	Research Associate	LMG NICHD
	E. Max	Research Associate	LMG NICHD
	J. Ravetch	Research Associate	LMG NICHD
	U. Siebenlist	Visiting Fellow	LMG NICHD
	C. Talkington	Staff Fellow	LMG NICHD

COOPERATING UNITS (if any) S. Korsmeyer and T. Waldmann, NCI, NIH; J. Seidman, NICHD, NIH, S.-P. Kwan and M. Scharff, Albert Einstein College of Medicine; F. Ruddle and P. D'Eustachio, Yale University; Department of Genetics, Harvard Medical School

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Gene Organization and Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	9.0	3.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We wish to understand the regulated expression of the globin and immunoglobulin genes of man and the mouse. In order to do this we have cloned the human and murine kappa and lambda light chain and heavy chain genes; the alpha and beta globin genes and pseudogenes of the mouse were also cloned. Detailed structural studies have been carried out that allow us to make certain critical inferences about mechanisms and nucleotide sequences that are involved in the transcriptional activation, somatic rearrangement and evolution of some of these genetic sequences. In particular we have established the arrangement of human immunoglobulin lightand heavy chain locus that accounts for the extraordinary diversity that can be generated by this system; its major source arises from the alternative assembly of genes from subgenic fragments of DNA. In addition, using the globin gene system, we have established an evolutionary mechanism that depends upon the amplification and conveyance of gene sequences on and between the different chromosomes.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Our objective is to understand, in molecular terms, the mechanisms which regulate the flow and exchange of genetic information during cell growth and development. We use two model systems, one involving the process of erythrodifferentiation. Specifically we ask how genetic elements corresponding to globin and immunoglobulin are organized in the genome, how they arise during evolution, what elements in the genome and what gene products regulate their expression and how this regulation and organization are relevant to other elements in the genome of higher organisms.

Methods Employed: These investigations have, naturally, involved an interdisciplinary approach. In addition to the usual techniques of cell growth and culture and the biochemistry of nucleic acids and proteins, special attention has been given to the development of specific techniques for the manipulation of recombinant DNA molecules and their detection.

Major Findings:

1. We have found that the alpha globin pseudogenes described in the last Annual Report are, in fact, not closely linked to the major alpha globin locus, but have been dispersed to two different chromosomes. This finding has important implications as to how new genes evolve and are moved about the host's genome. The observation suggests that DNA is in a highly dynamic state and is constantly amplifying and deleting copies of active genes.

2. We have established the physical maps of the alpha and beta globin loci of the mice and established the structure of embryonic beta-like globin genes.

3. We have completed the entire nucleotide sequence of the mouse kappa locus, including four active and one pseudo-J sites and indentified putative recombination signals within this locus.

4. We have cloned and sequenced an analogous region in the human genome and discovered five active J regions.

5. We have determined the structure and arrangement of the human lambda immunoglobulin locus, including a region of special instability that appears to be highly polymorphic in human populations.

6. We have cloned a large number of human heavy chain genes including μ, δ, γ, ε and α sequences. We have identified within these regions segments involved in the heavy chain switch that are conserved between mouse and man.

7. We have shown that sequences related to this recombinational switch exist outside this locus and therefore might be involved in recombination occurring in other genetic systems.

8. We have shown that there is a special order to gene rearrangment in the immunoglobulin system in which μ precedes κ and κ precedes λ.

Signficance to Biomedial Research and the Program of the Institute: These studies are directed towards understanding fundamental processes involved in the expression of genetic information. They are, thus, obviously relevant to achieving an understanding of a variety of inherited disorders that afflict man. By inherited disorders we refer not only to clearly identifiable conditions which involve mutations at a single genetic site, but also polygenic disorders such as diabetes, hypertension and susceptibility to oncogenic disease. For example, our studies in the past have led to techniques which have permitted a far more detailed understanding of the inherited anemia, thalassemia. Current studies involving gene cloning are obviously relevant to the possible production of valuable pharmacologic reagents as well as the diagnosis of certain inherited diseases such as thalassemia and sickle cell anemia.

Proposed Course of Research:

Efforts over the next year will involve experiments designed to understand the transposition of globin genes from their major locus. In particular the possibility that transposon or retrovirus may play a role in this process will be explored. Further work will be directed towards determining the nucleotide sequences of the remaining mouse globin genes. Next year our most intensive efforts will be directed toward completing the chromosomal map of the human light and heavy genes; determining their chromosomal location and establishing a biochemical basis for the recombinational processes they undergo and characterizing the human heavy chain J and D segments. We shall also explore the possibility that these recombinational signals might be involved in other human or murine genetic systems. Another major area of investigation will be directed towards isolating and cloning idiotype specific T-cell receptors.

Publications:

1. Hamer, D.H., Kaehler, M. and Leder, P.: A mouse globin gene promoter is functional in SV40. Cell 21: 697-708, 1980.

2. Max, E.E., Seidman, J.G., Miller, H. and Leder, P.: Variation in the crossover piont of kappa immunoglobulin gene V-J recombination: evidence from a cryptic gene. Cell 21: 793-799, 1980.

3. Seidman, J.G. and Leder, P.: A mutant immunoglobulin light chain is formed by aberrant DNA and RNA splicing events. Nature 286: 779-783, 1980.

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6. Leder, P., Hansen, J.N., Konkel, D., Leder, A., Nishioka, Y. and Talkington, C.: Mouse globin system: A functional and evolutionary analysis. Science 209: 1336-1342, 1980.

7. Ravetch, J., Kirsch, I.R. and Leder, P.: An evolutionary approach to the question of immunoglobulin heavy chain switching: evidence from cloned human and mouse genes. Proc. Natl. Acad. Sci. U.S.A. 77: 6734-6738, 1980.

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9. Hieter, P.A., Korsmeyer, S.J., Waldmann, T.A. and Leder, P.: Human immunoglobulin kappa light chain genes are deleted or rearranged in lambda producing B-cells. Nature 290: 368-372, 1981.

10. Max, E.E., Maizel, Jr., J.V., and Leder, P.: The nucleotide sequence of a 5.5 kb DNA segment containing the mouse kappa immunoglobulin J and C region genes. J. Biol. Chem. 256: 5116-5120, 1981.

11. Kwan, S.-P., Rudikoff, S., Seidman, J.G., Leder, P. and Scharff, M.D.: Nucleic acid and protein sequences of phosphocholine binding light chains. J. Exp. Med., in press.

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13. Hieter, P.A., Korsmeyer, S.J., Hollis, G.F., Max, E.E., Maizel, Jr., J.V., Waldmann, T.A. and Leder, P.: Immunoglobulin light chain genes of mouse and man. In: ICN-UCLA Symposia on Molecular and Cellular Biology, Immunoglobulin Idiotypes and Their Expression, edited by C. Janeway, E.E. Sercarz, H. Wigzell and C.F. Fox, Academic Press, New York, in press.

14. Weisberg, R.A. and Leder, P.: Molecular Genetics. In The Metabolic Basis of Inherited Diseases, edited by J.B. Stanbury, McGraw-Hill Book Co., New York, in press.

15. Leder, P., Max, E.E., Seidman, J.G., Kwan, S.-P., Scharff, M., Nau, M., and Norman, B.: Recombination events that activate, diversify and delete immunoglobulin genes. Cold Spring Harbor Symp. Quant. Biol. 45: 859-865, 1981.

16. Hansen, J.N., Konkel, D.A., and Leder, P.: The sequence of a mouse embryonic beta-globin gene: evolution of the gene and its signal region. J. Biol. Chem., in press.

17. Kirsch, I.R., Ravetch, J.V., Kwan, S.-P., Max, E.E., Ney, R.L. and Leder, P.: Multiple immunoglobulin switch region homologies exist outside the heavy chain constant region locus. Nature, in press.

18. Korsmeyer, S., Hieter, P.A., Ravetch, J.V., Poplack, D.G., Waldmann, T.A. and Leder, P.: A developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B cells. Proc. Natl. Acad. Sci. U.S.A., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 01000-02 LMG

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Molecular Genetics of the Major Histocompatibility Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.G. Seidman	Senior Staff Fellow	LMG NICHD
OTHERS:	G. Evans	Research Associate	LMG NICHD
	D. Margulies	Research Associate	LMG NICHD
	J. Parnes	Guest Worker	LMG NICHD
	B. Velan	Visiting Fellow	LMG NICHD

COOPERATING UNITS (if any)
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NICHD, NIH, Bethesda, Maryland 20205

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5.5	5.5	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are attempting to define the genetic basis of cell-cell recognition. As a first step, we plan to characterize the genes encoding the major histocompatibility antigens (transplantation antigens) and the Ia antigens (immune associated antigens) by the use of recombinant DNA technology. Our initial efforts have been directed towards obtaining cloned cDNAs corresponding to these genes. During the past year we have obtained cDNA clones for murine beta-2 microglobulin mRNA and H-2 mRNA and we have begun to search for I-A cDNA clones. The structure, evolution, and expression of the single murine beta-2 microglobulin gene and the multiple H-2-like genes are being studied with the aid of the cloned cDNAs.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Our objective is to understand the genetic basis for several cellular recognition events. Initially we will be concerned with the genes encoding the protein antigens that are responsible for transplant rejection. Although the function of these proteins has not been determined, a great deal has been learned about their structure. These proteins are very polymorphic, suggesting that their genes can alter rapidly during the course of evolution. We hope to understand the organization of these genes and their special features that allow for these extensive polymorphisms. Furthermore, we expect to be able to better define the family or related proteins that are responsible for this type of cell-cell recognition.

Methods Employed:

We are using recombinant DNA techniques to study the arrangement of genes encoding several cell surface proteins. The initial step in these procedures has required the generation of hybridization probes that will allow us to identify the genes of interest. These hybridization probes are made by cloning the mRNA sequences for these cell surface proteins. Unfortunately, the individual mRNA species are rare (1:1000-1:10,000). We have developed methods which will allow us to rapidly screen several thousand cDNA clones for the one clone containing the sequence of a particular protein. Using these procedures we expect to be able to clone the mRNA for any protein for which a good antisera is available. We used this procedure to obtain a cDNA clone for beta-2 microglobulin and we are using this procedure to isolate cDNA clones for the murine I-A mRNAs. cDNA clones for murine H-2 genes were isolated from a library of cDNA clones using an HLA cDNA clone provided by Dr. S. Weissmann.

The structure and evolution of the H-2-like genes and the beta-2 microglobulin genes are being studied by the application of the now standard recombinant DNA technologies and these cDNA clones.

Major Findings:

1. To isolate cDNA clones of interest we have devised a screening procedure which allows us to find a cDNA clone for any mRNA whose protein product can be identified by an antiserum. Using this procedure we have isolated a cDNA clone corresponding to beta-2 microglobulin mRNA.

2. The single mouse beta-2 microglobulin gene has been isolated from bacteriophage libraries and its structure analyzed. The gene contains at least four coding blocks separated by three intervening sequences. The structure of this gene confirms the model that beta-2 microglobulin, H-2 and immunoglobulin genes all derive from a common ancestral gene. There are two mRNAs made from the single beta-2 microglobulin gene.

3. Analysis of the mouse H-2-like genes indicates that:

   a) There are 10-30 H2-like-genes.

b) Some of these genes map to the Qa-2 and Tla regions of the mouse chromosome 1-2 map units from the major histocompatibility genes.

c) Each H-2-like gene has the same general structure--the extracellular segment is encoded in four coding blocks, and the membrane and intracellular segments are encoded in separate segments.

4. In collaboration with Dr. Carlo Croce, we have demonstrated that the beta-2 microglobulin and H-2-like genes have also conserved their regulatory signals since the expression of mRNA from these genes is coordinated. Teratocarcinoma cells normally do not make either H-2 or beta-2 microglobulin mRNA but retinoic acid induced differentiation of these cells leads to the expression of both H-2 and beta-2 microglobulin mRNA.

5. In order to isolate cDNA clones for the murine I-A genes we (in collaboration with Drs. R. Germain and M. Mescher) have developed an in vitro translation system for murine I-A genes. Using this system we have begun to screen for cDNA clones encoding the murine I-A alpha, beta, and invariant chains.

Significance to Biomedical Research and the Program of the Institute: These studies are directed towards understanding the fundamental processes in the development of living organisms at a molecular level. More immediately we hope to achieve a better understanding of the phenomena of transplantation rejection. The transplantation rejection phenomena has plagued various attempts to cure human disorders. Organ transplants and marrow transplants are all made much more difficult by the problems of matching donors and recipients. By understanding the genetic basis for transplantation rejections we may hope eventually to achieve better matching sytems and screening procedures. Also, as a result of DNA cloning experiments it may become possible to produce pharmacologic agents that will be useful in further studies of the transplantation antigens.

A second benefit of these experiments may derive from studies on the regulated expression of transplantation antigens. Mammals have developed systems which prevent maternal rejection of the fetus. Immunologists have attempted to explain this phenomenon in terms of blocking or masking of transplantation antigens. However, our preliminary results suggest that mammals control the expression of embryonic transplantation antigens at the transcriptional level. Understanding the molecular basis for the mother's failure to reject the fetus might eventually prove to be useful in the management of these important physiological events.

Proposed Course of Research:

Efforts over the next year will be directed towards understanding the organization and expression of the beta-2 and H-2 genes. We hope to understand the molecular and physiological basis for the two beta-2 microglobulin mRNAs that we have identified. We plan to isolate the mouse H-2D and K genes, from the 20 or so H-2-like genes and study their structure and evolution. Finally, we hope to begin to clone the murine I-A genes and examine their structure.

Publications:

1. Seidman, J.G. and Leder, P. A mutant immunoglobulin light chain is formed by aberrant DNA and RNA splicing events. Nature 286, 779-783, 1980.

2. Seidman, J.G., Nau, M.M., Norman, B., Kwan, S.-P., Scharff, M. and Leder, P. Immunoglobulin V/J recombination is accompanied by deletion of J and V region segments. Proc. Natl. Acad. Sci. U.S.A. 77: 6022-6027, 1980.

3. Hieter, P.A., Max, E.E., Seidman, J.G., Maizel, J.V., Jr. and Leder, P. Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. Cell 22: 197-207, 1980.

4. Levin, J.G. and Seidman, J.G. Effect of polymerase mutations on packaging of primer $tRNA^{Pro}$ during murine leukemia virus assembly. J. Virol. 38, 403-408, 1981.

5. Parnes, J.R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. and Seidman, J.G. Mouse beta-2 microglobulin cDNA clones: a screening procedure for cDNA clones corresponding to rare mRNAs. Proc. Natl. Acad. Sci. U.S.A. 78, 2253-2257, 1981.

6. Kwan, S.-P., Rudikoff, S., Seidman, J.G., Leder, P. and Scharff, M. Nucleic and protein sequences of phosphocholine binding light chains. J. Exp. Med., in press.

7. Parnes, J.R., Maizel, J.V. and Seidman, J.G. The beta-2 microglobulin gene: evolutionary relationships. ICN-UCLA Symposium, 1981, in press.

8. Croce, C.M., Linnenbach, A., Huebner, K., Parnes, J.R., Margulies, D., Appella, E. and Seidman, J.G. Control of the expression of histocompatibility antigens (H-2) and beta-2 microglobulin in F9 teratocarcinoma stem cells. Proc. Natl. Acad. Sci. U.S.A., in press.

NICHD Annual Report
October 1, 1980 through September 30, 1981
Child and Family Research Branch

Summary of Research Activities

The Child and Family Research Branch continues it focus on early development, with particular emphasis on the early environment and its role in psychological development. Current studies are concerned with several major problems: 1) the development of methods for studying the early interactions of the child in the family setting; 2) constructing techniques for investigating other than purely cognitive aspects of the child's functioning, i.e., methods for studying the child's motivation to be competent and to master the environment; 3) the development of methods for assessing the very earliest period of life, the experiences of childbirth. Within these studies on method development, we have been concerned with substantive issues such as the relationship between early experience and the development of mastery motivation, its role in cognitive development and in the formation of parent-child attachment. Sensitive methods are being developed for studying family interaction, and the view of the family environment has been broadened to include fathers. We are also assessing parent-infant interaction as a dynamic process, using techniques that enable us to analyze sequential interactions. As part of our studies of the origins of individual differences in development, we are examining neonatal temperament and the parent's perceptions of the child's temperament.

These issues which are basic to understanding early development have important implications. For example, studies of the transition to parenthood will increase our understanding of the roots of parents' feelings towards their children and the impact of parenthood on husband-wife relationships. Studies of the effects of the mother's return to work after the birth of a child will add to our knowledge of the variety of ways in which families make constructive adaptations to parenthood and employment.

Our research on mastery motivation has three basic objectives: 1) to develop and refine measures for assessing its manifestations during infancy; 2) to investigate its relationship to early cognitive development; 3) to study the impact of the early environment and the mediating role of temperament in the development of mastery behaviors. The findings of low, but significant interrelations among the measures of mastery at 6 and at 12 months suggest that the measures have some concurrent validity, but they are not redundant. Although there is no simple continuity in mastery behaviors some of the transformations that occur appear to be theoretically meaningful. The contemporaneous and cross-age relationships of mastery to cognitive development suggest bidirectionality. A minimal level of cognitive development is necessary for mastery behavior; on the other hand, mastery motivation energizes the infant's first attempts at exploration and efforts to secure feedback from the environment. This process is similar to the processes of assimilation and accomodation described by Piaget.

In a parallel study of the development of mastery in infants with Down syndrome, data collection was completed this year and some initial analyses have been performed. By one year, infants with Down syndrome show less persistence and

have fewer successes on problem solving tasks than do nondelayed children. Inasmuch as Down syndrome infants show great variation in levels of competence, it is of great importance to be able to predict later developmental levels. Preliminary findings indicate some significant relationships between visual responsiveness at 3 months of age and overall performance on the Bayley Scales at one year.

Data collection has been completed in the follow-up studies of mastery at 2 1/2 years of age. This study was designed to investigate continuity in mastery behavior from infancy to the preschool years, and the relationship between parental behavior in infancy and the later development of mastery. Codes have been developed to identify several aspects of mastery at 2 1/2 years and videotapes of the mastery sessions have been coded.

Some analyses have been completed in the study of the psychological aspects of childbirth. Regarding the support women receive from husbands during labor, we find that husbands give similar amounts of verbal support as other hospital personnel, but they give much more tactile comfort to their wives. Comparing the types of support, it was found that the women receive much less practical than psychological support from both husband and nurse, although husbands give considerably more of this type of support than nurses, e.g. giving an icepack or modeling breathing to minimize pain.

In the longitudinal investigation of attachment we are continuing to collect data on children who had been studied previously in the childbirth, the mastery motivation, and the family interaction projects. It is assumed that on the basis of their early relationships with parents, infants develop expectations about the extent to which their needs will be met, and the extent to which their social initiatives will elicit responses from others. These expectations may be carried over to later relationships and influence later social competence. The ultimate goal of this research is to assess the relative importance of the parent-infant relationship for later socioemotional development. Within this objective, the present study is attempting to test the validity of the construct of attachment and to determine whether there are significant relations between independent measures of the quality of attachment.

In the studies of family interaction, three interrelated studies are in progress. One is investigating cesarean delivery and its impact on husband-wife interaction and their interactions with the infant. The second study is concerned with the impact on family interaction and parents' interactions with the child when the mother returns to work during the first year of life. The third is a cross-cultural study of Danish and American families, with focus on childbirth experiences and parent-child interaction. Data collection has been completed on all three studies, and analyses are in progress. Comparing patterns of child care in families where the mother returns to work with families in which the mother remains the full-time caregiver during the first year of the infant's life reveals some interesting differences. Infants in two wage-earner families received less physical contact and caregiving from their fathers while there were no differences in amount of stimulation they received from their mothers. Analyses of cesarean delivered infants show differential treatment by both mothers and fathers. Fathers of cesarean delivered infants showed an increase in caregiving and distance receptor stimulation relative to their wives, while both mothers and fathers of cesarean delivered infants showed less near-receptor stimulation. Longitudinal

followup on these subjects will yield data on whether these patterns of interaction persist.

The Branch has begun a collaborative study on precocious puberty with the Developmental Endocrinology Branch, NICHD. We are planning to assess various aspects of psychological functioning, observe parent-child interaction, and to interview the parents regarding the impact of this disorder on the families.

Professional Activities

Staff members of the Child and Family Research Branch have been members of boards of national associations such as the Easter Seal Research Foundation, the National Center for the Improvement of Child Care, the National Center for Clinical Infant Programs, and the professional advisory council of the Parent and Child Association. One staff member is currently serving on the Credentials Committee of the Division on Developmental Psychology of the American Psychological Association. Another member of the staff has served as a consultant on research design to the Max Planck Institut fur Bildungsforschung in Berlin. A member of the staff has been a consultant to Smithsonian magazine. Staff members have served on the editorial boards of the Merrill-Palmer Quarterly Journal of Behavior and Development, and Family Relations, they have been ad hoc reviewers for Child Development, Developmental Psychology, the Psychological Bulletin, Infant Behavior and Development, the International Journal of Behavioral Development, Science, and Journal of Abnormal and Social Psychology. They have served as ad hoc reviewers of research grant proposals for the Canada Council, the National Science Foundation, and the National March of Dimes. They have been members of the Clinical Review Subpanel of the National Institute of Mental Health and have served on a site visit team for review of Epilepsy Centers for the National Institute of Neurological Disorders and Stroke. They have been outside members of dissertation committees at the University of Maryland and at George Washington University.

Publications Not Directly Related to a Specific Study

Messer, D. J.: The identification of names in maternal speech to infants. Journal of Psycholinguistic Research, 10: 69-77, 1981.

Newcombe, N., and Zaslow, M.J.: Do 2 1/2 year olds hint? A study of hints and question directives in the speech of 2 1/2-year-old children to adults. Discourse Processes, 4: 1981.

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Vietze, P. M., and O'Connor, S.: Mother-infant bonding: a review. In Kretchmer, N., and Brassel, J. (Eds.): Biomedical and Social Bases of Pediatrics. New York, Masson Publishing, 1981, pp. 95-114.

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Yarrow, L. J., and Zaslow, M. J.: The ecology of human development: experiments by nature and design. American Journal of Orthopsychiatry, 51: 363-365, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00012-05 CFR

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Development of Mastery Motivation in Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. J. Yarrow	Chief, Child & Family Research	CFR, NICHD
	P. M. Vietze	Head, Mental Retardation Research Centers	MRRD, NICHD
OTHER:	R. H. MacTurk	Research Assistant	U. of Maryland
	M. E. McCarthy	Research Assistant	U. of Maryland
	S. McQuiston	Research Assistant	U. of Maryland
	R. P. Klein	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)

Institute for Child Study, University of Maryland

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.75	0.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective is to investigate the development of mastery motivation in the first year of life. In a previous study, (Z01 HD 00008-04) three aspects of mastery were distinguished: persistence in problem solving, in practicing emerging sensorimotor skills, and in obtaining feedback from the physical environment. The interrelationships among these components of mastery motivation are being explored, and the origins of mastery and its continuity over time are being studied. The sample includes 75 infants from middle income families. Several methods are being employed--twelve tasks designed to measure mastery motivation; observation of mother-infant and father-infant interaction in the home; Bayley Scales of Infant Development and parent perception of infant temperament. Analyses will focus on continuity in mastery behavior between 6 and 12 months, its relationship to early cognitive development, and the relationship between parent-infant interaction and the development of mastery.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: This longitudinal study of mastery motivation is concerned with several problems: 1) to develop techniques and measures for assessing mastery motivation in infancy; 2) to investigate the relationship between mastery motivation and cognitive development; 3) to study the role of early parent-infant interaction in the development of mastery motivation; and 4) to determine whether there are predictive relationships between mastery at 6 months and mastery at 1 year. This research builds on a previous investigation in which measures of mastery motivation were developed for 12-month-old infants.

Methods Employed: The subjects include 75 first-born infants from intact middle-income families (39 boys and 36 girls); only infants born after uneventful pregnancies and deliveries were included. Infants and parents were studied when the infants were 6 months of age and again when they were 12 months old. Data were obtained in several sessions at each age. During the first session, the infants were given the Bayley Scales of Infant Development; during the second laboratory visit, six of the mastery motivation tasks were administered; the other six tasks were given on the third visit. To allow for estimates of the internal consistency of the procedure, the order of presentation of the two sets of tasks was counterbalanced. Within a week of the laboratory sessions two observations of mother-infant interaction and one observation of father-infant interaction were made in the home. In addition, the parents were asked to keep a diary of the infant's activities for a week, and to complete an inventory designed to obtain their perception of the infant's temperament.

The three components of mastery motivation were assessed in a variety of ways. There were four effect production tasks; four practicing sensorimotor skills tasks and four problem solving tasks. The effect production tasks consisted of materials which enabled the infant to produce auditory or visual effects by operating manipulanda. For example, at 6 months one of the tasks was the activity center in which a variety of effects could be produced by turning knobs, spinning wheels or pushing levers. A similar task at 12 months was the Forest in which the child by turning a key or a dial, or pushing a button could move a cow's head or make a squirrel come out from behind doors. The practicing sensorimotor skills tasks required coordination of perceptual and fine motor skills. One of the materials consists of three plastic men in a small tub; the infant's task is to remove them from the tub and then replace them. A 6 months problem solving task consists of a toy beyond the infant's reach. The toy was on a piece of cloth and could be obtained only by pulling the cloth towards him/her. A 12 month problem solving task was a toy behind a plexiglass barrier, the task could be solved by reaching around the barrier. Each task was presented for three minutes and was videotaped for later scoring.

A number of measures of different aspects of mastery were derived. Latency to task involvement was the time from presentation of the item to the child's first task-related behavior. This measure is an indicator of the infant's eagerness or reluctance to become involved in goal-directed activities. Three measures reflected different levels of goal-directed behavior. Visual attention was assumed to be the lowest level, simple exploration of the materials

was intermediate, and persistent, goal directed behavior, the highest level of task involvement. As an index of the child's feelings on being confronted with a challenging task, expression of affect was recorded. We also recorded time the child spent off task.

To observe parent-infant interaction in the natural setting, an observational system which preserved the sequence of interaction between the parent and infant was developed. The observer focused on the dyad with reference to communicative behaviors, caretaking, and the object-related behaviors of each member. These observations were made without the observer having knowledge of the infant's performance on the Mastery Assessment or the Bayley Scales. Inter-observer reliability was established and maintained by having the two observers code families simultaneously at regular intervals during the study. The observers used an electronic digital recording device, the Datamyte; consisting of a small 14-button keyboard through which the coded data are entered and stored in the unit along with the time of each data entry.

Major Findings: Coding of the mastery behaviors has been completed; data reduction on the measures of mastery and on the home observation measures has also been completed.

With regard to the concurrent validity of the mastery measures, we found significant interrelationships among five of the six measures at 6 months. Latency at 6 months was positively related to visual attention, suggesting that hesitancy in attempting to solve difficult tasks is associated with a low level of mastery behavior. On the other hand, Latency is negatively related to exploratory behavior and persistence on the tasks, indicating a positive association between speed of becoming involved with difficult tasks and persistence in working on them. The intercorrelations at 12 months are similar to those at 6 months. There are, however, fewer significant interrelationships among mastery behaviors at 12 than at 6 months and they are, on the whole, lower, suggesting that mastery becomes more differentiated with age. With regard to the predictive validity of the mastery measures, there is no simple continuity in mastery behavior; rather the relationships suggest there may be some meaningful transformations. The contemporaneous and cross-age findings on the relationship between mastery motivation and developmental level, the Bayley M.D.I., suggest that it is not unidirectional, but reciprocal. There is probably a minimal level of cognitive development necessary for mastery behavior; on the other hand, mastery motivation powers the infant's first attempts at exploration and efforts to secure feedback from objects.

Significance to Biomedical Research and the Program of the Institute: The long-held assumption that all behavior is motivated by simple drive reduction is shifting to the view that there are many determinants of the motivation to be competent. Although there have been a number of studies in recent years of competence and mastery motivation, little progress has been made in operationalizing the construct and developing measures to investigate the beginning of mastery behavior in infancy. This study will add to our understanding of the roots of mastery, the relations between motivational and cognitive development, and the development of feelings of competence.

Proposed Course of Project: Data analyses will be completed and several papers will be prepared for publication in professional journals during the next fiscal year. Follow-up studies of these children will be continued in project Z01 HD 00013-05 CFR.

Publications:

Yarrow, L. J. Beyond cognition: the development of mastery motivation. Zero to Three, 1, (3), 1981.

Yarrow, L. J., Morgan, G. A., Jennings, K. D., Harmon, R. J., Gaiter, J. L. Infants' persistence at tasks: relationships to cognitive functioning and early experience. Infant Behavior and Development, in press.

Presentations at Professional Meetings:

Yarrow, L. J. Issues in measuring mastery/effectance motivation in infants and young children. Presented at the biennial meeting of the Society for Research in Child Development, Boston, April 1981.

McQuiston, S. and McCarthy M. E. The relationship between mastery motivation and cognitive development. Presented at the biennial meeting of the Society for Research in Child Development, Boston, April 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00013-05 CFR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Mother-Infant Relationship in Subsequent Socio-emotional Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. Klein	Research Psychologist	CFR, NICHD
Other:	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	M. E. McCarthy	Research Assistant	U. of Maryland
	N. F. Gist	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Child and Family Research Branch

SECTION

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.75	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to identify factors in infancy which are predictive of later socio-emotional development. This project will focus primarily on the aspects of the mother-infant relationship and infant functioning which are hypothesized to be important for healthy emotional development during early childhood. In the second study of this project we have obtained several measures of the mother-infant relationship at the beginning of the infant's second year. We are currently following these infants up through their fifth birthday. For one of the subsamples of this study we have also collected measures of the father-infant relationship.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: A major goal of this longitudinal study is to test the validity of the construct of attachment. Two major questions are being asked. Do measures of attachment in infancy predict later socioemotional development? Are independent measures of the quality of attachment significantly related?

Methods Employed: The two studies in this project have the following features in common: (1) The infant's response to one or both parents in Ainsworth's Strange Situation is assessed during the second year. (2) Follow-up information is obtained from parents every six months, starting at age two years, and from preschool teachers starting at age 3 1/2 years. In both cases standardized questionnaires are used. The studies vary in the sample used. All the samples were seen in one or more Branch studies prior to their enrollment in the project. The varying nature of the other studies led to use of slightly different procedures with the different samples. Sample 1 was seen in the mastery project (HD 00012). We assessed only the infant-mother relationship using three procedures: The Differential Social Reaction Procedure, free play behavior, and the Strange Situation. Sample 2 participated in the family study (HD 00015 and HD 00016). In order to coordinate with the measures of that study, these children were seen twice in the Strange Situation, once with their mother and once with their father. Sample 3 consists of families who volunteered for the family study but did not meet the criteria of that study (approximately 125 cases). These children are seen in the Strange Situation accompanied by their mothers. Finally, Sample 4 consists of Down syndrome children who were studied in the project "Mastery Motivation and Social Competence in Down Syndrome Infants" (HD 00019). They are seen in the Strange Situation at two years of age, accompanied by their mothers. These children will be followed until their fifth birthday. Every six months during this time we will obtain information from the parents regarding their child's development and stressful events which have occurred to their child. In addition we will obtain reports from nursery school teachers for those children who enter preschool. This follow-up information will allow us to examine the extent to which socioemotional development is dependent on the quality of the mother-infant relationship and the extent to which it is dependent on contemporaneous stresses the child experiences. Efforts are underway to supplement the follow-up information obtained by questionnaires with direct observation of the children's social skills in a preschool setting using the Peer Interaction Quality-Effectiveness Scale developed by Mary J. Wright of the University of Western Ontario.

Major Findings: The children in Sample 1 were three years old in August. Therefore we are now able to begin longitudinal analyses in this sample. Despite offering flexible hours, including both weekends and evenings only 60% of the fathers in sample 2 have agreed to participate. Approximately two thirds of sample 3 has agreed to participate. This relatively high refusal rate raises questions regarding bias in the sample. We will be able to compare those who have participated with those who did not on a number of background measures, to ascertain the nature and the degree of the bias.

Significance to Biomedical Research and the Program of the Institute On the basis of experience with emotionally disturbed individuals, clinicians from several different theoretical perspectives have affirmed that the early mother-infant relationship is crucial for later emotional and cognitive development. There has, however, been little consensus on how to assess this relationship meaningfully. This project has provided one method of assessment Future data collection in this project will subject this method and the other major method, the Strange Situation, to further validity testing by examining the correlates with later socioemotional development.

Proposed Course of Project: We will continue to collect follow-up information on children who have already been seen in the parent-infant phase of data collection using the procedures discussed in the section on methods. We will also complete the first series of longitudinal analyses, using the data collected during this fiscal year as well as data collected in project HD 001100.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00014-05 CFR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychological Aspects of Childbearing and the Neonatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Klein	Research Psychologist	CFR, NICHD
	K. Standley	Psychologist, Private Practice	
OTHER:	N. F. Gist	Research Psychologist	CFR, NICHD

COOPERATING UNITS (if any)

Department of Obstetrics and Gynecology, The Washington Hospital Center, Washington, D.C.

LAB/BRANCH
Child and Family Research Branch

SECTION

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

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Childbirth is a special interaction of physiology and psychology which may have important consequences for infant development. The objective of this research is to gain understanding of psychological aspects of the childbirth experience and their impact on the well-being of the child, the new parents, and the family unit.

The goals of this research are to develop instruments for objective and comprehensive measurement of childbirth events. A method has been developed for recording the physical state of the woman in labor and medical and social interactions with her. A measure of delivery room events has also been developed. Prenatal and postpartum interviews with parents provide data on their expectations and recollections of the birth process which can be compared with the researcher's observations. Parent's perceptions of the newborn are compared with the researcher's assessment of infant behaviors.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The objective of this research is to develop methods and gather data which contribute to an understanding of the psychological factors in childbirth.

Methods Employed: The subjects are forty middle-income, Caucasian couples and their first-born infants. Data were obtained at three points in the perinatal period. The subjective and objective measures are designed to contribute to a more comprehensive view of the psychology of childbearing than has previously been available. In the last month of pregnancy, the expectant mother was interviewed about her pregnancy experience and her expectations of birth and parenting. Demographic and historic data were also obtained.

Observations were made of the woman and her husband during labor for one hour. Labor room events were recorded using a time-sampling method developed for this purpose. The observation system taps various dimensions of the woman's physical state and all medical and interpersonal interactions. The system has provided a comprehensive record of the labor room experience. Information was also acquired from hospital records.

One week after the birth, another member of the research staff visited the new parents in their home and interviewed them about their recollections of the birth and their new parenting experiences. These data are being compared with the observations of the labor. Parents also completed the Newborn Behavior Inventory to obtain their perceptions of the baby's temperamental characteristics. The NBI, developed in the course of this project, has dimensions that correspond to the Neonatal Behavioral Assessment Scale which was given independently by a trained examiner. As with the measures of the childbirth setting, the parent's and the researcher's perceptions of the infant can be compared.

Major Findings: One series of analyses compared the support given during labor by husbands and by nurses. Fathers were present about three times as much as nurses. When in the room, fathers and nurses were equally likely to converse with the laboring woman, but fathers were about five times as likely to touch their wives as were nurses to touch their patients. Women received considerably less practical support (presenting comfort items and modeling breathing) than psychological support (conversing and touching) from both husbands and nurses, but presentation of comfort items was done significantly more by the fathers than the nurses.

Although all the women in our sample were anticipating a vaginal delivery, 15% experienced a cesarean delivery. Reasons for cesarean delivery included failure to progress, fetal position, and fetal distress. No differences between the two groups emerged for the two forms of psychological support; however fathers whose wives were to deliver vaginally were significantly more likely to offer both forms of practical support than fathers whose wives were to experience a cesarean delivery. Because our observations were carried out before the use of any medication, these differences can not be due simply to the fact that women who had a cesarean delivery were more medicated than women who had a vaginal delivery. Several comparisons on possible confounding variables yielded no significant differences between the groups. Thus, these results sults raise the intriguing possibility that father support has the potential

for forstalling the use of cesarean delivery.

Significance to Biomedical Research and the Program of the Institute: Childbirth is increasingly recognized as a critical event in the early psychological development of the child and the new family. Childbirth represents an interface of physiology and psychology which may have important consequences for the physical and emotional health, not only of the infant, but the parents as well. It is hoped that the data of this research program and continuing research in behavioral perinatology will contribute significantly to pediatric and obstetric health care and to parents' understanding of their childbearing experiences.

Proposed Course of Study: We plan to complete analysis and write up the data from this study.

Publications:

Standley, K. Research on childbirth: Toward an understanding of coping. In P. Ahmed (Ed.), Coping with pregnancy, in press.

Papers Presented at Professional Meetings:

Nicholson, J. and Gist, N. Styles of father involvement in pregnancy and childbirth. Presented at the biennial meeting of the Society for Research in Child Development, Boston, April 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00015-04 CFR

PERIOD COVERED October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Parent-Infant Interaction Subsequent to Cesarean Delivery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Pedersen	Research Psychologist	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	R. L. Cain, Jr.	Research Psychologist	CFR, NICHD
	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	B. A. Rabinovich	Research Assistant	U. of Maryland
	E. Kramer	Student Scientist	CFR, NICHD

COOPERATING UNITS (if any)

Parent and Child, Childbirth Education Associates, University of Maryland

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to examine the effects of cesarean childbirth on family interaction and on the parent-infant relationship. The sample consists of 46 families with a medically normal, first-born infant, 23 having had a cesarean delivery and 23 a vaginal delivery. When the babies are three months old, and again when they are a year old, two observations are made of mother, father and baby interacting, and one observation is made of mother and baby alone. At each age parents are interviewed, asked to complete a Q-sort assessing their perceptions of their baby's temperament, and to record their participation in household and childcare tasks on a weekday and a day on the weekend. Cross-sectional and longitudinal analyses will assess differences in parents' reports of their experiences during childbirth, in family interaction, and in their adjustment subsequent to different birth experiences.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Although the rate of cesarean delivery among middle-class mothers has shown a 3-fold increase in the past decade, there have been relatively few focused investigations of the psychological impact of this experience on family relationships. The purpose of the present study is to obtain longitudinal data on the mother-infant, father-infant and spouse relationships in families where there was a cesarean delivery. Three major research questions are addressed: (1) what is the range of variation in adaptation to cesarean childbirth shown by parents of medically normal infants? (2) Compared to families that had a vaginal delivery, is cesarean birth associated with different behavioral patterns in relationships among mother, father, and infant? (3) If there are areas of difference, what is the longitudinal course of these patterns during the first year of life?

Methods Employed: The present investigation seeks to avoid some of the methodological deficiencies in the few previous investigations on this topic. The study is unique in several respects: (1) It addresses cesarean childbirth specifically rather than as an accident of a research design directed to other problems, thus yielding more focused information. (2) It studies a broad range of effects, including relationships that might be influenced indirectly by the birth experience, e.g., father-infant or husband-wife interaction. (3) Methods include self-report and observational techniques, allowing comparisons of effects at a behavioral level as well as in the cognitive constructions of the experience. (4) The investigation is longitudinal, allowing some appraisal of the time boundaries of effects. (5) The infants are screened to eliminate cases with postnatal complications, which might confound the effects of different birth experience. (6) The design includes a methodological safeguard so that the observers of family interaction have no knowledge of the birth experience.

The sample consists of 23 families with a medically normal, cesarean delivered infant and a comparison group of 23 families with a normal vaginally delivered infant (drawn from another study - Z01 HD 00016 CFR). The groups are comparable on all pertinent background variables, e.g., parental age and education, years of marriage, maternal employment status, the sex of the infant, and the hospitals in which delivery occurred. All infants are first-born.

Observational procedures from a previous study (Z01 HD 00003 CFR) of mother-infant, father-infant, and mother-father interaction have been revised for use in the present study. In addition an extensive parental interview has been developed which addresses the following areas: (1) the birth experience and adjustment to parenthood; (2) division of household and child care tasks and (3) employment history, attitudes and plans about employment. A previously developed method for studying parents' perceptions of their babies' temperament has been revised and extended for the present study.

A time log has been developed to record each parent's participation in household and child care tasks on a weekday and a day on the weekend. Finally, an interview has been developed to determine the frequency of mother-infant separations and arrangements for substitute care during the infant's first year.

Families are seen when the infant is 3 months old and again when the baby is 12 months old. At each age there are two observations of mother, father, and baby together, and one observation of mother and baby. At each age parents are interviewed, asked to complete the perception of infant temperament measure and the time log. When the infants are 6, 9, and 12 months old the interview concerning mother-infant separations is carried out by telephone.

Major Findings: All data collection and preliminary analyses of the 3-month evaluations have been completed. Findings fall in 3 major areas: (1) With regard to the birth experience itself, there appears to be a cohort effect; compared to a pilot study completed a few years ago, cesarean delivery now appears a more benign medical procedure. At the same time, there are significant differences between "optimal" cesarean and vaginal deliveries in the duration of hospital stay, likelihood that the father and mother would be together when birth occurs, and in the duration of time before the mother is able to assume full responsibility for child care. (2) On the basis of self report (interview) measures, cesarean delivery is viewed as a more negative emotional experience. There also is significantly greater likelihood of separations between mother and baby during the hospital stay and less opportunity for immediate contact between mother and baby after delivery. (3) With regard to home observations, 3 findings occurred that show differential treatment of cesarean delivered infants by their parents. There is a redistribution of distance receptor stimulation (talking, maintaining eye contact and smiling) and caregiving behavior. Fathers of cesarean delivered infants show greater amounts of these behaviors relative to their wives than is the case with vaginally delivered infants. Near receptor stimulation (touching, holding, or vigorous physical handling of the child) appears less for both mothers and fathers of cesarean delivered infants. The results generally confirm the major hypothesis developed in the pilot investigations and provide insight into the possible dynamics behind these changes in adaptation.

Analyses are also in process to examine variability in response within the cesarean group. Our impression is that the father's presence at the birth had a strong impact on how the parents evaluated the hospital experience.

The extensive interview developed for this study has served as the basis for investigation of issues other than cesarean delivery. A set of questions in the interview focused on the parents mood in the months following the birth of the baby. While depressed mood is a widely acknowledged phenomenon for new mothers, little is known about whether new fathers have such feelings. Analyses have been carried out for 37 families, all of whom had vaginal deliveries, addressing three questions: (1) what percentage of men as opposed to women report depressed mood? (2) to what do fathers attribute their feelings? (3) are there differences in family interaction according to the father's report of mood? Preliminary results indicate that 62% of the fathers report depressed mood, and they attribute their feelings largely to changes in the spouse relationship after the birth of the baby. In families in which the fathers reported 8 or more full days of depressed mood, the fathers showed diminished proximity and interaction with the baby.

Significance to Biomedical Research and the Program of the Institute: A cesarean childbirth is a potentially stressful surgical procedure that may have important psychological sequelae. It is important to determine whether distinctive patterns of care and interactions with the baby emerge as a consequence of this experience. The present study will clarify the range of adaptations which occur following cesarean childbirth, and determine whether differences persist throughout the infant's first year. The study, by identifying areas of maternal and paternal behavior that may be affected, will have important implications for intervention and prevention programs.

Proposed Course of Project: More definitive analyses of the 3-month data and the longitudinal analyses will be completed in the next fiscal year. It is anticipated that several reports of different aspects of the overall problem will be available within the coming year.

Publications: Pedersen, F.A., Zaslow, M.J., Cain, R.L., and Anderson, B.J. Cesarean childbirth: Psychological implications for mothers and fathers. Infant Mental Health Journal, 2, 1981.

Papers: Zaslow, M.J., Pedersen, F.A., Kramer, E., Cain, R., Suwalsky, J., and Fivel, M. Depressed mood in new fathers: interview and behavioral correlates. Paper presented at the Society for Research in Child Development, Boston, April 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00016-04 CFR

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Integration of Child Care and Work Roles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Pedersen	Research Psychologist	CFR, NICHD
	M. J. Zaslow	Staff Fellow	CFR, NICHD
OTHER:	R. L. Cain, Jr.	Research Psychologist	CFR, NICHD
	J. T. D. Suwalsky	Research Psychologist	CFR, NICHD
	M. W. Fivel	Research Psychologist	CFR, NICHD
	B. A. Rabinovich	Research Assistant	U. of Maryland
	E. Kramer	Student Scientist	CFR, NICHD

COOPERATING UNITS (if any)

Parent and Child, Childbirth Education Associates, University of Maryland

LAB/BRANCH

Child and Family Research Branch

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INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	1.0	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to obtain longitudinal descriptive data on various adaptation patterns which families use to integrate child care and employment in the infant's first year of life. Four groups of families are being studied: those in which the mother is the full time caregiver for the infant during its first year; families in which the mother is employed before the baby's first birthday, but is not employed when the baby is 3 months old; and two groups of families in which the mother is employed by the infant's 3 month birthday (one group less than 20 hrs/wk. and one group greater than 20 hrs/wk.). At 3 months and again at 12 months, two observations are carried out in the families' homes when mother, father and baby are together and one observation is carried out when mother and baby are alone. Parents are also interviewed and asked to complete a Q-sort to measure their perceptions of their infant's temperament. Data analysis will consist of cross-sectional and longitudinal comparisons of the four groups.

Project Description:

Objectives: Mothers with very young children are in the paid work force in unprecedented numbers. This study attempts to address a number of questions about mothers' employment decisions in families with first born infants:

1. What factors are related to mothers' decisions to seek employment or to be full-time caregivers in the infant's first year of life?

2. Are there systematic differences in the parental behavior of mothers with different employment patterns, i.e., mothers who are already employed when the infant is 3 months old, mothers who expect to be employed by their baby's first birthday, and mothers who plan to provide full time child care over the same period of time?

3. Is the father-infant relationship affected by variation in the mother's employment role?

4. What is the longitudinal course of each parent's relationship with the baby when there are different maternal employment patterns?

Methods Employed: The sample consists of 47 families with first born infants, divided into four groups according to employment plans: mothers who plan to be employed before the baby's first birthday, but are not yet employed when the baby is 3 months old; mothers who plan to be the baby's caregiver throughout its first year; and two groups of mothers who are employed when the baby is 3 months old but differ as to amount of hours in the paid work force. All groups are comparable in background characteristics. Families were seen when the baby was 3 months old and again when the baby was 12 months old.

Data on mother-infant, father-infant, and husband-wife interaction were obtained by means of direct observation in the natural setting of the home. A parent interview was developed which addresses several topics: the experience of becoming a parent; the division of child care and household tasks between mother and father; history, plans and attitudes of the mother and father about employment. The parents' perceptions of their baby's temperament were also assessed. Finally, an interview was utilized to determine the extent of mother-infant separations and arrangements for substitute care over the infant's first year.

Major Findings: All data collection and preliminary analyses of the 3-month evaluation have been completed. Interesting differences have emerged in comparisons of the home observations for families in the which the mother intends to be a full-time caregiver through the baby's first year with those for families in which the mother is at 3-months already employed more than 20 hrs/wk. Both mothers and fathers in the high employment group spent less time in the same room with the baby, but these mothers appear to compensate by interacting at higher rates during the periods they are with the baby, while fathers whose wives are employed do not. Thus, a major difference for infants in two wage-earner families is that they received less physical contact and caregiving from their fathers while there were no differences in amount of stimulation they received from their mothers. These results also mean that fathers and mothers in the two groups have established different patterns of behavior with the

baby relative to each other. On two measures of physical contact with the baby, fathers in two wage-earner families engaged the baby less than mothers, while, in single wage-earner families, rates of father behavior were higher than those of mothers. These findings partially replicate the findings from a pilot study, with the patterning of mother relative to father behavior in the two groups being the same. They indicate that the behavior of both parents is affected by variation in the mother's caregiving and wage-earner roles.

The longitudinal data from this investigation will yield data on some questions which have had little previous study. We wonder whether the initial adaptations of two wage-earner families, which show an attenuated rate of father interaction with the baby, are an enduring pattern that persists through the first year, or, as the range of the child's social behavior increases, is there some new accomodation that promotes more sharing of parenting between father and mother.

Significance for Biomedical Research and the Program of the Institute: This study will permit a more differentiated analysis of how maternal employment influences interaction of both parents with an infant. It includes variation in both the extent of the mother's participation in the work-force and the timing of the transition to work requiring integration of child-care and work roles. The longitudinal course in the first year of various adaptational patterns will also be illuminated. These findings will help assess the impact on families of mothers' employment decisions.

Proposed Course of Project: In the next fiscal year, further analyses will be performed. The 12-month assessments will be tabulated and longitudinal analyses will be completed. Reports of findings will be available in this period.

Publications: Pedersen, F.A., Cain, R.L., Zaslow, M.J. and Anderson, B.J. Variation in infant experience associated with alternative family roles. In L. Laosa and I. Sigel (Eds.) Families as Learning Environments for Children. New York: Plenum, in press.

Zaslow, M.J., and Pedersen, F.A. Sex role conflicts and the experience of childbearing. Professional Psychology, 12: 47-55, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00019-03 CFR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Mastery Motivation and Social Competence in Down's Syndrome Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. J. Yarrow	Chief	CFR, NICHD
	P. M. Vietze	Head, Mental Retardation Research Centers	MRRD, NICHD
OTHER:	M. E. McCarthy	Research Assistant	U. of Maryland
	S. McQuiston	Research Assistant	U. of Maryland
	R. H. MacTurk	Research Assistant	U. of Maryland

COOPERATING UNITS (if any)
Institute for Child Study, University of Maryland, Children's Hospital; Children's Brain Research Clinic

LAB/BRANCH
Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .50	PROFESSIONAL: .25	OTHER: .25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this research is to study social competence and mastery motivation in the first year of life of infants with Down syndrome and to examine the relationship between the mother-infant interaction and infant's mastery behaviors. Down syndrome infants are being studied longitudinally at 3, 6 and 8 months, and a cross-sectional sample is being studied at 12 months of age. Mastery motivation is being assessed using 12 tasks developed in this laboratory. An observational system which preserves the sequence and continuity of the transactions between mother and infant is being employed to examine the mother-infant interaction. In addition, the relationship between the infants' social and mastery behaviors and specific cognitive, and temperamental variables is being examined. Infants with Down syndrome show patterns similar to normal infants in the distribution of visual attention, exploratory behavior and goal-directed behaviors in the second half of the first year; by the end of the first year, they show less persistence in and success on problem solving tasks. Patterns of mother-infant interaction for the Down syndrome children differ from those of normal children.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: The major purpose of this investigation is to examine the development of mastery motivation and communicative competence in social interaction in Down syndrome infants. The infants with Down syndrome are being compared with normal children. In addition, we are interested in examining the relationship between specific cognitive and temperamental variables, and mastery and social interactive behaviors of infants with Down syndrome.

Methods Employed: Infants with Down syndrome and their parents were recruited through two physicians and through parent-infant education programs in the Washington, D.C. metropolitan area. Both the longitudinal and cross-sectional samples consist of 30 infants. Data on a comparable number of normal infants are also being obtained. Participants in the longitudinal phase of the study are seen in the laboratory at 3, 6, and 8 months of age. At 3 months, observations of mother-infant interaction are made, the Bayley Scales of Infant Development are administered, and the mother's perception of her infant's temperament is obtained through a Q-sort technique. At 6 months, mother-infant interaction is observed, and the infants are administered the mastery motivation tasks and the Bayley Scales of Infant Development. At 8 months, the mastery tasks and Bayley Scales are administered. Infants in the cross-sectional portion of the study visit the laboratory on three occasions at 12 months of age and are administered the mastery motivation tasks and the Bayley Scales. The infant's performance on the mastery tasks is videotaped for later coding using observational procedures which have been developed for another study (Z01 HD 00012-04).

The mother-infant interaction observations consist of a laboratory procedure in which the infant and mother are videotaped while facing one another, with the infant in an infant seat.

Major Findings: Data collection has been completed and some analyses have been carried out. Observations of the mastery behavior of Down syndrome infants provide some validation for our working conceptualization of the components of mastery: effect production, practicing sensorimotor skills, and problem solving. They also confirm our other findings on the developmental course of mastery motivation in infancy. In the second half of the first year, infants with Down syndrome show patterns similar to normal infants in the distribution of visual attention, exploratory behavior and goal-directed behaviors on the mastery tasks. Like nondelayed infants, mastery behavior in Down syndrome infants shows a developmental progression from producing effects to practicing sensorimotor skills. By the end of the first year, Down syndrome infants show less persistence in and success on problem solving tasks than do normal infants.

Inasmuch as Down syndrome children show great variation in their level of competence, it is especially important to be able to make predictions during infancy about later levels of functioning. Using clusters of items from the Bayley Scales, a significant relationship was found between visual responsiveness at 3 months and overall performance on the Bayley at one year. Significant relationships were also found between the mother's perception of the child's temperament and the mastery behaviors of the infants. Further

significant relationships were found between mothers' attitudes toward child-rearing and mastery behaviors of infants with Down syndrome. All these findings require replication on a larger sample.

Significance to Biomedical Research and the Program of the Institute: Since we have found in the Down syndrome sample patterns in the development of mastery motivation similar to those found among normal infants, our research suggests that similar environmental influences might facilitate the development of mastery in this group. In contrast to most research on developmentally delayed infants which has focused on the achievement of developmental milestones, this study is concerned with developmental processes and the motivational underpinnings of competence.

Proposed Course of Project: Data analyses will be completed and several papers will be prepared for publication in professional journals during the next fiscal year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00020-03 CFR

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Impact of Childbirth Setting on Infant Behaviors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. F. Gist	Research Psychologist	CFR, NICHD
	K. Standley	Psychologist, private practice	

COOPERATING UNITS (if any)

Dept. of Obstetrics & Gynecology, Washington Hospital Center, Wash., D.C.
Maternity Center Associates, Bethesda, Maryland

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.25	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project investigated the importance of the childbirth environment for newborn functioning. The Brazelton Neonatal Behavioral Assessment Scale was administered to healthy, one-week-old infants born in planned home deliveries. These infants were compared with a group of hospital born infants drawn from a study of hospital births ("Psychological Aspects of Childbearing and the Neonatal Period," Z01 HD 00014-04). An effort was made to determine whether there are differences in the two birth environments, and to identify aspects of these environments, such as specific obstetric practices, which may account for differences in home and hospital born infants. Of special interest are the effects of obstetric medications on the behavioral and neurological functioning of the newborn.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Because of the increasing numbers of expectant parents who are electing to have planned home deliveries, this study was designed to investigate the relative advantages and disadvantages of home and hospital births. The behavioral characteristics of infants born at home were compared with a comparable group of infants born in traditional hospital deliveries.

Methods Employed: The methods of this study are the same as those used in the study of "Psychological Aspects of Childbearing." The comparative data on the hospital-born sample are taken from that study.

Two measures were obtained on the behavioral characteristics of the newborn infant. When the infant was 7 to 10 days of age, the mother and father were given the Newborn Behavior Inventory, an instrument that obtains the parents' perceptions of the child, and the Brazelton Neonatal Behavior Assessment Scale which was administered by a trained examiner.

To control for differences that might be due to the use of analgesic drugs in the hospital, comparisons were made between the home-born infants and two hospital samples--one sample of infants born in entirely unmedicated births and one sample whose mothers were given epidural anesthesia.

Major Findings: During the past year we have carried out several analyses of the data.

Comparisons of reports of fathers of home-born babies and those of babies born in non-medicated hospital deliveries show no significant differences in perceptions of their babies at one week of age. For fathers of the two hospital-born groups, those whose babies were born to non-medicated mothers reported their newborns to be signicantly more responsive to animate stimuli, than did fathers of those born to mothers who received obstetric medication.

When responses of mothers in these three groups are compared, babies born in medicated deliveries were reported to be easier console than those born to non-medicated mothers. Babies born at home were reported to be more labile than those born in non-medicated hospital deliveries, by both their mothers and the examiner.

The examiner also rated the hospital no-medication group as significantly more responsive to animate visual and suditory stimuli than the babies born to mothers who received obstetric anesthesia.

Significance to Biomedical Research and the Program of the Institute: The home birth movement of health comsumers is not only making home births a viable alternative in several parts of the country, but it is creating social and economic pressures for change in more traditional obstetrics. This study addresses the issue of whether the birth setting makes a difference to the newborn.

Proposed Course of Study: Data analyses have been completed. This study will be terminated at the end of this fiscal year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 01100-02 CFR

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Follow-up Study of Mastery Motivation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. J. Yarrow	Chief, CFR Branch	CFR, NICHD
	D. J. Messer	Visiting Fellow	CFR, NICHD
OTHER:	M. E. McCarthy	Research Assistant	U. of Maryland
	B. C. Marcus	Research Psychologist	CFR, NICHD
	R. H. MacTurk	Research Assistant	U. of Maryland
	D. Rachford	Research Assistant	U. of Maryland

COOPERATING UNITS (if any)

Institute for Child Study, University of Maryland

LAB/BRANCH

Child and Family Research Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

In this study of the longitudinal course of the development of mastery motivation, the goals are to determine whether mastery motivation in infancy is predictive of later mastery behavior and whether specific aspects of parent-infant interaction influence mastery behavior at 2 1/2 years. The interrelationships between the development of cognitive functions and mastery motivation during the preschool years is also being studied. Methods have been developed to assess similar aspects of mastery at 2 1/2 years as were studied at 6 and 12 months of age. Data are being collected using these measures of mastery in a focused play session, an observation of the infant's free play with the mastery toys, and a brief observation of mother-child interaction with another set of toys. Data on cognitive development are being obtained by the use of the McCarthy scales.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: This study is concerned with the longitudinal course of cognitive development and mastery behavior during the first 2 1/2 years of life. The overall goals are to determine whether mastery motivation assessed in infancy is predictive of later mastery behavior and whether parent-child interaction in infancy is predictive of later manifestations of mastery behavior. The children in this sample were first studied at 6 and 12 months of age (Z01 HD 00012-03) and are now being assessed at 2 1/2 years.

Method Employed: The subjects are 53 first-born 2 1/2 year old infants. Procedures have been developed to assess aspects of mastery at 2 1/2 years similar to those studied at 6 and 12 months of age. Data were collected in two sessions. During the first session, the children were given a standardized intelligence test, the McCarthy Scales of Children's Abilities. Afterwards they took part in a task involving the operation of some simple levers which switch on a contingent display. During the first half of the second session six toys were presented by the examiner to the child one at a time, giving the child an opportunity to explore and attempt to master the toy. Four of the toys were then reintroduced and the child was allowed to play with them as he or she wished. In the second half of the session, mother and child were observed playing together with another set of toys.

Major Findings: During this year, data collection has been completed. In addition, codes for gaze, speech and the manipulation of toys in the focused mastery session have been developed. Inter-rater agreement on these codes has been obtained and coding for this session has been completed. The child's looking behavior in the contingency task has also been coded, and we are presently coding the child's exploratory behavior in this situation. Computer programs have been developed to obtain the frequency, duration, latency, and maximum duration of behaviors. From these data, we plan to identify the following behavioral dimensions: overall attention to task, persistence, exploration, reaction to failure, social initiative and social responsiveness.

Significance to Biomedical Research and the Program of the Institute: The child's motivation to explore and master the environment is an integral part of cognitive development. It is influenced by cognition, and in turn influences cognitive development. If we can ascertain factors in the infant and in the early interaction with parents that facilitate the development of mastery behavior, it might be possible to influence its course. This project also has implications for theories of continuity of behaviors and for early influences on personal-social and cognitive development.

Proposed Course of Project: In the next year we plan to complete the coding of the videotapes. Analysis of the data will also take place. From these results a number of reports will be submitted to professional journals.

Presentations: Messer, D. The measurement of mastery motivation. Presented at the biennial meeting of the Society for Research in Child Development, Boston, April 1981.

1981 ANNUAL REPORT
Pregnancy Research Branch

Project Numbers	Project Title	Principal Investigator
Z01 HD 00026-06 PR	Fertilization and Activation of Development in Animals	B. Gulyas
Z01 HD 00029-07 PR	In Vitro Studies on Primate Luteal Cells	B. Gulyas
Z01 HD 00031-08 PR	Hormonal Interrelationships Between Fetus, Mother and Placenta in Primates	G. Hodgen
Z01 HD 00045-06 PR	Regulation of Folliculogenesis in the Monkey	G. Hodgen
Z01 HD 00168-05 PR	Ovarian Xenobiotic Metabolism and Oocyte Toxicity	D. Mattison
Z01 HD 00900-03 PR	Factors Regulating Estrogen and Progesterone Receptors in Monkey Endometrium in the Fertile Menstrual Cycle	G. Hodgen
Z01 HD 00901-03 PR	Endocrine Assays Laboratory	W. Nixon
Z01 HD 00907-02 PR	Reproductive Toxicity of Drugs	D. Mattison
Z01 HD 00908-02 PR	Genetics of Ovarian Failure	D. Mattison
Z01 HD 00909-02 PR	Effects of Ethanol on the Mother and the Fetus	A. Mukherjee
Z01 HD 00910-02 PR	Role of Uteroglobin and Transglutaminase in Reproduction	A. Mukherjee
Z01 HD 00911-02 PR	Gene Transfer by Lipochromosome	A. Mukherjee
Z01 HD 00912-02 PR	Gene Regulation and Cellular Differentiation	J. Chou
Z01 HD 00913-02 PR	Primate Models for In Vitro Fertilization and Alternatives	G. Hodgen
Z01 HD 00914-02 PR	Fallopian Tube Dysfunction and Endometriosis	G. Hodgen
Z01 HD 00915-02 PR	Antenatal Diagnosis and Intrauterine Surgical Restoration of Neural Tube Defects and Limb Anomalies	M. Michejda
Z01 HD 00916-01 PR	Studies of Corpus Luteum Function in the Cycling and Pregnant Monkey: Relaxin Secretion	W. Nixon

ANNUAL REPORT
of the
Pregnancy Research Branch
National Institute of Child Health and Human Development
October 1, 1980 through September 30, 1981

SUMMARY

The current research program of the Pregnancy Research Branch focuses upon the physiology of gestation (including genetic and developmental factors which contribute to perinatal fetal and maternal morbidity and mortality), fertility regulation, toxicological aspects of ovarian function, as well as fertilization and implantation. Both basic and clinical aspects activities, the Branch trains Associates and Visiting Fellows in laboratory and clinical investigation by participation in the combined program in Reproductive Endocriniology that has been established with the Armed Forces Institute, specifically for career development in obstetrics and gynecology. Also, the combined program with NIAMDD is available to Clinical Associates interested in Endocrinology. In addition, the Pregnancy Research Branch has applied jointly with the Department of Obstetrics and Gynecology, Georgetown University to establish sub-specialty certified Fellowships in Maternal-Fetal Medicine and Gynecologic Endocrinology.

We have continued to study cellular differentiation and gene regulation in placenta and liver, to isolate and characterize selected enzyme systems and other proteins from normal placenta, choriocarcinoma cells, SV40-transformed placental cells and SV40-transformed rat liver cells, and to characterize the hormonal regulation of glycogenolysis and glyconeogenesis in liver. Ectopic production of these fetal proteins may serve as an early signal of malignant transformation and serve to elucidate the control mechanisms for the synthesis of these proteins.

Certain polycyclic aromatic hydrocarbons destroy oocytes in rodent ovaries. The mechanism of oocyte toxicity appears to depend on ovarian metabolism of these hydrocarbons to reactive intermediates which are toxic but is now being expanded to nonhuman primates. Preliminary results show susceptibility of monkey oocytes to polycyclic aromatic hydrocarbons, re-enforcing the probability of significant human risk from these reproductive toxins. Other studies of reproductive toxins include: 1) the metabolic affects on steroids and xenobiotic compounds by microsomal monooxygenases; and 2) side effects of medicinal agents on reproduction, such as cyclophosphamide.

The state of immune privilege in pregnancy, whereby the non-rejection of the fetus is accomplished, may now be less of an enigma, especially in the rabbit model. To ascertain the immunosuppressive role of uteroglobin in the rabbit, its affects on the incorporation of H^3thymidine into maternal lymphocytes in a mixed culture with mitomycin-C inactivated blastomeres was assessed. The mixture of maternal lymphocytes with rabbit blastomeres suggested maternal recognition of fetal antigens, but which is fully suppressed in the presence of uteroglobin and transglutaminase beforehand. The uteroglobin affect persisted after inhibition of transglutaminase, suggesting a uteroglobin crosslinkage to H-2 antigen (embryonic) which masks maternal recognition. Similarly, recent findings indicate that transaminase and uteroglobin of prostatic orgin may render ejaculated sperm non-antigenic.

Fertilization, and the sperm-egg interaction which is permissive to it, were investigated in both rodent and primate models. In rodent studies, the corticol reaction and its dependence on ovoperoxidase to achieve zona hardening was studied. The results indicate the presence of an ovoperoxidase, perhaps of cortical granule origin, on the surface (histochemically detected) of activated mammalian eggs. In primate studies on in vitro fertilization, we aspirated oocytes from the preovulatory follicle, achieved in vitro fertilization and modest success as embryo transfer. An alternative to in vitro fertilization, low tubal ovum transfer, was also developed, allowing upper tubal obstruction to be by-passed. These approaches may enhance clinical investigations aimed at alleviating infertility due to irreparable tubal dysfunction. These studies have continued, showing induced corpus luteum dysfunction after follicular aspiration. Also, preliminary results, using an intra-abdominal egg-embryo chamber to sustain timely embryonic cleavage, has been obtained. Comparatively, embryos grown in vitro progress more slowly than their in vivo counterparts.

The physiological control of ovarian function and endocrine regulation during the reproductive cycle in pregnancy was extensively analyzed in the monkey as a model for understanding of ovarian function in the primate; these studies continue. The regulation of ovarian folliculogenesis, and selection of the dominant ovarian follicle, was examined during the primate ovarian cycle. Circulating patterns of gonadotropin and ovarian steroids were measured by radioimmunoassay before the after manipulations that included follicle cautery, luteectomy, and hemiovariectomy; during the monkey menstrual cycle. In addition to these studies on follicle growth during the non-fertile menstrual cycle, the resumption of follicle growth was investigated in these animals during the puerperium. The relationship between ovarian function and the conceptus, as well as the cessation of follicle growth during pregnancy, were also studied during the fertile menstrual cycle. Among newer interests is relaxin. Current studies are aimed at understanding its effects on ovarian function.

The ovarian follicle destined to ovulate was selected by the mid-follicular phase, at the expense of other follicles in the original cohort. The time-course of new follicle growth after removal of the dominant follicle was unaffected by the concurrent removal of one ovary. The next follicle destined to ovulate was not selected until after the removal of the reigning dominant follicle. In the presence of the dominant follicle, the contralateral ovary contributes little if anything to the regulation of follicle growth, ovulation, corpus luteum function, or gonadotropin secretion. Investigations included the inhibitory anti-estrogen effects of high dose clomiphene in failure of ovulation induction.

Recently, it was found that charcoal-treated bovine and porcine follicular fluid (PFF1) reduced peripheral plasma FSH levels, with little alteration in LH levels, in gonadectomized rats and in cycling female rats and monkeys. The data suggested the presence of a nonsteroidal substance (inhibin) in follicular fluid thought to be responsible for regulation of FSH secretion. Administration of porcine follicular fluid to suppress circulating FSH offers a convenient, novel system for evaluating the actions of FSH during the ovarian cycle. In current studies we are testing the effects of PFF1 administration during the first half of the menstrual cycle on follicle development in the rhesus monkey. The goal of this research is to test the hypothesis that the formation and steroidogenic activity of luteal tissue may be impaired, when the corpus luteum orginates from a developing follicle exposed to less-than-normal FSH. Even greater and more lengthy suppression of serum FSH is associated with anovulation.

The major cause of human infertility in the U.S.A. is tubal dysfunction. Endometriosis and other causes of failure to achieve satisfactory gamete transport are major contributing factors. We sought to examine how these infertilities arise and how to better treat them medicinally and surgically. The cause of infertility when endometrial tissue grows ectopically is unknown; even its hormonal support is poorly understood. We studied: 1) the role of estrogens and progesterone in the maintenance of ectopic endometrial plaques; and 2) the effect of ectopic endometrium on ovum and sperm transport. Current projects are aimed at determining if ovum pick-up at ovulation and tubal transport of both egg and sperm are inhibited by ectopic endometrium. Also, we are evaluating GnRH analogues as a preferred treatment for endometriosis.

As illustrated by the studies summarized in this report, the investigators within the Pregnancy Research Branch have developed a broad range of studies on normal processes and disorders which affect fertility and gestation. A variety of animal models and clinical interests are being pursued with the expectation that useful information will be derived about the basic causes and optimal treatment of disorders adversely affecting the reproductive process, especially pregnancy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00026-06 PR

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Fertilization and Activation of Development in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Bela J. Gulyas	Senior Investigator	PRB, NICHD
Other:	Eli D. Schmell	Senior Staff Fellow	PRB, NICHD
	Lydia Yuan	Chemist-Technician	PRB, NICHD
	Jennifer Wilbanks	Summer Student	PRB, NICHD

COOPERATING UNITS (if any)

R.B.L. Gwatkin, Merck Institute for Therapeutic Research

LAB/BRANCH
Pregnancy Research Branch

SECTION
Secton on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sperm binding to the zona pellucida of the unfertilized egg is not strictly species specific because heterologous binding between gametes occurs.

To study the sperm's role in sperm-egg interaction monoclonal antibodies were prepared toward mouse sperm surface antigens. Four hybridoma cell lines were isolated that produced monoclonal antibodies (MCA) to mouse sperm surface antigens. The MCA were tissue and species specific and were targeted against integral sperm antigens. Furthermore, the antigens were localized in distinct domains of the mouse sperm, specifically: acrosomal cap, mid-piece and tail, in contrast with polyclonal antibodies which were localized on the entire sperm surface. Sperm binding to the zona pellucida was inhibited by tail and acrosome directed antibody and tail directed antibody immibolized sperm.

To study the egg's role in sperm-egg interaction antibody was made against zona pellucida. Initial results on active immunization of female monkeys with porcine zona pellucida indicate that they produce anti-zona antibody and they failed to conceive when mating was started after the maximal levels of serum titer was reached. The anti-sperm will be used to investigate the ovum aspect of sperm-egg recognition.

Objective

This project focuses upon physiological, ultrastructural and immunological aspects of mammalian gametes, with particular emphasis at this time on sperm-egg recognition during fertilization. The objective of the project is to characterize sperm and egg surface components involved in recognition during fertilization. To this end, two separate approaches were taken. First, characterize sperm surface antigens by preparing monoclonal antibodies to them. Second, prepare antibodies directed against zona pellucida antigens and utilize them in active and passive immunization and other characterization.

Methods Employed

In an initial study the species specificity of sperm-egg recognition was examined in three rodents utilizing living and fixed eggs. An in vitro system was utilized for quantitative assessment of homologous and heterologous sperm-egg binding.

To identify mammalian sperm surface proteins and study their function in sperm-egg recognition, monoclonal anti-mouse sperm antibodies were produced. Rats were immunized with epididymal sperm of C3H mice, and spleen cells from the immune rats were fused with mouse myeloma cells. Antibodies in immune serum and in hybridoma culture fluids were detected using a solid phase, antibody-protein A assay. Briefly, immobilized sperm were incubated sequentially with rat serum or hybridoma supernatant, followed by rabbit anti-rat IgG, then finally with fluorescein-conjugated protein A for visual localization, or ^{125}I-labeled protein A for quantitation. To determine the molecular nature of the target antigens sperm surface components were radioiodinated and analyzed by immunoprecipitation and gel electrophoresis. For localization of antigenic targets at the electron microscopic level horseradish conjugated protein A was utilized.

In the zona pellucida antibody studies female monkeys were immunized with isolated and purified pig zonae. At a predetermined time, the immunized females were placed with fertile males. Pregnancy was determined by a rapid hemagglutination inhibition test. Serum LH, FSH, P and E_2 patterns were determined for one menstrual cycle 60 days after initial immunization and toward the end (18 months) of the trial. To determine serum monkey anti-porcine zona pellucida antibody levels a simple and rapid noncompetitive solid-phase radioimmunoassay was developed utilizing ^{125}I-protein A.

Major Findings

Qualitative observations of sperm-egg interactions in both homologous and heterologous mixtures of guinea pig, mouse, and hamster gametes revealed that sperm initially attached in a loose reversible manner to homologous eggs and subsequently, a stable irreversible binding was observed. Mouse and hamster sperm attached and bound to both mouse and hamster eggs with similar affinities. Guinea pig sperm attached and bound only to homologous eggs, and attachment or binding to heterologous eggs was not observed.

Quantitative assessment of sperm binding to mouse and hamster eggs gave similar results. When the kinetics of mouse, hamster and guinea pig sperm

binding to eggs was measured using a 100-fold variation in sperm concentrations, mouse and hamster sperm showed nonspecific binding, whereas guinea pig sperm did not bind. Similar results were obtained when sperm binding was studied with living or fixed eggs.

In a related study, six stable hybridoma cell lines secreting monoclonal antibodies were isolated. Four of the hybridomas, AMS IV-25,-33,-54 and -76 produced monoclonal antibodies to integral sperm antigens. The remaining antibodies, AMS IV-28 and -50, were directed to non-integral sperm antigens or epididymal fluid components. Binding of monoclonal antibodies to unwashed, washed, or fixed sperm was similar, indicating that the target antigens were probably localized on the sperm surface. Immunofluorescence studies with polyclonal antibodies of immune rat serum showed antibody binding over the entire sperm cell. In contrast, monoclonal antibodies of the individual cloned hybridomas bound to localized regions; AMS IV-33 to the acrosome; AMS IV-25 to the midpiece; and AMS IV-54 and -76 to the midpiece and tail. By combining immunoprecipitation and immunoperoxidase techniques it was determined that one such monoclonal antibody, AMS IV-33, recognized a 200,000 dalton protein localized on the acrosomal cap of the sperm cell. Two other monoclonal antibodies (AMS IV-54 and -76) bound to a 68,000 dalton component on the surface of the sperm tail. Quantitative binding assays using a solid phase antibody-protein A assay revealed that both antigenic targets were species and tissue specific but were present in about equal amounts on sperm from several different strains of mice. These results indicate that species-specific differentiation antigens are localized in distinct domains on the surface of the mouse sperm cell. In vitro fertilization experiments revealed that the midpiece and tail directed antibodies immobilized sperm cells and both the tail and acrosomal cap directed antibodies inhibited sperm-egg binding. The antibodies to the non-integral sperm antigen(s), in contrast, had no effect on sperm motility or sperm-egg binding. These results indicate a spatial differentiation of antigens on the sperm surface. The monoclonal antibodies provide probes to be used for immunochemical characterization of sperm antigens and for elucidating the role of the antigens in sperm function.

The long term study on active immunization of monkeys with porcine zona pellucida is still in progress. The initial results are as follows. The immunized monkeys responded within 2 weeks after immunization producing measurable quantities of antibody against zona. Maximal serum antibody levels were reached between four and six weeks after immunization and maintained at this level as long as monthly boosters were given. Monkeys that were mated prior to reaching maximal serum titer levels conceived, whereas those that were bred at the time or after they reached maximum serum antibody levels have not conceived for 16 months. Upon discontinuation of the boosters serum antibody titers started to decline gradually, but conceptions have not occurred.

Significance

The results of sperm binding studies indicate that initial sperm-egg recognition is not strictly species-specific as it was originally thought. The occurrence of heterologous binding between gametes does not imply cross-fertilization because the zona pellucida is a distinct block to cross-species fertilization, with a few exceptions of natural hybrids.

The monoclonal antibodies which have been described in the rodent model system should prove useful in assessing the correlation between the structure and function of sperm surface antigens. Such information and eventual application of monoclonal technology to the study of human sperm should prove useful in analyzing the role of gamete surface antigens in normal sperm function and aid in understanding the role of autoantibodies to human spermatozoa.

Studies on rodents indicate that the zona pellucida of mammalian eggs contain tissue specific common antigens that shows crossreactivity between different species. Because this system is considered to hold encouraging promise for development of oocyte specific fertility control it is essential to evaluate this system in primates. In particular, reversibility and long term effects on the ovaries must be carefully evaluated.

Future Course

Utilizing the antibodies prepared in this lab (monoclonal antibodies to sperm antigens and monkey anti-porcine zona pellucida antibody) the initial interaction between sperm and egg at fertilization will be further investigated. The work shall concentrate on the utilization of these antibodies for passive immunization of rodents to determine their effect on fertility. Special attention will be given to complete evaluation of the effects of active immunization of monkeys paying particular attention to 1) effects on menstrual cycle and hormonal patterns; 2) reversibility of antifertility effect; and 3) long term effects of the zona antibodies on the oocyte population of immunized monkeys. The monkey antizona serum will be further characterized with respect to 1) its cross-reactivity with other species; 2) zona hardening; 3) interference with sperm-egg binding, and 4) configurational changes of zona surface as observed with SEM; 5) in vitro fertilization.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00029-07 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

In Vitro Studies on Primate Luteal Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	B.J. Gulyas	Senior Investigator	PRB, NICHD
Other:	L.C. Yuan	Chemist-Technician	PRB, NICHD
	G.D. Hodgen	Chief	PRB, NICHD
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1/2	1/2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The morphological changes occurring in monkey luteal cells in culture were correlated with the concomitant decline in steroidogenic activity. The results showed that under our culture conditions, despite the presence of hCG, the luteal cells gradually lost their original morphological appearance and assumed a new flattened phenotypic appearance. These changes in cell shape paralleled observations on declining progesterone synthesis by luteal cells in culture. In a separate study we demonstrated that horseradish peroxidase conjugated hCG (HRP:hCG) when purified binds specifically to LH/hCG receptors of incubated monkey luteal cells. Upon binding with the receptor the HRP:hCG is internalized by the luteal cells.

In vitro studies on dissociated monkey luteal cells was continued, and drawn to a conclusion, by examining the morphological changes that occur concomitant with decline in steroidogenic activity during culture. The objective of the study was to define the cell shape-related cytoplasmic changes as examined with the SEM and TEM. In a related study, the objective was to localize LH/hCG receptors of monkey luteal cells utilizing a hormone-horseradish peroxidase conjugate.

Methods Employed

The corpora lutea were obtained at mid luteal phase of the menstrual cycle of rhesus monkeys and were dissociated in collagenase. Dispersed luteal cells were cultured in Costar dishes, with or without cover glass. The culture medium, Ham's F10 and 10% fetal calf serum, was supplemented with 100 ng/ml hCG in half of the cultures. Subsequently, cells were prepared for SEM and TEM observations.

hCG was covalently linked to dinitrophenylated horseradish peroxidase (HRP) to produce a conjugate that contained HRP and hCG in a 1.6:1 molar ratio. The conjugate was separated from free hCG and HRP by gel chromatography. The conjugate was used in conjunction with electron microscopy to determine binding affinity to receptors and internalization by luteal cells.

Major Findings

Luteal cells in the presence and absence of hCG were nearly spherical during the first day of culture. The surface of the luteal cells consisted almost exclusively of small, nearly uniform-size blebs and some filopodia. Three types of protrusions were present with respect to content: (1) zyotic vesicles with ribosomes and/or agranular endoplasmic reticulum; (2) aggregates of small vesicles, and (3) lipid inclusions. During the second day of culture the spherical cells began to flatten, their ribosome content increased, and short bundles of microfilaments and a few microtubules appeared in the cytoplasm. Luteal cells in culture for 2 days and longer were flattened and assumed typical epithelioid morphology. The number of cell surface blebs was reduced and those present varied in size. Some ruffling of the extenuated cells' border was present. Unlike the spherical cells at the onset of culture, the flattened cells contained microfilaments. The formation of the filamentous cytoskeleton occurred concomitant with the morphological transition from rounded to flattened cells. For the most part, the microfilaments occurred in arrays of bundles and sheaths; they were particularly prominent at the marginal edges of the cells running parallel to the substratum. Filopodia and flat extensions consisted of membrane-enclosed bundles of filaments oriented in the long axis of these structures. Occasionally, microtubules were observed running parallel with the microfilaments.

In a separate study the isolated HRP:hCG had a biological activity that elicited dose-dependent enhancement of progesterone secretion by dispersed monkey luteal cells in vitro. The specificity of the conjugate to LH/hCG receptors was demonstrated by the insignificant nonspecific binding of HRP:hCG to dissociated cells that were previously saturated with native hCG. Electron microscopic studies revealed that the HRP:hCG was distributed uniformly on the surface of luteal cells previously fixed with aldehyde, and to some extent on the surface of those cells that were exposed to the conjugate at 4°C. In those cells labelled at 4°C, rinsed and warmed to 37°C, a rapid redistribution of HRP:hCG into small

clusters occurred. When dissociated cells were incubated with the hCG conjugate at 37°C, it was localized in small clusters on the cell surface. Internalization of small aggregates of HRP:hCG occurred within 5 min at 37°C. This process occurred through invaginations of smooth segments of the plasma membrane. Once internalized, the conjugate was located within membrane bound vesicles of the cytoplasm. After 120 min at 37°C the HRP:hCG was located in large cytoplasmic vesicles near the periphery of the cells, but they were not seen in association with Golgi complexes or lysosomes.

Significance

In view of the ultrastructural observations it appears that our inability to maintain high and steady levels of P synthesis by luteal cells in culture may not necessarily be due to the lack of steroid precursors and other nongonadotropin hormones in the culture medium. Instead, it is quite evident that the luteal cells, once introduced into culture, disposed of their lipid content and agranular endoplasmic reticulum through an apocrine-like secretory process, both of which are essential for steroidogenesis. Subsequently, these monkey luteal cells undergo a gradual phenotypic transformation which is incompatible with steroidogenesis and is manifest in diminished steroidogenic activity.

The HRP:hCG conjugate is an effective tool for examining the events involved from the initial binding of hCG to the specific cell surface receptor all the way through the internalization of hormone:receptor complexes.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00031-08 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hormonal Interrelationships Between Fetus, Mother and Placenta in Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Gary D. Hodgen	Chief	PRB, NICHD
Other:	Robert F. Williams	Senior Staff Fellow	PRB, NICHD

COOPERATING UNITS (if any)

None

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Section on Endocrinology

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0.5	0.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Over the past nine years, a continuum of studies has been directed at understanding the homonral interrelationships between the fetal, placental and maternal compartments. Most of these studies have utilized primate models, where the similarities to equivalent events in human pregnancies are most extensive. During previous years, we concluded a series of experiments which employed fetal hypophysectomy in rhesus monkeys to establish the importance of fetal hypophysical secretion for gonadal development in utero; particular attention was given to ovarian and testicular development.

During the past year we have demonstrated a synergistic action of feto-placental estrogens and progesterone in regulating maternal prolactin secretion, and we have developed a fetal cannulation procedure which allows for repeated sampling of fetal blood for determining the relationship between pulsatile LH and FSH secretion and gonadal differentiation.

PHS-6040
(Rev. 2-81)

To evaluate interrelationships between steroid hormone secretion of the feto-placental unit and the secretion of prolactin from the maternal pituitary.

To characterize the episodic secretion of gonadotropin during fetal development which is associated with gonadal differentiation.

Methods

Peripheral prolactin concentrations have been determined by radioimmunoassay as a measure of maternal pituitary secretion of prolactin in intact, fetectomized, and fetectomized pregnancies supplemented with steroids, and in non-pregnant monkeys with exogenously produced steroid levels which mimic the concentrations of pregnancy.

Major Findings

Previous experiments have demonstrated that the significant rise in maternal prolactin concentrations which occur during the last month of pregnancy in monkeys is of maternal pituitary origin, not from the fetal pituitary origin, nor from the fetal pituitary or membranes, nor the maternal decidua.

We have concluded that estrogens secreted by the feto-placental unit have a significant role in stimulating the secretion of prolactin during the last month of pregnancy, since the suppression of estrogen production by the feto-placental unit, caused by fetal ablation, prevented the rise of prolactin, and the fetal ablation plus estrogen therapy elevated prolactin concentrations. In these experiments an association between prolactin and progesterone concentrations was also noted, which suggested a possible role for progesterone in the regulaton of prolactin secretion.

Utilizing non-pregnant monkeys with induced steroid levels, similar to those of pregnancy, we have demonstrated that progesterone and estrogens act synergistically to stimulate prolactin secretion, but alone neither steroid enhances prolactin secretion.

Proposed Course

Having demonstrated the estrogen-progesterone synergy which regulates prolactin secretion during pregnancy, we will now set forth to determine the sites and mechanisms of action of these steroids in promoting prolactin secretion and the precise nature of the estrogen-progesterone interaction.

With the development of procedures for fetal cannulation, we will characterize the secretion of LH and FSH during fetal life. Gonadal differentiation is dependent upon gonadotropin secretion from the fetal hypophysis; therefore, we will evaluate relationships between pulsatile gonadotropin secretion and gonadal development.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00045-06 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Folliculogenesis in the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Gary D. Hodgen	Chief	PRB, NICHD
Other:	Wilbert E. Nixon	Senior Investigator	PRB, NICHD
	Robert F. Williams	Senior Staff Fellow	PRB, NICHD
	Gere S. diZerega	Clinical Associate	PRB, NICHD
	Edward L. Marut	Clinical Associate	PRB, NICHD
	Lawrence B. Werlin	Clinical Associate	PRB, NICHD
	Bryan D. Cowan	Guest Worker	PRB, NICHD
	Robert Stillman	Guest Worker	PRB, NICHD
	Robert Schenken	Guest Worker	PRB, NICHD

COOPERATING UNITS (if any)

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Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

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4	2	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endocrine factors contributing to the regulation of folliculogenesis (selection of the dominant follicle) during the primate ovarian cycle were examined in two non-human primate models, the rhesus monkey, and the cynomolgus monkey. Circulating patterns of gonadotropins and ovarian steroids (estradiol and progesterone) were monitored by radioimmunoassay before and after follicle cautery, luteectomy and during the menstrual cycle in monkeys. In addition to examining follicle growth during the nonfertile menstrual cycle, the resumption of follicle growth was studied during the puerperium. Ovary conceptus interrelationships, as well as the cessation of follicle growth in pregnancy were studied during the fertile menstrual cycle. Salient findings include: 1) usefulness of the cynomolgus monkey as alternative non-human primate model, 2) asymmetrical function of the two ovaries during the menstrual cycle, 3) evidence that the dominant follicle is selected during the first of the menstrual cycle, 4) onset of asymmetrical ovarian function prior to the onset of ovulatory cycles in juvenile monkeys, and 5) the enhanced biological activity of LH associated with the onset of the first ovulatory cycles and at mid-cycle in adult ovulatory monkeys.

PHS-6040
(Rev. 2-81)

Objectives

To examine the regulation of follicle growth during the primate ovarian cycle, its cessation during pregnancy, and its resumption post partum, using non-human primate models. To examine functional capacities of the hypothalamic-pituitary-ovarian axis in neonates.

Methods

Peripheral and ovarian venous concentrations of gonadotropins and ovarian steroids were determined by radioimmunoassay before and after ablation of the cyclic structure (dominant follicle, or corpus luteum). Episodic secretion of LH and FSH was evaluated in monkeys bearing a femoral cannula which permitted frequent blood collection. Treatments of GnRH, steroids, porcine follicular fluid, and gonadotropins were utilized to evaluate the functional status of the H-P-O axis and interrelationships within the axis.

Major Findings

The utilization of a fluorescein isothiocynate-hCG conjugate permitted the specific localization of LH receptors in the ovaries of cycling monkeys. This technique identified the follicle destined to ovulate at an earlier stage than other morphological procedures permit.

Studies in cycling female monkeys have demonstrated that in the presence of the dominant follice, the responsiveness of other follicles to gonadotropic stimulation is transiently attenuated. The dominant follicle requires continued FSH support even in the immediate preovulatory interval.

Pregnancy induces a hiatus in folliculogenesis culminating in ovulation. This hiatus continues into the postpartum interval, lasting 3-4 months in non-nursing females and up to 7 months in nursing monkeys. The lesion within the hypothalamic-pituitary-ovarian axis which prevents progression of folliculogenesis is neither induced by the elevated steroid levels of pregnancy, nor products directly attributable to the fetus. During the anovulatory interval in non-nursing monkeys the pituitary is responsiveness to GnRH, the hypothalamic-pituitary unit to estrogen positive feedback, and the ovaries to gonadotropic stimulation. Thus, refractoriness of these endocrine organs do not contribute to the lesion which blocks folliculogenesis. Following weaning of infant monkeys after 5 months of lactation, the onset of ovulatory menstrual cycles by the mothers is rapid. Pulsatile secretion of gonadotropins is suppressed during lactation, and is dramatically enhanced after weaning; thus, suggesting that an alteration in the frequency of secretion may be a limiting step in folliculogenesis during the postpartum interval and even during the menstrual cycle.

In normal cycling monkeys we have found an increasing frequency and amplitude of gonadotropin secretion as the follicular phase progresses. Also during midcycle there is a marked divergence in the concentrations of LH as measured by RIA and bioassay. Perhaps the discrepancy in the determinations by these assays are actual reflecting an enhanced biological activity of the LH molecule rather than a recognition of different species of LH molecule.

In studies during the pubertal process, when the hypothalamic-pituitary-ovarian axis is developing competency for supporting ovulatory menstrual cycles, we have found that an asymmetrical secretion of steroids by the ovaries occurs long before the first ovulation is achieved. We have concluded that folliculogenesis is progressing in these immature monkeys to a stage in which the dominant follicle is selected, but that developemnt cannot progress to ovulation. Concurrently we have found an increased pulsatile secretion of FSH associated with maturation, and increased LH biological activity with the onset of ovulatory cycles. These changes in gonadotropin secretion may account for the inability of folliculogenesis to proceed to ovulation immediately after menarche.

Proposed Course

We will continue to investigate the mechanism regulating follicular growth in the primate ovary. Investigations will focus upon the relationship between gonadotropin secretion and ovarian function, specifically the pulsatile secretion of LH and FSH and the role of enhanced biological activity of LH. The investigations of these processes will continue in adult cycling monkeys, anovulatory postpartum individuals, and juvenile monkeys. Studies in postpartum and juvenile individuals will lead to delineation of specific lesions within their hypothalamic-pituitary-ovarian axis which prevents ovulation, and these results will provide insights into mechanisms regulating ovarian function in normal cycling adults.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00168-05 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Ovarian Xenobiotic Metabolism and Oocyte Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Donald R. Mattison	Medical Officer	PRB, NICHD
Other:	Maria Nightingale	Chemist	PRB, NICHD
	Kenji Shiromizu	Visiting Fellow	PRB, NICHD
	Lucy Chang	Biological Aide	PRB, NICHD
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COOPERATING UNITS (if any)
Nathanial White, Statistician, NICHD; David Longfellow, Asst. Chief CPCB, NCI; Carol Henry, Director, Dept. Exptl. Oncology, Microbiological Associates; James Felton, Lawrence-Livermore Laboratories

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Certain polycyclic aromatic hydrocarbons destroy rodent oocytes. Oocyte toxicity follows ovarian metabolism of polycyclic aromatic hydrocarbons to reactive metabolites. Experiments exploring the role of metabolic activation using ovarian aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity (AHH) suggest that AHH activity determines the rate of ovotoxicity but not the extent of oocyte destruction which occurs. The metabolic pathway to the proximate ovotoxin(s) is being explored using oxygenated derivatives of benzo(a)pyrene (BP). The 3-,6-,7-, and 9-hydroxy- BP derivatives, the 4,5-oxide, both 4,5-dihydrol-BP derivatives, and the 7,8-oxide were less ovotoxic than BP. The 2-hydroxy, and the 7,8-dihydrodiol derivatives were more ovotoxic than BP. As the 2-hydroxy is not formed in vivo the 7,8-dihydrodiol-BP appears to be a proximate ovotoxin. Intraovarian injection of BP destroys oocytes, indicating that ovarian monooxygenases and epoxide hydratases are sufficient to metabolize polycyclic aromatic hydrocarbons to ovotoxic metabolites. Experiments utilizing Rhesus Monkeys suggests that non-human primates are less sensitive than rodents to the ovotoxic effects of BP.

Objectives

Polycyclic aromatic hydrocarbons are ubiquitous environmental pollutants. Some of these compounds are also reproductive toxins; destroying oocytes, altering meiosis, and impairing reproduction.

The goal of this research is to elucidate the mechanism(s) and site(s) of reproductive toxicity of polycyclic aromatic hydrocarbons in rodents, and non-human primates.

Detailed investigation of the role of ovarian metabolic activation in oocyte destruction by polycyclic aromatic hydrocarbons is proceeding in four directions: 1) ovotoxicity of benzo(a)pyrene and oxygenated derivatives; 2) high pressure reverse phase liquid chromatographic characterization of benzo(a)pyrene metabolites produced by ovarian microsomes in two inbred murine strains and their F_1 heterozygote cross, 3) evaluation of the temporal evolution of dose-response curves for oocyte destruction in inbred murine strains, 4) use of intraovarian injection techniques to determine ovarian metabolic capability, and identify proximate and ultimate ovotoxins.

Major Findings

Three prototype polycyclic hydrocarbons (benzo(a)pyrene, 3-methylcholanthrene, 7,12-dimethylbenz(a)anthracene) destroy primordial oocytes in a time, dose, strain, species, and metabolism dependent manner. Characterization of the time and dose response curves for oocyte destruction in C57BL/6N and DBA/2N mice demonstrates that strain differences in metabolic activation as measured by the aryl hydrocarbon (benzo(a)pyrene) hydroxylase assay, determines the rate of oocyte destruction but not the ultimate extent of oocyte destruction.

At short times (5 to 7 days) after intraperitoneal treatment the ED_{50}'s for oocyte destruction in inbred C57BL/6N and DBA/2N mice are widely divergent. By 14 days after treatment the ED_{50}'s of both of these strains comes together and are of the same order of magnitude. These experiments also demonstrate that primordial oocytes, oocytes in resting follicles, are most sensitive to destruction by polycyclic hydrocarbons while the growing and pre-ovulatory oocytes/follicles are not destroyed over this dose range and during the 28 day observation time. These experiments, in comparison with similar less extensive experiments conducted several years ago also demonstrate the stability of the ovotoxicity assay in that ED_{50}'s for primordial ooctye destruction have not changed over this time period.

The metabolic pathways for formation of proximate and ultimate ovotoxin(s) are not known. Experiments comparing the ED_{50}'s for oocyte destruction of benzo(a)pyrene and ten oxygenated derivatives have recently been completed. These experiments demonstrate that the order of ovotoxicity of these compounds is: 3-hydroxy, 6-hydroxy, 7-hydroxy, 9-hydroxy 4,5-oxide, cis-4,5-dihydrodiol, trans-4,5-dihydrodiol, 7,8-oxide $<<$ BP $<$ 2-hydroxy, 7,8-dihydrodiol. As the 2-hydroxy derivative is not formed in vivo or in vitro the 7,8-dihydrodiol is apparently the only proximate ovotoxin among the ten derivatives tested. This is also consistent with the 7,8-dihydrodiol-9,10-epoxide being an ultimate ovotoxin. The parallels between mutagenicity, carcinogenicity and ovotoxicity are

striking and suggest that for the polycyclic aromatic hydrocarbons, at least, ovotoxicity may represent another useful in vivo screen for genotoxicity.

The respective roles of ovarian, hepatic, or other extraovarian metabolism of polycyclic hydrocarbons to ovotoxic metabolite(s) is unknown. Intraovarian injections of small volumes of polycyclic aromatic hydrocarbons induces ovarian microsomal mixed function oxidases and produces oocyte destruction. These experiments demonstrate that ovarian microsomal Cytochrome P450 dependent monooxygenases and epoxide hydratases are fully capable of metabolizing exogenous xenobiotic substrates like the polycyclic aromatic hydrocarbons to ovotoxic intermediates.

Previous comparative (SD rat vs C57BL/6N mouse) analysis of benzo(a)pyrene metabolites using reverse phase high pressure liquid chromatography suggested that differences in the rate of production of metabolites in the 7,8,9,10 region correlated more closely with extent of ovotoxicity than the fluorescent phenolic metabolites. Experiments, still in progress, evaluating the profile of benzo(a)-pyrene metabolism in DBA/2N, C57BL/6N and their F_1 heterozygote are consistent with those observations.

Although oocyte destruction occurs after treatment with polycyclic aromatic hydrocarbons, additional reproductive toxicity is also possible. Breeding experiments suggest that polycyclic aromatic hydrocarbons may decrease fertility in the presence of sufficient stores of oocytes, and without altering ovarian response to gonadotropins. A long term breeding study conducted over the full reproductive life span of DBA/2N, C57BL/6N, and their F_1 heterozygotes has just been completed. Computer analysis of this data is in progress.

Preliminary experiments suggest that the ovaries of non-human primates are less susceptible to adverse effects of polycyclic aromatic hydrocarbons, than rodents.

Significance to Biomedical Research and the Program of the Institute

Reproduction does not take place in a chemically pristine environment. Understanding mechanism(s) of action, site(s) of toxicity, and species susceptibility to reproductive toxins is necessary in determining risks to human reproduction. These experiments provide the basis for elucidation of the mechanism of action of one class of reproductive toxins and will therefore increase our understanding of reproductive and genetic toxicology.

Proposed Course

Further characterization of proximate and ultimate ovotoxins is planned. Computer analysis of the reproductive performance studies will be completed during the coming year.

The characterization of the benzo(a)pyrene metabolite profile in ovarian microsomes from DBA/2N, C57BL/6N and their F_1 heterozygote will be completed during the coming year. This will complete a major investigation into the relationship between metabolic activation and oocyte destruction. Further investigation of the role of detoxification in ovotoxicity is planned to follow this characterization of benzo(a)pyrene metabolism.

1. Mattison, D.R.: Smoking and Fertility. In, The Health Effects of Smoking for Women, Report of the Surgeon General, 1980, pp.

2. Mattison, D.R.: The effects of biologically foreign compounds on reproduction. In Drugs in Pregnancy, Ed. R.W. Abdul-Karim. pp. 101-125, G.F. Stickley Co., Philadelphia, 1981.

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5. Mattison, D.R., Nightingale, M.S.: Murine ovarian benzo(a)pyrene metabolism and primordial ooctye detruction: A survey of nine inbred strains. In, Dynamics of Ovarian Function. Eds. N.B. Schwartz, M. Huunziger-Dunn. Raven Press pp. 89-94, 1981.

6. Mattison, D.R.: Drugs, xenobiotics and the adolescent: implication for reproduction. In, Drug Metabolism in Immature Human. Eds., L.F. Soyka, G. Redmond. Raven Press, in press.

7. Mattison, D.R., Ross, G.T.: Oogenesis and ovulation. In, Laboratory Methods for Evaluating and Predicting Specific Reproductive Dysfunctions. In press.

8. Shiromizu, K., Mattison, D.R.: Oocyte destruction by intraovarian injection of benzo(a)pyrene. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00900-03 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Factors Regulating Estrogen and Progesterone Receptors in Monkey Endometrium in the Fertile Menstrual Cycle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	B. Kreitmann	Guest Worker	PRB, NICHD
	D. Barber	Technician	PRB, NICHD

COOPERATING UNITS (if any)

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Pregnancy Research Branch

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Section on Endocrinology

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NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Appropriate methods for repeated surgical collection of endometrial tissue from monkeys and characterization of cytosol and nuclear estrogen and progesterone receptors have been developed. Equilibrium dissociation constants of estradiol and progesterone were 2.1×10^{-10}M and 3.6×10^{-9}M, respectively. When estrogen or estrogen + progesterone replacement therapy was given to castrate monkeys, we found that progesterone receptor synthesis was induced by estrogens. Further, in the fertile menstrual cycle, progesterone secretion from the corpus luteum preserved the endometrium, induced a shift toward enhanced nuclear receptors for estrone.

PHS-6040
(Rev. 2-81)

Objectives

To develop surgical and other methodologic procedures for studying factors influencing endometrial receptors for estrogens and progesterone in monkeys. To investigate the action of these receptors in facilitating implantation. To exploit these findings for the purposes of fertility regulation, either to resolve infertility or provide contraception.

Methods

At abdominal transfundal hysterotomy, we were able to collect endometrial tissue repeatedly. Standardized techniques and characterization of steroid receptors in other species were adapted to optimal circumstances. By giving estrogen and progesterone replacement therapy to castrate monkeys in a pattern which mimicks the normal ovulatory menstrual cycle, follicular phase and luteal phase factors influencing receptor formation were evaluated.

Major Findings

Simulated menstrual cycles were produced in 20 castrate monkeys by sequential treatment with estradiol and progesterone in silastic capsules. RIA of E_2 and P, and gonadotropins in peripheral serum provided assuredness of the hormonal status of each monkey under treatment. Cytosol and nuclear receptors for E_2 and P were measured in the endometrium after different intervals of the treatment. E_2 receptor (E_2R) levels were not changed during the estrogen sequence, but were lowered by progesterone therapy in both cytosol and nuclear components. Progesterone receptor (PR) synthesis in cytosol was induced by exogenous estrogen. The total concentration of PR decreased with the uptake of P by the cell; meanwhile, the ratio of cytosol to nuclear P receptors declined. These data suggest that this sequential estrogen-progesterone regimen induces the changes in E_2R and PR patterns in endometrium of ovariectomized monkeys which occurs due to ovarian cyclicity in the normal menstrual cycle.

Proposed Course

Current studies center on the changes in endometrial steroid receptors that accompany implantation in the fertile menstrual cycle and the abnormal characteristics of endometrial receptors for these steroids during short luteal phase infertility. We are defining the minimal level of progesterone necessary to sustain early pregnancy.

References

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2. Kreitmann-Gimbal, B., Bayard, F. and Hodgen, G.D.: Changing Ratios of Nuclear Estrone to Estradiol Binding in Endometrium at Implantation: Regulation by Chorionic Gonadotropin and Progesterone During Rescue of the Primate Corpus Luteum. J. Clin. Endocrinol. Metab. (in press).

3. Kreitmann, O., Bayard, F. and Hodgen, G.D.: 17β-hydroxysteroid Dehydrogenase in Monkey Endometrium: Characterization of Enzyme Activity and Effect of Estradiol Alone and in Combination with Progestrone. Steroids 34: 693-703, 1980.

4. Kreitmann-Gimbal, B., Goodman, A.L., Bayard, F. and Hodgen, G.D.: Characterization of Estrogen and Progesterone Receptors in Monkey Endometrium: Surgical and Biochemical Methodology and Effects of Estradiol and/or Progesterone on Castrate Monkeys. Steroids 34: 749-770, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00901-03 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Endocrine Assays Laboratory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Wilbert E. Nixon	Senior Investigator	PRB, NICHD
Other:	Rudolph Reid	Technician	PRB, NICHD
	Jeffrey Gelblum	Stay-in-School	PRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.2	.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Endocrine Assays Laboratory generated, titered and distributed sheep anti-rabbit gamma globulin sera (second antibody) to NICHD Intramural Investigators, mostly in PRB, ERRB and DEB. Rigid quality control and efficient harvesting procedures were combined to provide a high quality reagent at a fraction of the commercial cost. Specific antisera against steroid hormones were provided to investigators at the University of Maryland Medical School, Baltimore, MD and at Eastern Virginia Medical School at Norfolk, VA. Preliminary studies were initiated in collaboration with Dr. Griff Ross and Dr. Donald Mattison to investigate neonatal and fetal ovarian function in vitro using a rat model. Determination of the optimal model system with respect to age, species, culture conditions, biochemical and morphological markers is in progress.

PHS-6040

Second antibody has been provided NICHD Intramural investigators on a continuous basis. The production, titering, storage and distribution of this reagent provide large monetary savings to the Institute while providing a very high quality product to investigators. Commercial cost of antiserum produced is in excess of $200,000 while production cost are estimated to be only a fraction of this cost.

Specific, high titer antiserum to steroid hormones of importance in reproduction (estradiol, progesterone, androstenedione and testosterone) were provided on request of Drs. Ed Albrecht and Gerald Pepe of the University of Maryland Medical School and of Eastern Virginia Medical School, respectively.

The investigation of neonatal and fetal ovarian function in vitro requires solutions to a number of specific methodologic problems.

1. Preparing ovarian tissue in appropriate medium.
2. Attaining successful growth in culture.
3. Determination of viability and function by biochemical and morphological endpoints.
4. Demonstration of experimentally related alterations in marker systems.

Solutions to the first 3 problems appear imminent. The fourth will then be testable.

Directions:

The Endocrine Laboratory will actively seek to interface with the various Branches of the Intramural program. Where Branch programs and laboratory capabilities can be matched, it is anticipated that collaborative efforts will be established contingent on level of resources availability.

Investigations of neonatal and fetal ovarian function in vitro are dependent on progress of the preliminary studies. Continued favorable results in the crucial areas of culture viability and marker development (both biochemical and morphological) will influence and determine the experimental approaches possibly with this system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00907-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Reproductive Toxicity of Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Donald R. Mattison	Medical Officer	PRB, NICHD
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	Kenji Shiromizu	Visiting Fellow	PRB, NICHD
	Lucy Chang	Biological Aide	PRB, NICHD
	Helene Fedde	Nurse	CC, NIH
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COOPERATING UNITS (if any) Snorri Thorgeirsson, Head, Biochem. Pharm. Sect. NCI; Anthony Fauci, Head, Clin. Physiol. Sect., Lab. Clin. Invest., NIAIDD; Susan Fabro, Head, Pharm. & Exper. Thera. Sect., NCI; Thomas Shawker, Chief, Ultrasound, CC; L.F. Soyka, Dept. Pharmacology, Univ. Vermont.

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Section on Endocrinology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	.5	1.5

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Certain drugs used in the treatment of neoplastic and some non-neoplastic diseases produce human reproductive toxicity. Detailed studies of these patients before, during and after treatment may help elucidate the mechanisms of, and sites of reproductive toxicity. Parallel experimental studies in rodents and non-human primates will allow a more specific dissection of the mechanism of reproductive toxicity of these drugs as well as the sensitive reproductive processes. Cyclophosphamide a commonly used alkylating agent has been demonstrated to destroy rodent primordial oocytes in a strain and species dependent fashion. Single intraperitoneal injections of cyclophosphamide do not destroy the growing or large oocytes/follicles in rodents. This appears in marked contrast to their action in humans. Azathioprine and 6-mercaptopurine do not destroy oocytes in weanling mice.

Objectives

Cyclophosphamide, a widely used alkylating agent has been demonstrated to produce gonadal toxicity. We are exploring the mechanism of action of reproductive toxicity of cyclophosphamide in rodents, non-human primates and women. The women studied are patients at the clinical center, being treated for diseases for which cyclophosphamide is the drug of choice. The goal of this research is to elucidate mechanisms, sites of action, and species susceptibility of drugs producing reproductive toxicity. Other drugs studied in the past year have inclined azathioprine, and its metabolite 6-mercaptopurine.

Major Findings

Studies in rodents, in collaboration with Snorri S. Thorgeirsson, NCI, (ZO1 CM 06137-03 LCHP) have demonstrated that cyclophosphamide and several of the antitumor agents destroys primordial oocytes in rodents in a time, and dose dependent manner. Little toxicity is seen in growing or pre-ovulatory follicles. Strain and species differences in sensitivity to oocyte destruction have been observed but are not understood at the present time.

Observations on women treated with cyclophosphamide suggest further species differences in follicle sensitivity to the drug. Women chronically treated with cyclophosphamide generally develop amenorrhea shortly after starting therapy, suggesting effects on growing and pre-ovulatory follicles. Pelvic ultrasound offers an indirect measure of ovarian size and follicular proliferation. In these patients on cyclophosphamide it is frequently difficult to "see" the ovary with pelvic ultrasound, similar to postmenopausal women. In several patients switched from cyclophosphamide to azathioprine the ovary has become visible and grown in size, further supporting the suggestion that cyclophosphamide predominantly effects the growing and pre-ovulatory oocytes/follicles. This is in contrast to primordial oocyte destruction which occurs in rodents treated with cyclophosphamide.

Studies in other laboratories have demonstrated that prenatal treatment with the immunosuppressive azathioprine (or its metabolite 6-mercaptopurine) produces sterility. We have extended those observations by demonstrating that both drugs are not toxic to oocytes/follicles in weanling mice at doses large enough to depress weight gain and produce mortality in the treated animals. This suggests that the reproductive toxicity produced by these drugs is secondary to disruption of oogenesis.

Preliminary investigations suggest that cyclophosphamide does not alter hypothalamic-pituitary control of gonadotropin secretion in castrate female Rhesus monkeys.

Significance to Biomedical Research and the Program of the Institute

Reproductive toxicity of drugs used in the treatment of human disease is a significant side effect. With increasing success in producing prolonged remissions in patients with cancer, premature ovarian failure is a major concern of the patient and her family. Understanding the mechanism of reproductive toxicity of these drugs may allow the design of alternate treatment schedules, or use of

different drugs to decrease this side effect. An additional gain from these studies is the knowledge of comparative mechanism of action of reproductive toxins in humans and experimental animals.

Proposed Course

Further research will explore in greater detail the gonadal and reproductive toxicity of cyclophosphamide and other antitumor agents to determine effects on ovarian and pituitary function, as well as follicle susceptibility. Detailed studies will continue in rodents to elucidate the mechanisms of activation, and pathways for detoxification. We will also continue our observations of patients treated with cyclophosphamide, as well as other known or suspect reproductive toxins.

Publications

1. Mattison, D.R., Chang, L., Thorgeirsson, S.S., Shiromizu, K.: The effects of cyclophosphamide, azathioprine, and 6-mercaptopurine on oocyte and follicle number in C57BL/6N mice. Research communications in Chemical Pathology and Pharmacology. 31, 155-161, 1981.

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4. Mattison, D.R., Ross, G.T.: Oogenesis and ovulation. In, Laboratory Methods for Evaluting and Predicting Specific Reproductive Dysfunctions. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00908-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Genetics of Ovarian Failure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Joseph D. Schulman	Medical Officer	NPMB, NICHD
	Lucy Chang	Biological Aide	PRB, NICHD
	Y.T. Chen	Clinical Associate	DPB, NICHD

COOPERATING UNITS (if any) Carol Henry, Director, Dept. Exptl. Oncology, Microbiological Associates; Nathanial White, Statistician, NICHD; Blanch McFarland, Biomedical Mathematical Modeling Consultant; Bev. White, Senior Investigator, NIAMDD

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Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

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XX (a) HUMAN SUBJECTS XX (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS XX (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The genetics of atresia is being explored in several inbred strains of mice and their F_1 heterozygote crosses. Initial experiments have identified differences in the rates of atresia in several inbred mice strains as well as their F_1 heterozygotes. Two families with familial human premature ovarian failure are being studied. The mechanism of premature ovarian failure in galactosemia is being explored. Experimental animal studies demonstrate that exposure to galactose during pregnancy can decrease oocyte number.

PHS-6040
(Rev. 2-81)

Objectives

Investigations in other laboratories have demonstrated differences in the rates of oocyte loss with age among different inbred strains of mice. The genetics of the differences in these rates have not been explored in detail. These experiments have been designed to elucidate genetic aspects of atresia using inbred mice and their heterozygote crosses. Familial spontaneous premature menopause and premature ovarian failure in galactosemia are also being studied.

Major Findings

The spontaneous rate of loss of primordial oocytes is more rapid in C57BL/6N than DBA/2N mice or their F_1 heterozygotes. The rate of primordial oocyte loss is more rapid in C3H/AnfCum than C57BL/6Cum mice or their F_1 heterozygotes. DBA/2J and DBA/2N mice have similar rates of atresia, as do C57BL/6N and C67BL/6Cum mice. Two families with premature ovarian failure have been identified. Prenatal exposure to a high galactose diet alters oogenesis and results in a decreased complement of oocytes at weaning.

Significance ot Biomedical Research and the Program of the Institute

The ultimate event of reproductive senescence in the female is the depletion of oocytes from the ovary. The age at which the ovary is depleted of oocytes depends in part upon the initial complement of oocytes, the rate of recruitment and atresia, as well as the effects of exposure to ovotoxins. Characterization of the genetics of oocyte loss in mice may provide insight into the biological nature of these processes. Additionally, these studies may also help clarify the interrelationship between oocyte toxicity and oocyte loss.

Proposed Course

Further characterization of the rates of ooctye loss in these inbred strains and their crosses will continue. A computer model is being prepared to provide cross analysis of data in this project as well as projects Z01 HD 00907-02 PR and Z01 HD 00168-05 PR. Further study of women with familial premature ovarian failure will continue. Additional experiments are in progress to characterize the mechanism of action of galactose in decreasing ooctye number.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00909-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Ethanol on the Mother and the Fetus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Anil B. Mukherjee	Senior Investigator	PRB, NICHD
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	Lindsey Golden	Technician	
	R. Manjunath	Visiting Fellow	PRB, NICHD
	Marcy Comly	Biological Aide	PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of ethanol on the pregnant rats and their progeny have been evaluated from the standpoint of β-endorphin receptor in the brain and β-endorphin level in the blood. It was found that β-endorphin receptor level increased following ethanol administration in the mother. Endorphin receptor level in the fetal brain also went up but in a slightly delayed fashion compared to the mother. This indicates that β-endorphin receptor in the brain as well as its ligand (β-endorphin) is affected in the adult and in fetal rats following ethanol administration.

PHS-6040
(Rev. 2-81)

Objectives

Z01 HD 00909-02 PR

Ethanol abuse is on the increase throughout the world. In this country alone there are at least 10-15 million people who are alcoholics. Teenage alcoholism is on the rise. Only recently an effort is being made to delineate whether or not a biological or biochemical marker for alcoholism could be identified in individuals who are prone to alcoholism. In Netherlands studies with identical twins from alcoholic parents (both parents alcoholic) have shown that these twins eventually became alcoholics even if they were raised in a non-alcoholic family environment. The presence of a genetic factor for predisposition to alcoholism has been proposed. Our studies were directed towards identifying possible biological/biochemical factor(s) that may be predictors of alcoholism at an early age.

Major Findings

It was found that adult rats treated with ethanol had higher levels of β-endorphin receptor in the brain. This higher receptor level was observed at six days post ethanol feeding. The receptor level in the 16-19 day fetuses were also higher in the ethanol treated rats compared to normal. The receptor level was from day 6 of ethanol feeding was significantly higher than controls. Plasma β-endorphin level, on the other hand, increased immediately (24 hr) after ethanol feeding and slowly decreased to lower levels which were slightly above normal control values at all time periods studied.

Significance of the results

Our results indicate that chronic ethanol feeding increases β-endorphin receptor level in the rat brain. Plasma β-endorphin level, however, is increased only when alcohol is administered acutely. The relationship between plasma β-endorphin level and its receptor level in the brain is now being established.

Proposed Course

We plan to measure β-endorphin levels in the plasma of the mother and of the fetus. The mother will either be alcohol fed or on normal diet. This will establish the effect of ethanol on the fetal plasma as affected by maternal alcohol consumption.

The enzymes alcohol dehydrogenase and acetaldehyde dehydrogenase are now being assayed in both ethanol fed animals compared to the controls.

Publications

1. Mukherjee, A.B., Fisher, A. and Golden, L.: Influence of ethanol feeding on β-endorphin and its receptor level in the rat. (In preparation).

2. Mukherjee, A.B., Tomorkin, L. and Fisher, A.: Use of inhibitors in the preservation of plasma samples for β-endorphin assay. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00910-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Uteroglobin and Transglutaminase in Reproduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Anil B. Mukherjee	Senior Investigator	PRB, NICHD
Other:	R. Manjunath	Visiting Fellow	PRB, NICHD
	D. Cunningham	Biological Aide	PRB, NICHD
	A. Tichivakunda	Technician	PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Last year we reported that uteroglobin (UG) in conjunction with transglutaminase can render implanting embryonic cells non-antigenic to maternal lymphocytes. We proposed at that time that the presence of uteroglobin and transglutaminase in the prostate may be responsible for creating a non-antigenic state of the male gamete in the female organism. We have now tested the hypothesis by mixing epididymal sperm with female lymphocytes with various treatment combinations with uteroglobin, transglutaminase or its inhibitors and antibodies. Our results indicate that epididymal sperm is highly antigenic to female lymphocytes. However, treatment of these sperm with prostatic fluid, or uteroglobin and transglutaminase makes them non-antigenic. Inhibition of transglutaminase abolishes the immunosuppressive role of uteroglobin and prostatic fluid. These findings further substantiate our hypothesis that protein such as UG in the prostate may crosslink with antigens of the sperm surface to mark their antigenicity.

PHS-6040
(Rev. 2-81)

The present investigations were carried out to delineate a) whether or not rabbit prostatic fluid is capable of suppressing immunogenicity of epididymal sperm when mixed with lymphocytes of female animals, and b) whether or not the active factors present in the prostatic fluid (e.g. UG & TG) are responsible for induction of nonimmunogenic state of the ejaculated sperm.

Major Findings

It was found that epididymal sperm are very antigenic to female lymphocytes in a mixed culture. This was suggested by the increased incorporation of ^{3}H-thymidine into maternal lymphocytes in a mixed culture of sperm and lymphocytes. However, when epididymal sperm were treated with either prostatic fluid or uteroglobin in combination with transglutaminase they did not stimulate ^{3}H-thymidine incorporation into maternal lymphocytes. Inhibition of transglutaminase led to the elimination of this suppressive effect. Treatment of UG and TG with their antisera prior to the treatment of sperm with these substances abolished the immunosuppressive effects. Non-specific proteins such as myoglobin did not result in any immunosuppression. This suggests the specificity of UG and TG in masking the antigenicity of sperm in the inseminated female.

Significance in Biomedical Research

Although male gametes express alloantigens on their surface, they are not rejected by the female in most instances. The mechanism of such non-rejection was not well understood. The results of our experiments may explain a mechanism by which male gametes become non-antigenic to the females. There are instances, however, when a female goes into anaphylexis following coitus. This may be due to a lack of either UG or TG or both in the prostate. Experiments now could be conducted to delineate this. Furthermore, a nontoxic inhibitor of transglutaminase could be used as a male contraceptive. Some of our preliminary experimental results indicate that inhibitors of TG such as cystamine prevents implantation in the mouse. This whole idea of masking of cell surface antigens by another protein is a novel one and this may have implications not only in transplantation tolerance at pregnancy but also in tumor biology, especially, how tumor cells escape immunological assault by the host.

Proposed Course

Uteroglobin (UG) is a progesterone stimulated endometrial protein. Progesterone agonists or antagonists could be easily tested if an in vitro assay system was available. At present we have developed highly specific antibodies to UG to be utilized in a radioimmunoassay for quantitation of this protein. We also are in the process of establishing a permanent rabbit endometrial cell culture which will produce UG in response to progesterone. This will enable us to determine progestogenic properties of substance in vitro.

Studies on the inhibition of transglutaminase in the male and its effect on pregnancy are now being investigated.

It has been demonstrated recently, that the gene for UG in the endometrium, the lung and in the prostate as the same. However, the factors that control the expression of this gene is different in difficult organs. In the endometrium it

is progesterone. We are interested in delineating what factor(s) controls this gene in the prostate and in the lung.

Publications

1. Mukherjee, A.B., Laki, K. and Agrawal, A.K.: Possible mechanism of success of an allotransplantation in nature: Mammalian pregnancy. Med. Hypoth. 6(10): 1043-1055, 1980.

2. Mukherjee, A.B., Ulane, R.E., and Agrawal, A.K.: Role of uteroglobin transglutaminase and progesterone in the non-rejection of a natural allotransplant: Mammalian pregnancy. Soc. Gyn. Invest. 1981. Abst. 238.

3. Mukherjee, A.B., Ulane, R.E. and Agrawal, A.K.: Possible mechanism of non-rejection of the developing mammalian embryo: The role of uteroglobin and transglutaminase. Ped. Res. 15: 486, 1981.

4. Mukherjee, A.B. and Manjunath, R.: Uteroglobin and α-fetoprotein are substrates of plasma transglutaminase. Polymerization in solution and on the cell surface. J. Biol. Chem. (In preparation).

5. Mukherjee, A.B., Manjunath, R. and Cunningham, D.: Uteroglobin and transglutaminase in the prostatic fluid render sperm non-antigenic to the female rabbits. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00911-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Gene Transfer by Lipochromosome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Anil B. Mukherjee	Senior Investigator	PRB, NICHD
Other:	William Rizzo	Clinical Associate	NPMB, NICHD
	M. Rampertaap		

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using liposome-entrapped SV40 DNA as a model system to infect monkey kidney cells the carrier potential of liposomes in gene transfer has been tested. The frequency of plaque formation corresponds to the frequency of successful DNA transfer and this was quantitated. The results of these experiments have shown that liposome-entrapped SV40 DNA becomes infectious when incubated with monkey kidney cells, that the frequency of plaque formation can be modulated by lipid composition of the liposomes and various treatments of the cells and that the addition of carrier DNA inhibits infectivity. Preliminary results of some experiments indicate that liposome-entrapped SV40 DNA can be used to transform mouse cells, an event which requires integration of viral DNA into these cells.

PHS-6040
(Rev. 2-81)

Objectives ZO1 HD 00911-02 PR

Research in gene transfer has centered around developing various vectors to transfer the desired target cells. As more genes are isolated and purified the need for a suitable gene transfer mechanism will be required if the biological functioning of these isolated genes are to be tested. Our efforts are directed toward developing a method of gene transfer which would be nontoxic to the target cells and yet most efficient in transferring the desired genes.

Major Findings

The results of our experiments suggest that liposome entrapped SV40 DNA becomes infectious when incubated with monkey kidney cells and that the frequency of transfer is dependent on the lipid compositon of the liposomes. Phosphatidyl serine containing liposomes are the most efficient from the standpont of increased frequency of gene transfer. Use of carrier DNA with the gene inhibited the transfer frequency. Liposome entrapped SV40 DNA could be transferred to mouse cells (not its natural host) with a high frequency of transformation.

Significance in Biomedical Research

The advantage of liposome mediated gene transfer over other methods (i.e. Calcium phosphate, DEAE-dextran etc.) resides in 1) the versatility of modifying either liposomes, DNA or recipient cells, 2) lack of requirement for carrier DNA which integrates into the recipient cell along with the desired gene(s) to be transferred, (3) the potential for using the system as a functional assay for various purified viral proteins or chromosomal proteins and 4) potential for targeting gene containing liposomes to desired tissues by crosslinking antibody or other tactic agents on the liposome surface.

Proposed Course

At present, we are attempting to reconstitute SV40 DNA with cellular histones to produce chromatin. Then chromatins will be loaded into liposomes to assay its effectiveness in gene transfer. Crosslinking of liposome surface with low density lipoprotein (LDL) for targeting these liposomes for specific binding on the cell surface is now being attempted.

Publications

1. Rizzo, W., Schulman, J.D. and Mukherjee, A.B.: Ped. Res. 15: 568 (1981).

2. Rizzo, W., and Mukherjee, A.B.: Transfer of SV40 genoma into homo and heterologous cells via liposome entrapment. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00912-02 PR

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Gene Regulation and Cellular Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Janice Y. Chou	Senior Investigator	PRB, NICHD
Other:	Toshiaki Mano	Visiting Fellow	" "
	Fumiyuki Ito	Visiting Fellow	" "
	Richard Staton	Technician	" "

COOPERATING UNITS (if any)

S. Rosen, NIAMDD, NIH

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS XXX(b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Topics of current interest are: 1) control of the expression of human chorionic gonadotropin (hCG), pregnancy-specific β-1-glycoprotein (SP1) and alkaline phosphatase in placenta, SV40 tsA-transformed placental, choricarcinoma, and nontrophoblastic tumor cells; 2) control of the expression of α-fetoprotein (AFP), albumin, and transferrin in liver, SV40 tsA-transformed liver, and hepatoma cells; 3) biosynthesis and processing of AFP, albumin and transferrin in liver cells; 4) biosynthesis and processing of hCG, SP1 and alkaline phosphatase in placental cells.

Project Description: Z01 HD 00912-02 PR

Objectives: The main objectives of this project are: (1) to study cellular differentiation and gene regulation in placenta and liver; (2) to isolate and characterize selected enzymes and other proteins from normal placenta, choriocarcinoma cells, SV40-transformed placental cells and SV40-transformed rat liver cells; and (3) to study the biosynthesis and processing of selected enzymes and other proteins in placental and liver cells.

Methods Employed: The principal methods employed are: (1) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, ion-exchange chromatography, and affinity chromatography; (2) enzyme assays, which are usually based on spectrophotometric or radiometric methods; (3) radioimmunoassays of hormones, liver proteins, and cAMP; (4) transformation of placental cells and liver cells with SV40; and (5) Immunoprecipitation, gel electrophoresis, and autoradiography.

Major Findings:

(1) 8BrcAMP and Bt_2cAMP greatly induced the synthesis of hCGα with little or no stimulation of the synthesis of hCG in SV40 tsA-transformed placental cells grown at 33C or 40C. On the other hand, 8BrcAMP stimulated the synthesis of hCG preferentially in choriocarcinoma cells. Our data indicate that adenosine cyclic nucleotides have different effects on the production of hCG but not on hCGα in SV40 tsA-transformed placental cells and choriocarcinoma cells.

(2) In the SV40 tsA-transformed human first trimester placental cells, glucocorticoids (cortisol or dexamethasone) greatly inhibited hCG synthesis but induced hCGα synthesis. The glucocorticoids, however, did not affect the synthesis of hCG or hCGα either in tsA-transformed human term placental cells or in choriocarcinoma cells. These selective effects in cultured first trimester placental cells suggest that glucocorticoids may play an important role in the regulation of hCG production during early pregnancy.

(3) Using newly developed radioimmunoassay for pregnancy-specific B_1-glycoprotein (SP1), we found that the tsA-transformed placental cells synthesized low levels of SP1 at 33°C. At 40°C, SP1 production was greatly increased. Sodium butyrate and BrdUrd which affect the synthesis of hCG, hCGα, and alkaline phosphatase also induced the synthesis of SP1 in the tsA-transformed placental cells. Therefore, the tsA-transformed placental cells should be useful in studying SP1 synthesis in human placentas.

(4) Clonal rat fetal liver cell lines that expresses the functions of differentiated liver cells under controllable conditions has been established. Normal fetal liver cells were transformed by tsA mutants of SV40. These transformed cells are temperature sensitive in maintenance of the transformed phenotype. The transformed fetal liver cells synthesized AFP, albumin, and transferrin. At 33°C, the levels of these liver proteins were relatively low. At 40°C, the transformed phenotype was lost, and the levels of AFP, albumin, and transferrin were greatly increased. In addition, these SV40 tsA-transformed liver cells, like primary cultures of hepatocytes, responded to glucagon with markedly elevated levels of cAMP. Thus, it appears that glucagon receptors characteristic of hepatocytes are retained in the SV40 tsA-transformed fetal liver cells.

Significance to Biomedical Research and the Program of the Institute: The study of the mechanisms of mammalian cell differentiation is of importance because it may lead to an understanding of normal and abnormal cellular functions.

The investigations of hormonal regulation and gene expression in placenta and liver are essential for an understanding of fetal development and embryogenesis. The study of the control of gene expression of alkaline phosphatase, hCG, SP1, and AFP is of importance because these proteins are derepressed in many tumors. Extopic production of these fetal proteins may serve as an early signal of malignant transformation. The establishment of human placental cells and rat fetal liver cells which approximate normal placental and liver cells in vivo respectively should help us to elucidate the control mechanisms for the synthesis of these proteins.

Proposed Course of Research Project: (1) To study the synthesis and processing of hCG, SP1, and alkaline phosphatase in placental cells. (2) To study the synthesis and processing of α-fetoprotein, albumin, and transferrin in liver cells. (3) To study the molecular mechanisms regulating the levels of the placental and liver proteins in these conditionally transformed mammalian cells. To determine: a) whether the levels of expression of these proteins are regulated by transcription and/or; b) whether translational control is a factor in the control of gene expression. (4) Delineate the mechanism regulating the synthesis of these placental and liver proteins by hormones and other effectors.

Publications

1. Chou, J.Y. (1980): Regulation of human chorionic gonadotropin synthesis in placenta, choriocarcinoma, and non-placental tumors. "Chorionic Gonadotropins" in Topics in Population Sciences, (S.J. Segal, Editor) Plenum Press, New York, p. 317-344.

2. Chou, J.Y. (1980): Effects of sodium butyrate and 5-bromo-2-deoxyuridine on the synthesis of human chorionic gonadotropin in human placental cells transformed by tsA mutants of simian virus 40. Endocrinology 107: 1327-1333.

3. Chou, J.Y. (1980): Effects of adenosine cyclic nucleotides on the synthesis of human chorionic gonadotropin in transformed human placental cells. Cancer Research 40: 4025-4030.

4. Chou, J.Y., and Schlegel-Haueter, S.E. (1981): Study of liver differentiation in vitro. J. Cell. Biol. 89: 216-222.

5. Sakiyama, T., Mano, T., and Chou, J.Y. (1980): Synthesis of first trimester placental alkaline phosphatase in cultured human term placental cells. J. Biol. Chem. 255: 9399-9403

6. Mano, T., and Chou, J.Y.: Regulation of human chorionic gonadotropin synthesis in cultured human placental cells by glucocorticoids. Endocrinology, accepted for publicaton.

7. Chou, J.Y., Rosen, S.W., and Mano, T.: Production of pregnancy-specific β_1-glucoprotein by cultured placental cells. J. Clin. Endocrinol. Metab., accepted for publication.

8. Schlegel-Haueter, S.E., and Chou, J.Y.: Characterization of human chorionic gonadotropins secreted by human cell lines. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00913-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Primate Models for In Vitro Fertilization and Alternatives

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief	PRB, NICHD
Other:	O. Kreitmann	Guest Worker	PRB, NICHD
	B. Cowan	Guest Worker	PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

We have developed procedures for oocyte aspiration from the preovulatory follicle, in vitro fertilization, embryo culture and an alternative low tubal ovum transfer surgical procedure allowing in vivo fertilization. Restrospective analysis demonstrated that aspiration of the preovulatory follicle often induced corpus luteum dysfunction. Further, we have assessed cleavage rates of monkey embryos in vitro, finding that their retarded progression may be a major obstacle in successful in vitro fertilization with embryo transfer.

Project Description Z01 HD 00913-02 PR

Objectives

Collection of the "ripe" oocyte from the preovulatory follicle is difficult to schedule appropriately. The fertilizability of the "unripe" egg is poor, but writing too long will permit spontaneous rupture of the dominant follicle and loss of the oocyte. We seek to maximize the skill of "timing" aspiration of the oocyte.

Major Findings

Aspiration of the preovulatory follicle induced luteal dysfunction, probably due to removal of granulosa cells.

We have succeeded in achieving in vitro fertilization of monkey oocytes.

After low tubal ovum transfer, we obtained 5 pregnancies after 31 attempts.

Proposed Course

Two major problems remain priority studies: 1) Development of replacement therapy regimens that correct induced luteal dysfunction after follicular aspiration; and 2) overcoming the asynchrony between retarded embryo development in vitro and maternal uterine status at the time of embryo transfer. To this end, we have initiated development of an egg-embryo chamber, taking advantage that the intra-abdominal milieu is supportive of timely embryonic cleavage.

Publications

1. Kreitmann, O. and Hodgen, G.D.: Low Tubal Ovum Transfer: An Alternative to In Vitro Fertilization. Fertil. Steril. 34: 375-378, 1980.

2. Kreitmann, O. and Hodgen, G.D.: Induced Corpus Luteum Dysfunction After Aspiration of the Preovulatory Follicle in Monkeys. Fertil. Steril. 35: 671-675, 1981.

3. Hodgen, G.D.: In Vitro Fertilization and Alternatives. JAMA 246: 590-597, 1981.

4. Kreitmann, O. and Hodgen, G.D.: Retarded Cleavage Rates of Preimplantation Monkey Embryos In Vitro. JAMA 256: 627-629, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00914-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Fallopian Tube Dysfunction and Endometriosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	G.D. Hodgen	Chief	PRB, NICHD
Other:	G.S. diZerega	Clinical Associate	PRB, NICHD
	E.L. Marut	Clinical Associate	PRB, NICHD
	L.A. Werlin	Clinical Associate	PRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major cause of human infertility in the U.S.A. is tubal dysfunction. Endometriosis and other causes of failure to achieve satisfactory gamete transport are major contributing factors. We sought to examine how these infertilities arise and how to better treat them medicinally and surgically. On-going studies have compared the effectiveness of Danazol vs GnRH agonists/antagonists in reducing pelvic lesions arising from induced endometriosis.

PHS-6040
(Rev. 2-81)

Project Description ZO1 HD 00914-02 PR

Objectives

The cause of infertility when endometrial tissue grows ectopically is unknown; even its hormonal support is poorly understood. We studied: 1) the role of estrogens and progesterone in the maintenance of ectopic endometrial plaques; and 2) the effect of ectopic endometrium on ovum and sperm transport.

Major Findings

Either estrogen or progesterone, alone or in combination, support survival of endometrial plaques. Removal of steroids suppresses long existing ectopic endometrium within 4 to 6 weeks.

Proposed Course

Current projects are aimed at determining if ovum pick-up at ovulation and tubal transport of both egg and sperm are inhibited by ectopic endometrium.

Publications

1. Marut, E.L. and Hodgen, G.D.: Serial Evaluation of Danazol Action on Ectopic Endometrium in Monkeys. Fertil. Steril., in review.

2. Werlin, L.B. and Hodgen, G.D.: Endometriosis II. Effects on Ovulation, Ovum Pick-up, and Gamete Transport in Monkeys. Fertil. Steril., in review.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00915-02 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Antenatal diagnosis and intrauterine surgical restoration of neural tube defects and limb anomalies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Maria Michejda	Special Consultant	PRB, NICHD
Other:	Gary D. Hodgen	Chief	PRB, NICHD
	Linda Wilmot	Part-time-student	PRB, NICHD

COOPERATING UNITS (if any)

J. Bacher, Surgery Unit, VRB, DRS

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX(c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The first stage of our study was concerned with the immunobiological properties of bone allografts and their usefulness in intrauterine repair of fetal bone defects.

Allogeneic bone transplantation in utero in the Macaca mulatta model has been carried out successfully. Two techniques were applied. The first consisted of correcting a surgically created loss of a bone segment of the fetal humerus with bone-plug taken from a different fetus. The second was the application of a bone-paste, consisting of allogeneic bone particles suspended in an agar-base culture medium to a surgically ablated humerus of the host. Both techniques were very successful and provided firm evidence for the permissiveness of the fetal immune surveillance system in the acceptance of allogeneic bone transplants in primates. It was determined that this permissiveness persists for up to eight weeks of postnatal life.

The second stage of our study, which is now in progress, involves the diagnosis and in utero surgical correction of skeletal defects, such as hydrocephaly, meningocele, encephalocele and spina bifida. These conditions are being induced at an early stage of gestation by treating monkeys with teratogens.

Objectives

The research project which was initiated more than a year ago consists of four major lines of investigations.

1. Allogeneic bone transplantation *in utero*
2. Induction of skeletal anomalies
3. Intrauterine diagnosis of birth defects
   a. determination of AFP levels in maternal serum
   b. roentgenography
   c. real-time ultrasound scanning
   d. fetoscopy
4. *In utero* surgical repair or treatment of anomalies
   a. hydrocephalus
   b. spina bifida
   c. limb bud regeneration

While the first study (1) of allogeneic bone transplantation is completed, the induction of neural tube defects such as hydrocephalus, crania bifida (associated with meningocele or encephalocele), spina bifida and limb malformations, their antenatal diagnosis and the treatments (4) are currently being carried out.

Methods Employed

1. The details of two surgical techniques of allogeneic transplantation *in utero* were described in References 1 and 2.

2. Induction of neural tube defects:

   Successful attempts have been made to induce bone defects in monkey fetuses by treating the pregnant females with a synthetic cortico-steroids in the early gestation period (18-25 days). This method has been shown to induce various defects such as spina bifida, hydrocephaly, encepholocele or meningocele.

3. Antenatal diagnosis of induced birth defects.

Intrauterine detection of various anomalies are being made, utilizing ultrasonography, roentgenography, AFP determination in the maternal serum and fetoscopy.

4. *In utero* surgical repair of various anomalies.

Successful attempts were made for antenatal treatment of hydrocephalus. We have designed and successfully used a pressure sensitive prosthesis which when placed into the subarachnoid space and/or lateral ventricle *in utero*, relieves these hydrocephalic fetuses of excessive CSF throughout the remainder of gestation. Thus, after intrauterine ultrasonography and measuring excessive intracranial CSF pressures in hydrocephalic fetuses, the indwelling prosthetic valve was applied - the hydrocephalic antenatal vent for intrauterine treatment (HAVIT). The HAVIT was surgically implanted in the fetal skull so that when CSF pressures exceed 60 mm of H_2O, the ports opened to excess vent CSF from the fetal ventricular system into the amniotic fluid. A potent application has been filed

for the HAVIT prosthesis. On-going research is directed at evaluating the postnatal cognitive and neurological well-being of infant monkeys that received this prosthesis antenatally. In all cases of hydrocephalus associated with the herniation of the brain tissue (due to increase of intracranial pressure) resulted in cranium bifidum associated with encephalocele or meningocele the hypertrophic tissue was surgically removed. Moreover, the cranium bifidum caused by extensive hypoplasia of occipital bone was repaired (cranioplasty) using techniques applied in the allogenec bone transplantation.

Major Findings

1. Allogeneic bone transplantation.

Our findings indicated that the immune surveillance system of fetal monkeys was tolerant of bone allografts (1,2,3). The roentgenographic and histologic results demonstrated that both of the transplantation procedures employed achieved restoration of the fetal long bones. Importantly, the use of bone-paste allowed us to sculpt the allograft to the desired conformation. These results encouraged us to continue investigation of fetal allogeneic bone transplantation, because of its ultimate potential for intrauterine repair of skeletal anomalies in the human fetus.

2-4. Induction of neural tube defects, their antenatal diagnosis and treatment in utero.

The early diagnosis of hydrocephalus enabled us to determine the mangitude of induced anomalies and treat them in utero. Our findings clearly indicated that whereas nontreated hydrocephalic neonates seldom survived more than 10 to 14 days (manifesting progressive muscular weakness and frequent seizures) fetal monkeys treated the HAVIT demonstrated markedly superior postnatal development of motor skills and weight gain after decompression of the hydrocephalus. Although additional laboratory studies are indicated, ultimately, clinical investigations combining early diagnosis and in utero insertion of the HAVIT or similar prosthesis may significantly enhance the prognosis of children who develop severe antenatal hydrocephalus (3). Moreover, in cases of hydrocephalus associated with meningocele or encephalocele and cranium bifidum the meningeal or cortical brain herniation was surgically corrected and cranium bifidum was repaired in utero using allogeneic bone transplants. This neurosurgical treatment was also applied postnatally (5); the outcome of these latter studies is not yet available. Likewise, early results demonstrate some degree of limb bud re-differentiation of monkey embryos.

Significance

Neural tube defects are among the most common major congenital malformations. Although the specific etiologies of neural tube defects are not well understood, these malformations may occur as a result of either genetic aberrations or environmental teratogens exposure to toxins, irradiation or medicinal agents. The moral and economic burden arising from these congenital defects is staggering, since it includes: fetal wastage, mental retardation, severe physical handicaps and overwhelming psychological stress within affected families. Of course, skeletal deformaties encompass an array of anomalies. Our current studies focus

principally on antenatal diagnosis and treatment of hydrocephalus and spina bifida, two common and devastating neural tube defects, as well as limb malformations of the extremities.

Proposed Course

The in utero surgical correction of certain induced congenital bone defects will be continued (6,7,8). The initial studies will be focused on the anomalies such as hydrocephalus caused by the steroid treatment; the studies of spina bifida induced with thalodimide have been initiated. The utilization of the allogeneic bone-paste method may be particularly useful in the correction of spina bifida because the paste can be molded into any desired shape and applied before the spinal effusion is irreversible. Additionally, induction of fetal limb defects by treatment of the mothers with thaladimide are being attempted. These defects are principally those of the long bones, whose growth in utero is stunted or arrested completely (9).

Publications

1. Michejda, M., Bacher, J., Kuwabara, T. and Hodgen, G.D.: In utero allogeneic bone transplantation in Primates: Roentgenographic and histologic observation. Transplantation, 32:96-100, 1981.

2. Michejda, M. and Hodgen, G.D.: Postnatal onset of immune rejection after allogeneic bone transplantation in monkeys. IRCS J. Med. Sci. 9(6):524, 1981.

3. Michejda, M. and Hodgen, G.D.: In utero diagnosis and treatment of fetal skeletal anomalies. I. Hydrocephalus. (in press - J.A.M.A.).

4. Hodgen, G.D.: Antenatal diagnosis and treatment of fetal skeletal malformations: With emphasis on in utero surgeries for neural tube defects and limb bud regeneration. JAMA 246:000-000, 1981.

5. Michejda, M., Bacher, J., and Hodgen, G.: Bone transplantation in utero and application of allogeneic bone-paste (submitted for publication, Proc. Int. Congress Primat.)

6. Michejda, M., Milhorat, T.H. and Hodgen, G.D.: Postnatal neurosurgical repair of crania bifida and encephalocele; allogeneic transplantation of bone-paste. (in preparation).

7. Michejda, M. and Hodgen, G.D.: Significance of elevated alpha-fetoproteins in the antenatal diagnosis of NTD. (in preparation).

8. Michejda, M. and Hodgen, G.D.: Postnatal neurological follow-up of hydrocephalic rhesus monkeys treated in utero. (in preparation).

9. Michejda, M. and Hodgen, G.D.: Intrauterine repair of induced neural tube defects. (in preparation).

10. Michejda, M. and Hodgen, G.D.: Limb bud regeneration in utero; preliminary studies (in preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00916-01 PR

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Corpus Luteum Function in the Cycling and Pregnant Monkey: Relaxin Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Bodour M. Abou-Hozaifa	Guest Worker	PRB, NICHD
	Robert Williams	Sr. Staff Fellow	PRB, NICHD
	Richard Stouffer	Univ. Arizona Med. Sch.	
	Gary D. Hodgen	Chief	PRB, NICHD

COOPERATING UNITS (if any)
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University of Arizona Medical School

LAB/BRANCH
Pregnancy Research Branch

SECTION
Section on Endocrinology

INSTITUTE AND LOCATION
NICHD, Bethesda, MD 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Secretion of relaxin by the cynomolgus monkey has been studied during the menstrual cycle and during pregnancy. In vivo, relaxin was found in peripheral and left and right ovarian vein blood during the luteal phase of the menstrual cycle. Relaxin was found in blood of pregnant monkeys from 40 days gestation to term. Peripheral, left and right utero-ovarian and uterine vein blood contained relaxin. In vitro, luteal cells taken from the corpus luteum of the menstrual cycle secreted relaxin in short term cultures (4-48 hrs). Luteal cells from the corpus luteum of early pregnancy and at term delivery secreted relaxin in long term cultures, up to 12 days, with a medium changes each second day.

PHS-6040
(Rev. 2-81)

Rationale

The corpus luteum has a critical role in the cyclic nature of the reproductive process in primates. Understanding its role in the non-fertile cycle as well as during gestation can be studied as a function of its hormonal control and secretory products derived from it. Relaxin and progesterone are 2 such secretory products. The role of progesterone has been extensively investigated, that of relaxin is not understood at this time.

Methods

In vivo studies were conducted with both cycling and pregnant monkeys. Peripheral, left and right ovarian vein blood was harvested from cycling monkeys during the entire luteal phase of the menstrual cycle prior to luteectomy. Blood was obtained from the peripheral, utero-ovarian and uterine veins of pregnant monkeys at various periods during gestation and at term when animals were subject to Cesarean section, luteectomy or fetectomy.

In vitro studies were conducted utilizing collagenase-dispersed luteal cells from both cycling and pregnancy corpora lutea. HCG and other stimulatory substances were added to incubation medium in short term (3-24 hours) or long term (up to 12 days) cultures.

Relaxin was determined by a heterologous porcine radioimmunoassay system. Progesterone was quantitated by a specific laboratory radioimmunoassay.

Results

Blood obtained from the three compartments sampled in the cycling monkeys contained relaxin in 6 of 30 animals. Most sampling came from early and middle luteal phase (days 13 to 20 of menstrual cycle), however only 2 animals secreted relaxin at this early time period. Late luteal phase sampling resulted in 4 animals secreting relaxin at 24 and 25 days of the menstrual cycle. Asymmetry of secretion with respect to the ovary containing the corpus luteum was confirmed by progesterone secretion. Relaxin was found in blood of pregnant monkeys from day 40 of gestation to term. Blood taken from the peripheral circulation showed wide variations in concentration but no pre-term rise was noted. Secretion of relaxin in the uteroovarian or uterine veins was not greater than that in the periphery, however uterine vein levels were lower than femoral vein or utero-ovarian vein concentrations.

In vitro secretion of relaxin by luteal cell cultures obtained from the corpus luteum of the cycle substantiated the finding of relaxin in the blood of 6 cycling animals. In contrast to the six noted animals, other corpora lutea obtained on day 18 or 19 of the menstrual cycle secreted no relaxin in short term culture or in long term culture up to 12 days.

Luteal cell cultures prepared from corpora lutea obtained during early pregnancy or at term secreted relaxin for a period of 4 to 10 days in control and hCG stimulated cultures. Although hCG enhanced progesterone production, its major effect on relaxin was to extend secretion in the medium for 2 days longer than control cultures.

Relaxin secretion is another function of the corpus luteum. The role of relaxin in the reproductive process is under active investigation to determine its source, possible stimulants to secretion and tropic effects of this hormone at traditional as well as other possible target tissues. Although a role for relaxin at parturition has been propounded for a number of species, the lack of a pre-parturition rise in primates brings that role into question. Similarly, pregnancy has been maintained in monkeys following ovariectomy at 21-25 days gestation. While the ovary may not be the sole source of relaxin in the monkey, other sources remain obscure at this time. These investigations seek to understand the sources of relaxin, the stimulus to secretion, its physiologic role in the reproductive process, its target organs and the biochemical sequelae to exposure of target tissue to the hormone.

Directions

Investigate anatomical/structural source of relaxin in both pregnant primates and during the menstrual cycle.

Determine relationship between demise of corpus luteum of menstrual cycle and secretion of relaxin.

Determine biochemical events accompanying relaxin action on target tissue.

Study tropic stimulation leading to relaxin secretion.

Investigate relaxin secretion with respect to physiological requirements.

Manuscripts in Preparation

1. Nixon, W.E., Abou-Hozaifa, B.M., Reid, R. and Hodgen, G.D. Immunoreactive Relaxin in the Cynomolgus Monkey (Macaca fascicularis). In preparation.

2. Nixon, W.E., Stouffer, R.L. and Hodgen, G.D. Relaxin and Progesterone Secretion In Vitro by Monkey Luteal Cells in Short Term and Long Term Cultures. In Preparation.

NICHD ANNUAL REPORT
October 1, 1980 through September 30, 1981
Developmental Pharmacology Branch

SUMMARY

The DEVELOPMENTAL PHARMACOLOGY BRANCH studies the molecular mechanisms underlying genetic differences involved in the stimulation of enzymes which metabolize drugs, carcinogens, and other environmental pollutants. Why does the same dose of the same drug or other foreign compound given to two different individuals (with the exception of monozygotic twins) evoke different responses? The responses not only include therapeutic effectiveness of a drug, but also undesirable side effects such as tissue necrosis, cancer, and birth defects. Our studies are conducted with a variety of biological systems, each chosen so as to provide unique experimental access and unequivocal answers to the questions being asked. From results obtained with such experimental systems as inbred strains of mice, established cell culture lines, and recombinant DNA technology, we can form hypotheses which can be quickly tested in clinical patients. The Branch presently comprises one Section and three Units.

A. The Section on Pharmacogenetics and Molecular Teratology, under the direction of Daniel W. Nebert, M.D., studies the relationship between the etiology of birth defects and the genetic regulation of drug-metabolizing enzymes. The teratogenic, carcinogenic, or toxic effects of certain drugs and other foreign compounds (xenobiotics) may reflect important genetically mediated differences between individual laboratory animals or individual humans. Our laboratory has developed experimental model systems for studying drug metabolism in cell culture and in a colony of inbred strains of mice. We have determined that the induction of at least two dozen drug-metabolizing enzyme "activities" is regulated at one or two genetic loci, called the [Ah] complex for aromatic hydrocarbon responsiveness. The relative "aromatic hydrocarbon responsiveness" among siblings in the uterus or as children results in genetic differences in: chemical-initiated tumors, aplastic anemia, or leukemia; paralysis times when given a certain muscle relaxant; survival times when dosed with several environmental pollutants; hepatic necrosis and cataract formation when given acetaminophen; number of fetal resorptions, stillbirths, and malformations when the pregnant mother is given certain chemicals; and ovarian toxicity and infertility. There is ample evidence for the [Ah] complex in the human. Because of these genetically mediated dissimilarities in xenobiotic metabolism, we have suggested it is possible to explain in the human why "drug-induced syndromes" occur in one child although the mother has received the same dose of the same drug for two or more pregnancies. We have developed an assay for the Ah receptor, which appears to be the main regulatory gene product for this complex. We have developed antibodies to several of the structural gene products (membrane-bound forms of cytochrome P-450) for use as probes to understand further the genetic regulation. With the aid of these antibodies, we have been able to "size" mRNA associated with several P-450 proteins, through the use of translation in vitro. Knowing the size, we have enriched for this mRNA and have isolated and characterized four cDNA clones of different P-450 structural genes. We also have cloned the mouse genomic P_1-450 gene from rat, rabbit, and human. With these cloned genes, we should know soon the answers to such questions as genetic linkage, mechanisms of regulation of P-450 induction, and

evolutionary phylogeny of this enzyme system (which exists in plasmids, apparently all eukaryotes). With these clones, we also hope to develop sensitive assays for predicting individual humans at increased risk for birth defects, other drug toxicities, or cancers.

B. The Unit on Genetic Regulation of Drug-conjugating Enzymes, under the direction of Ida S. Owens, Ph.D., is interested in genetic regulation of basal and inducible glucuronide-conjugating enzyme activities among inbred strains of mice and in cell culture. Detoxification of certain highly lipophilic endogenous substrates and noxious foreign compounds, such as environmental pollutants, requires a multistep process involving phase I and phase II enzyme systems. With inbred strains of mice, research in this Branch has established that certain monooxygenases (phase I enzymes) are regulated at a few genetic loci and that expression of enzyme induction is dominant or codominant in response to aromatic hydrocarbon treatment. Since certain enzymatic products of the monooxygenase system are often toxic intermediates which can serve as substrates for conjugation (detoxification) via the UDP glucuronosyltransferase system (phase II enzymes), we are eager to determine the extent of coupling between these two enzyme systems in various tissues. We have shown that induction of several hepatic UDP glucuronosyltransferase activities by aromatic hydrocarbons is genetically linked to induction of the monooxygenase system. Furthermore, our studies concerning mutagenicity have shown that induced transferase activity can prohibit the further metabolism and conversion of hydroxylated metabolites of the monooxygenase system to mutagenic products. In order to characterize the substrate specificity of the transferases involved we have begun purification studies with liver from aromatic hydrocarbon- and phenobarbital-treated mice. We have purified to homogeneity, as determined by SDS polyacrylamide gel electrophoresis, a transferase preparation which catalyzes the conjugation of 3 endogenous and 6 exogenous substrates. Phospholipid reconstitution of transferase is required for certain of these activities. Transferase antibodies which are currently being produced will be used to further characterize and localize transferase activity(s) in the membrane as well as embark on isolating transferase mRNA.

C. The Unit on Physical Biology, under the direction of Jack S. Cohen, Ph.D., examines the structure and interactions of biological molecules in detail in solution using several nuclear magnetic resonance spectroscopic methods. An alternating conformation has been shown to characterize the phosphodiester backbone of double-stranded poly(dAdT) in solution from the observation of a bifurcated ^{31}P NMR signal. This result may indicate the presence of sequence-specific local variations in the helical structure of DNA, which may have implications for protein-DNA recognition. To further characterize the nature of the sequence-dependent secondary structures of DNA in solution extensive salt-dependence studies of synthetic DNAs of different sequences are in progress. In addition extensive S1 nuclease treatments of these DNAs indicate differences in their solution conformations, notably an exposed phosphodiester group for d(AT).d(AT) not present in other sequences. The conformation of the Z form of DNA in solution is being investigated using poly(dGm^{5}dC).poly(dGm^{5}dC). It should be noted that m^{5}C has important genetic functions. The mobility of groups in protein active sites are being investigated using the information (^{13}C T_1 and NOE relaxation times) which has been accumulated on the ^{13}C enriched ribonuclease S system. The use of

^{31}P NMR as a noninvasive technique for the study of phosphate metabolism has been extended to large and sensitive cell systems using gel embedding. Preliminary results indicate that the application of ^{31}P NMR to study membrane ghosts of neurosecretory particles should provide accurate quantitative information on their ion and proton translocation functions.

D. The Unit on Genetic Expression, under the direction of Howard J. Eisen, M.D., studies the mechanism of action of glucocorticoid hormones. The glucocorticoid receptor is purified by DNA-cellulose chromatography, and anti-receptor antibodies are prepared for use in determining the chemical structure and biochemical function of the receptor. Antibodies directed against human glucocorticoid receptors have been produced and are being used to investigate the genetics of human glucocorticoid receptors and the mechanisms of glucocorticoid resistance in human diseases. We have used immunochemical methods and affinity labeling to isolate ^{3}H-labeled glucocorticoid receptors from rodent and human cells. These studies provide the first covalently labeled receptor preparations suitable for detailed chemical analysis of the glucocorticoid receptor.

E. Clinical Research Protocols in this Branch include (i) attempts to develop a bio-assay for the determination of "breast milk jaundice factor," (ii) family studies in an assessment of allelic differences at the Ah locus with: idiopathic aplastic anemia, solid tumors, childhood leukemia, and retinitis pigmentosa.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00136-13 DP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

PHARMACOGENETICS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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W.F. Dodge	Guest Worker	DP	NICHD
M.G. MacDonald-Ginzburg	Guest Worker	DP	NICHD
N.M. Gordon	Chemist	DP	NICHD
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
97/12	87/12	10/12

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In certain instances, human pharmacogenetic disorders cause individuals to react very differently to the same dose of the same drug. The teratogenic, carcinogenic, or toxic effects of certain drugs and other foreign compounds (xenobiotics) also may reflect important genetically mediated differences among individuals. Accordingly, our laboratory has developed experimental model systems for studying drug metabolism with recombinant DNA technology, in cell culture, and among inbred strains of mice. We have found that the induction of more than two dozen drug-metabolizing enzyme activities is regulated by a genetic system called the [Ah] complex. Among the structural gene products are multiple forms of P-450 induced by way of the Ah receptor. Cloned cDNA associated with four distinctly different forms of P-450 has been isolated and characterized. These mouse genes cross-hybridize to the corresponding genes from rat, rabbit, and human. With such clones we hope to understand the mechanisms of P-450 induction by drugs, to gain insight into the evolution of P-450 (present in plasmids and all eukaryotes), and to develop sensitive tests to determine clinically who is at increased risk for various drug-induced birth defects, other forms of drug toxicity, and environmentally-caused malignancies.

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Project Description:

Objectives: Membrane-bound multisubstrate monooxygenases are important in the metabolism of drugs, carcinogens, and insecticides, as well as many endogenous lipophilic substrates such as steroids, fatty acids, cholesterol, indoles, biogenic amines, and thyroxine. We have found that the "induction," or stimulation, of a group of drug-metabolizing enzyme activities in response to aromatic hydrocarbon treatment is regulated by only a few genetic loci in the mouse. We have employed cell culture techniques for studying the mechanism of monooxygenase induction in an experimental system which is more rigidly controlled than similar studies in the intact animal. Understanding the mechanism by which drug-metabolizing enzymes are induced may elucidate important concepts in the fields of drug metabolism, developmental pharmacology, steroid biochemistry, chemical carcinogenesis and mutagenesis, teratology, entomology, and limnology. The main objectives of this project are (1) to exploit genetic differences in drug metabolism among various inbred strains of mice in order to understand better the potential importance of pharmacogenetics; (2) to use tissue culture techniques in answering questions that would be difficult or impossible with studies in the intact animal; (3) to use recombinant DNA technology to understand better the genetic linkage and evolutionary development of these drug-metabolizing enzymes; (4) to combine the genetic differences with the tissue culture and recombinant DNA techniques in order to elucidate certain mechanisms related to mammalian gene regulation; and (5) to apply certain of these techniques to human tissues so that extrapolations can be made from rigidly controlled experimental systems to human pharmacogenetic differences.

Methods Employed: The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) cell culture techniques, (5) differential centrifugation, (6) phase microscopy, (7) polyacrylamide gel electrophoresis, (8) isoelectric-focusing electrophoresis, (9) column- and thin-layer chromatography, (10) magnetic resonance techniques, (11) high pressure liquid chromatography, (12) gas chromatography, (13) gas chromatography-mass spectrometry, (14) *in vitro* translation of mRNA, (15) peptide mapping *via* partial proteolysis, and (16) recombinant DNA and ancillary technologies.

Major Findings: A. Receptor Studies. (1) The effective doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for inducing half-maximally (ED_{50} values) aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity (EC 1.14.14.1) in Hepa-1, H-4-II-E, HTC, and VERO continuous cell culture lines are about 0.45, 0.23, >200, and 110 nM in the growth medium, respectively. The maximally induced hydroxylase activity is more than 100 times greater in "responsive" TCDD-treated Hepa-1 and H-4-II-E than in "nonresponsive" TCDD-treated HTC and VERO lines. The *Ah* receptor required for this induction process was assessed in these lines by sucrose density gradient analysis following dextran-charcoal treatment. With this assay we showed (a) a temperature- and time-dependent cytosol-to-nucleus movement of the inducer receptor complex in Hepa-1, H-4-II-E, and HTC cultures; (b) a TCDD concentration of about 0.28 nM required to saturate half of the nuclear specific binding sites in Hepa-1 cells; (c) of the total TCDD retained by cells in culture, an amount of less than 1%

specifically bound to the receptor; (d) the lack of induction of receptor by benz[a]anthracene in the growth medium; (e) a greater thermolability of the receptor in the cytosol than the receptor in the nucleus; (f) sensitivity of both the cytoplasmic and nuclear receptor to proteolytic enzymes such as trypsin but not to nucleases; (g) the lack of detectable receptor in the nucleus of cells not exposed to TCDD in culture; and (h) a size of about 6 S for both the cytosolic and nuclear receptor, as estimated on sucrose density gradients in the presence of 0.4 M KCl. The presence of the Ah receptor in the cytosol and its apparently normal translocation into the nucleus, such as in HTC cells, does not guarantee that the induction process will proceed normally. (2) [1,6-^{3}H]TCDD or [^{3}H]3-methylcholanthrene binding to the Ah receptor and other moieties in hepatic cytosol was examined by gel permeation chromatography, velocity sedimentation (sucrose density gradient centrifugation), dextran-charcoal adsorption, and anion exchange chromatography. In the liver of Ah-responsive C57BL/6N and the Ah^b/Ah^d heterozygote, both radioligands bind to three major components: peak I, a large aggregate which is eluted in the void volume of Sephacryl S-300 columns and which sediments as a residue to the bottom of sucrose density gradients; peak II, an asymmetric protein ($M_r \cong 245{,}000$) with a Stokes radius of about 75Å; and peak III, a globular protein ($M_r \cong 87{,}000$) with an estimated Stokes radius of 40Å. The peak I aggregate is not adsorbed by dextran-coated charcoal and therefore represents the large proportion of nonsaturable radioligand binding measured by dextran-charcoal adsorption. The peak II protein has a size of about 9.0 S in low ionic strength and 7.5 S in high ionic strength, high affinity for TCDD, and saturability at TCDD concentrations greater than about 1.0 nM. The peak II protein is not detectable in the liver of Ah-nonresponsive DBA/2N and the Ah^d/Ah^d homozygote and therefore represents the Ah receptor. The peak III protein has an estimated size of 5.0 S, is not saturable with either TCDD or 3-methylcholanthrene under the under the conditions of these experiments, and is not associated with the Ah^b allele. 3-Methylcholanthrene binds to the peak III protein to a greater extent than TCDD. These data explain the discrepancies between the dextran-charcoal adsorption and sucrose density gradient assays. (3) The capacity of nineteen polycyclic aromatic compounds and fifteen benzo[a]pyrene metabolites to displace [1,6-^{3}H]TCDD from the mouse liver cytosolic Ah receptor was examined. An excellent correlation is seen between the capacity to displace the radioligand from the Ah receptor and: the capacity of seven polycyclic hydrocarbons to induce aryl hydrocarbon hydroxylase activity in human cell cultures, the capacity of ten polycyclic hydrocarbons to induce azo dye N-demethylase activity in rat liver, the capacity of six polycyclic hydrocarbons to shorten zoxazolamine paralysis times in the intact rat, and the capacity of fifteen benzo[a]pyrene metabolites to induce aryl hydrocarbon hydroxylase activity in rat hepatoma H-4-II-E. Differences in the rate of cellular uptake and formation of alkali-extractable metabolites of dibenzo[a,h]anthracene, 3-methylcholanthrene, and benzo[a]anthracene in Hepa-1 mouse hepatoma cell cultures do not account for differences in the capacity of these three polycyclic hydrocarbons to displace [^{3}H]TCDD from the Ah receptor.

B. Structural Gene Studies. (1) Total cytochrome P-450 content, the Soret maximum of the reduced P-450·CO complex, NADPH-cytochrome c reductase activity, and 20 monooxygenase activities were determined in liver microsomes

from each of 12 individual Heterogeneic Stock (HS) mice at varying times after 3-methylcholanthrene treatment. The choice of genetically very heterogeneous mice and the possibility of different turnover rates (synthesis/degradation) during the 3-methylcholanthrene induction process provided us with a strong likelihood of detecting differences in drug-metabolizing enzyme activities. Such diversity would also be expected among the human population. The substrates included benzo[a]pyrene, ethoxyresorufin, biphenyl, p-nitroanisole, 2-acetylaminofluorene, 7-ethoxycoumarin, zoxazolamine, estradiol-17β, testosterone, progesterone, pregnenolone, dehydroepiandrosterone, and aldrin. By two-factor analysis of variance, 18 unique liver microsomal monooxygenase activities appear to be distinctly different from one another, out of the 20 activities chosen for study. These data with intact microsomes illustrate the complexity of the problem with multiple P-450-mediated monooxygenases. (2) Cytochrome P-450 from cholate-solubilized liver microsomes prepared from 3-methylcholanthrene-treated genetically "responsive" C57BL/6N mice (Ah^b/Ah^b) was partially purified by amino-octyl Sepharose 4B column chromatography with an elution buffer that included Emulgen 911, followed by hydroxylapatite column chromatography. The P-450 was separated into 16 fractions: 15 fractions principally associated with the Ah^b allele and induction by 3-methylcholanthrene; and one fraction not associated with the Ah^b allele, i.e. predominantly constitutive form(s) of P-450. The metabolism of benzo[a]-pyrene, 7-ethoxycoumarin, biphenyl, ethoxyresorufin, acetanilide, 2-acetylaminofluorene, phenacetin, estradiol-17β, and testo-sterone was investigated. With these nine substrates, we examined the rates of formation of 17 products, i.e. 17 P-450-mediated reconstituted monooxygenase "activities," in each of the 16 fractions. By two-factor analysis of variance, our data for 17 catalytic activities can be explained by a minimum of 19 unique groups of monooxygenase activities: twelve induced by 3-methylcholanthrene and seven control (endogenous). Almost every reconstituted monooxygenase activity, as is true also of each activity in intact liver microsomes, therefore appears to be unique and probably represents the aggregate activity from numerous forms of P-450.

C. Recombinant DNA and Ancillary Technologies. (1) Total cellular RNA was isolated from 3-methylcholanthrene and control C57BL/6N and DBA/2N mouse liver and enriched for poly(A)-containing mRNA. Using the reticulocyte lysate system in vitro, we examined total translation products and translated products immunoprecipitated by antibodies to mouse liver microsomal cytochrome P_1-450 and mouse serum albumin. Sucrose density gradient centrifugations under denaturing conditions (5 mM methylmercury hydroxide) yield two mRNA species associated with the antibody to P_1-450, one about the same size as preproalbumin mRNA, the other larger. These two mRNA species associated with anti-P_1-450 correspond to about 2,700 and 3,500 nucleotides, respectively; the size of an mRNA required for translation into a M_r = 55,000 subunit is ~1,500 base pairs. (2) Using partially purified mouse liver 23 S mRNA known to be associated with 3-methylcholanthrene-induced cytochrome P_1-450, we synthesized double-stranded cDNA by the successive action of reverse transcriptase (RNA-directed DNA nucleotidyltransferase) and the Klenow A fragment of Escherichia coli DNA polymerase I. The double-stranded cDNA was inserted into pBR322 plasmid DNA by Pst I cleavage and homopolymeric "tailing" and cloned in E. coli LE392. Clone 46 hybridized with [^{32}P]cDNA made from 23 S mRNA from Ah-responsive C57BL/6N mice but did not hybridize with similarly prepared [^{32}P]cDNA from Ah-nonresponsive DBA/2N mice. By translation-arrest

experiments, clone 46 DNA was shown to be associated with anti-P_1-450-precipitable material. By agarose gel electrophoresis of Pst I digests, the clone 46 DNA insert was shown to be 1100 base pairs in total length and to contain one internal Pst I site. The cDNA made from total mRNA isolated from 3-methylcholanthrene-treated C57BL/6N mice hybridized to the two fragments of Pst I-digested DNA from clone 46, whereas similarly prepared cDNA from 3-methylcholanthrene-treated DBA/2N and control C57BL/6N and DBA/2N mice did not. Of 11 restriction endonucleases used, two (Pst I and Xba I) had sites within the clone 46 DNA insert. After hybridization of clone 46 ^{32}P-labeled nick-translated DNA to EcoRI fragments from A/HeJ mouse genomic DNA and fractionation by RPC-5 chromatography and gel electro-phoresis, only one positive band (3-4 kilobase pairs appeared. These data demonstrate conclusively that pBR322 clone 46 DNA is associated with mRNA controlled by the murine Ah locus, presumably the structural gene encoding 3-methylcholanthrene-induced P_1-450. (3) Liver DNA from control, 3-methylcholanthrene-treated, and TCDD-treated C57BL/6N and DBA/2N mice was digested with HindIII, EcoRI, or BamHI. The restriction fragments were electrophoresed on agarose gels, fixed to diazobenzyloxymethyl paper, and hybridized to nick-translated [^{32}P]DNA from clone 46. No differences in the intensity or in the restriction profile exist among any of the variously treated mouse DNA preparations, suggesting (a) that the structural gene for P_1-450 is quite similar in C57BL/6N and DBA/2N mice, and (b) that gene duplication or some gross form of DNA rearrangement does not occur during the P_1-450 induction process. (4) Liver poly(A^+)-enriched RNA was electrophoresed on agarose gels under denaturing conditions, fixed to diazobenzyloxymethyl paper, and hybridized to nick-translated [^{32}P]DNA from clone 46. Striking increases in a 23 S mRNA--believed to be P_1-450 mRNA--and in an intranuclear larger molecular weight precursor RNA are both closely associated with the induction of aryl hydrocarbon hydroxylase activity (and its associated P_1-450) in 3-methylcholanthrene-treated and TCDD-treated C57BL/6N (Ah^b/Ah^b) and in TCDD-treated but not 3-methylcholanthrene-treated DBA/2N (Ah^d/Ah^d) mice. Among the F_1 hybrids (Ah^b/Ah^d) and nine progeny examined of the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross, 3-methylcholanthrene-treated Ah^b/Ah^d but not Ah^d/Ah^d mice always exhibit the increased 23 S mRNA. The data demonstrate that the P_1-450 induction process is under transcriptional control.

D. Membrane Biochemistry Studies. (1) The subcellular distribution of aryl hydrocarbon hydroxylase, NADPH-cytochrome c reductase, NADH-cytochrome c reductase, and NADH-cytochrome b_5 reductase activities in mouse liver was studied using the biochemical membrane markers microsomal glucose-6-phosphatase, mitochondrial cytochrome c oxidase, and plasma membrane 5'-nucleotidase. The nuclear fraction contains less than 1% of the total cellular activities of the hydroxylase and all three reductases. All detectable basal activity of aryl hydrocarbon hydroxylase in the nuclear fraction of control C57BL/6N and DBA/2N and 3-methylcholanthrene-treated DBA/2N mice and all detectable activities of NADPH-cytochrome c, NADH-cytochrome c, and NADH-cytochrome b_5 reductase in the nuclear fraction of control and 3-methylcholanthrene-treated C57BL/6N and DBA/2N mice can be completely accounted for by the degree of microsomal fragment contamination (as assessed by the microsomal marker glucose-6-phosphatase). These data raise doubts about certain previous reports of "nuclear" enzyme activities in which microsomal contamination was not taken into account. However, there is more induced activity of aryl hydrocarbon hydroxylase in the nuclear fraction

of 3-methylcholanthrene-treated C57BL/6N mice than can be accounted for by the degree of microsomal membrane contribution. (2) Liver microsomes from 3-methylcholanthrene-treated and phenobarbital-treated and control adult mice and TCDD-treated adult and fetal mice were examined. With antibodies against mouse liver microsomal cytochromes P_1-450 and P-448, two polycyclic aromatic-inducible cytochromes, immunoprecipitable radioactivity was measured, following labeling with pyridoxal phosphate/NaB[^{3}H]$_4$ or with ^{125}I-labeled p-aminosulfobenzoic acid/$NaNO_2$ in vitro or with [^{3}H]leucine, [^{14}C]glucosamine, or [^{32}P]O_4 in vivo. (a) Induction of cytochrome P_1-450 occurs developmentally earlier in gestation than induction of cytochrome P-448 when the mother is treated with polycyclic aromatic compounds. (b) There appears to be a basal form of cytochrome P-448 but no cytochrome P_1-450 in control liver microsomes; inducibility of cytochrome P-448 thus ranges between 5-12-fold, whereas that of P_1-450 is infinite. (c) Phenobarbital pretreatment induces no detectable P_1-450 or P-448. (d) P-448 appears to be either greater in concentration than P_1-450 in the membrane or more exposed than P_1-450 on the microsomal membrane surface. (e) By the radioimmunoassay methods used, TCDD-induced P_1-450 and P-448 in Ah-nonresponsive mice are indistinguishable from those in Ah-responsive mice; this is true in both the fetus and the adult. (f) Compared with P-448 expression, the expression of P_1-450 is more closely associated with 3-methylcholanthrene-induced aryl hydrocarbon hydroxylase activity, and these two structural gene products are apparently regulated independently. (g) P-448 but not P_1-450 appears to be a glycoprotein. (h) Neither P_1-450 nor P-448 appears to be a phosphoprotein.

E. Other Genetic and Molecular Biology Studies. (1) Hepatic cytosolic glutathione transferase activity with 1-chloro-2,4-dinitrobenzene as substrate was induced by 3-methylcholanthrene or β-naphthoflavone in C57BL/6N inbred mice and in (C57BL/6N)(DBA/2N)F_1 but not in DBA/2N inbred mice. High doses of TCDD induced the transferase activity in both C57BL/6N and DBA/2N mice. The glutathione transferase activity with benzo[a]pyrene 4,5-oxide as substrate was induced by 3-methylcholanthrene in C57BL/6N but not DBA/2N mice. The transferase activity with styrene 7,8-oxide as substrate was different from either of the above activities in that about twofold induction by 3-methylcholanthrene occurred in both C57BL/6N and DBA/2N mice. Among progeny from the (C57BL/6N)(DBA/2N)F_1 x DBA/2N backcross, however, no association was found between the transferase induction process by 3-methylcholanthrene and the presence of the Ah receptor [i.e., inducible aryl hydrocarbon hydroxylase activity]. It is therefore concluded that induction of glutathione transferase activity by polycyclic aromatic compounds is mediated by a gene(s) distinct from the Ah regulatory genes. These data emphasize the importance of examining progeny from the appropriate backcross before making conclusions about the genetic linkage of any two expressed traits. (2) Ornithine decarboxylase (ODC) activity is markedly stimulated in fetal rat primary hepatocyte cultures by the addition of fresh growth medium and in hepatoma-derived cell culture 2 to 6 hours following the addition of fresh serum-containing or serum-free medium. The three hepatoma-derived continuous cell lines were studied. No relationship is apparent between ODC induction and total cellular content of either cyclic 3':5'-adenosine monophosphate (cAMP) or cyclic 3':5'-guanosine monophosphate (cGMP). Whereas 3-isobutyl-1-methylxanthine stimulates cAMP levels, this inhibitor of phosphodiesterase does not enhance ODC activity. These results do not support the hypothesis that stimulation of monooxygenase and ODC activities by various inducers of

P-450 is mediated by the increased amount of cAMP. (3) ODC induction is never found to be greater in 3-methylcholanthrene-treated cultures than in control cultures. "Basal" ODC activities in nonstimulated liver- or hepatoma-derived cells in culture are two to 25 times greater than those in liver of the nonstimulated adult intact animal. A difference in ODC induction between 3-methylcholanthrene-treated and control cultures therefore (a) may be obscured by the already elevated ODC activity, (b) may not be a necessary prerequisite for the induction of aryl hydrocarbon hydroxylase to occur, or (c) may be necessary if the starting ODC activity is very low but unnecessary if the starting ODC activity is already high. These data point out the difficulties in using cell culture to study the relationship between drug-metabolizing enzyme induction and ODC induction. (4) Forty-four inbred and four randombred rat strains and 20 inbred mouse strains not previously studied were surveyed for their [Ah] complex phenotype by determining the induction of liver microsomal aryl hydrocarbon hydroxylase activity by intraperitoneal treatment with 3-methylcholanthrene. All 48 rat strains were found to be Ah-responsive. The maximally induced hydroxylase specific activities of the ALB/Pit, MNR/Pit, SHR/Pit, and Sprague-Dawley rat strains are the same order of magnitude as the basal hydroxylase specific activities of the ACI/Pit, F344/Pit, OKA/Pit, and MNR/N rat strains. Six of the 20 mouse strains were observed to be Ah-nonresponsive (i.e. lacking the normal induction response and presumably lacking detectable amounts of the Ah receptor). The basal hydroxylase specific activities of the BDL/N, NFS/N, STAR/N, and ST/JN mouse strains are more than twice as high as the maximally induced hydroxylase specific activity of the CBA/HT mouse strain. (5) To date, 24 Ah-nonresponsive mouse strains have been identified, out of a total of 68 known to have been characterized. The reasons for finding not a single Ah-nonresponsive inbred rat strain--as compared with about one Ah-nonresponsive inbred mouse strain found for every three examined--remain unknown.

Significance to Biomedical Research and the Program of the Institute: We have demonstrated that allelic differences at a genetic locus controlling concentration of a receptor in the mouse is associated with: (1) increased susceptibility to 3-methylcholanthrene-initiated tumors; (2) decreased zoxazolamine paralysis time; (3) increased susceptibility to 7,12-dimethylbenz[a]-anthracene-produced skin inflammation; (4) increased susceptibility to acetaminophen-induced hepatic necrosis and cataract formation; (5) shortened survival time, or (conversely) apparent protection, when large doses of polycyclic hydrocarbons, lindane, or polychlorinated biphenyls are administered intraperitoneally or orally; (6) apparent protection against aplastic anemia and leukemia caused by oral benzo[a]pyrene; (7) increased susceptibility to stillborns, fetal resorptions, and malformations caused by 3-methylcholanthrene or 7,12-dimethylbenz[a]anthracene given to the pregnant mother; (8) increased ovarian toxicity (loss of primary oocytes) following polycyclic hydrocarbon treatment; and (9) increased infertility in the female. An increased incidence of lung cancer in man has been correlated with living in highly polluted urban areas. It is therefore possible in the human that differences--caused by a very small number of genes--are related to increased susceptibility to cancers, birth defects, tissue necrosis, drug metabolism, bone marrow disorders, and decreased life span caused by exposure to foreign compounds.

These genetic differences in drug-metabolizing enzymes will soon be understood through the combination of further receptor studies and recombinant DNA-technology. All of these studies are relevant to the program of this Institute in the following areas: (1) possible effects of environmental pollutants (i.e., polycyclic hydrocarbons and insecticides) and various drugs on normal fetal growth and development and on the health of the pregnant woman, (2) mechanisms of "pharmacological immaturity" of the premature and newborn, (3) mechanisms of disease of the fetus and newborn concerning oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (4) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

Proposed Course of Project: (1) Delineate further differences in the mechanism(s) of monooxygenase induction by polycyclic aromatic compounds, phenobarbital, and numerous other drugs and environmental pollutants; (2) characterize specific binding of these various inducers to receptors (or other subcellular sites); (3) compare these genetic differences with contemporary models of mammalian gene expression and regulation; (4) explore evolutionary development and phylogenetic differences among genes associated with the metabolism of drugs and other environmental pollutants; (5) search for drug metabolism parameters in cultured human lymphocytes or fibroblasts or in various excreta that might aid in predicting a patient's response to a drug or class of drugs; and (7) extrapolate mouse data to clinical diseases by the development of sensitive tests for assessing individual human risk of drug-induced birth defects, toxicity, and environmental carcinogenesis and mutagenesis.

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detoxification studies using noxious lipophilic foreign and endogenous compounds, sometimes following metabolism by monooxygenase activity, have continued with conjugation reactions by genetically regulated (at the Ah locus) and phenobarbital inducible UDP glucuronosyltransferase activities. Substrates used for the transferases included 3 endogenous and 6 exogenous substrates. In attempts to distinguish the number of different transferases involved, UDP glucuronosyltransferase has been purified from phenobarbital-treated C57BL/6N mice by hydrophobic, anion exchange and affinity chromatography to homogeneity as defined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The purified protein conjugates at least 6 substrates and has a subunit molecular weight of about 51,000 daltons. In addition transferase studies have continued with established cell lines. Aryl hydrocarbon hydroxylase and 3-hydroxybenzo[a]pyrene transferase are induced or are not induced in parallel in the hepa-1 established cell line and 4 of its mutants by polycyclic aromatic compounds or phenobarbital.

Project Description:

Objectives: The removal of many foreign and endogenous lipophilic compounds from the body requires both hydroxylation by monooxygenase activity and subsequent conjugation by sulfotransferase(s) or UDP glucuronosyltransferase(s). With prototypic inbred strains of mice and their offspring from the appropriate backcrosses, research in this Branch has established that induction of several monooxygenase activities by aromatic hydrocarbon compounds is genetically regulated by a single gene designated the Ah locus. More recently, our studies have shown that induction of two different UDP glucuronosyltransferase activities is also genetically mediated and is linked to the Ah locus. The genetic linkage of these two functionally related membrane bound enzymes does not occur through a common intervening hydroxylated product of the monooxygenase activity which is also substrate for UDP glucuronosyltransferase activity. These observations are further substantiated in cell culture studies with benzo[a]pyrene metabolites which are generally less potent inducers than the parent compound. Furthermore, the genetic regulation for induction of bilirubin transferase activity was found to be genetically linked to the induction of monooxygenase but, the endogenously-derived bilirubin does not require monooxygenase. Since monooxygenases are important in the metabolism of drugs, carcinogens, and insecticides, as well as endogenous compounds such as, steroids fatty acids and cholesterol, it is of interest to investigate the extent to which monooxygenase is coordinated with UDP glucuronosyltransferase activity utilizing a wide range of endogenous compounds, such as tryptamine, corticosterone, aldosterone, testosterone, and estradiol and xenobiotics, such as 3-hydroxybenzo[a]pyrene, morphine, chloramphenical, naphthol, and phenolphthalein. A further understanding of the relationships of coordinate regulation of monooxygenase (a phase I enzyme) and a particular UDP glucuronosyltransferase (a phase II enzyme) should provide insight into critical requirements which allow this type of drug metabolism. In addition to defining the biochemical connection between monooxygenase and transferase, purification studies with transferase are underway to delineate possibly the number of different transferases. With purified protein and the production of antibodies, it should be possible to isolate and characterize transferase mRNAs, clone critical transferase structural genes, and, finally, the genomic DNA. Ultimately, one can determine the genomic linkage between monooxygenase and transferase. The primary objectives of this project are (1) to delineate further the genetically mediated induction of UDP glucuronosyltransferase (using substrates which are products of the monooxygenase enzyme and those which are not); (2) to exploit genetic differences in the conjugation of critical substrates, as bilirubin; (3) to study the functional relationship between UDP glucuronosyltransferase and various P-450-mediated monooxygenases in the detoxification of various drugs in vivo in genetically different mouse strains; (4) to purify genetically regulated and nongenetically regulated UDP glucuronosyltransferases and compare properties of these enzymes; (5) to make antibodies to purified transferases and attempt membrane localization studies of transferases; (6) to use a particular transferase antibody to isolate and characterize transferase mRNA with respect to size and translatable product; (7) to clone a transferase structural gene which utimately allows one to isolate and clone genomic DNA; (8) to attempt to use tissue culture techniques to study the regulation of mammalian UDP glucuronosyltransferase activities and the interdependence in regulation of the transferases and the monooxy-

genases at the molecular level; and (9) to apply tissue culture results and an in vitro bilirubin conjugation assay for the purposes of establishing a bioassay for "breast milk jaundice factor."

Methods Employed: The principal methods to be employed are: (1) enzyme assays using spectrophotometric, spectrophotofluorometric, radiometric determinations of product or disappearance of substrate, (2) in vivo radiometric measurements, (3) regular cell culture and hybridoma cell culture techniques, (4) differential centrifugation, (5) phase microscopy, (6) high-pressure liquid chromatography, (7) column chromatography, (8) chromatofocusing by protein isoelectric point, (9) gel electrophoresis, (10) antibody production in goat and rabbit, and (11) immunoprecipitation of UDP glucuronosyltransferase activity.

Major Findings: Since UDP glucuronosyltransferase is an integral part of the endoplasma reticulum, it is necessary for purification of transferase to develop the best detergent system to completely solubilize membranes such that the individual protein components can be disaggregated from each other and under conditions which will allow transferase activity to remain. To this end, various detergent systems consisting of combinations of cholate or a cholate derivative and a nonionic detergent, such as Emulgen 911 or Lubrol 12A9 were systematically analyzed for effective solubilization. As well, cholate or its derivative or one of the nonionic detergents was used alone. The extent of disaggregation of four crucial membrane proteins from each other was determined on solubilized membrane fractions eluted from a Sephacryl 300 chromatography system which gel filtrates proteins up to a molecular weight of 650,000 daltons. Analyses for cytochrome c reductase, cytochrome P-450, morphine- and naphthol-UDP glucuronosyltransferase indicated that morphine and naphthol transferases with most of the detergent systems remained constant at molecular weights of approximately 340,000 daltons while cytochrome c reductase remained constant at 272,000 daltons. On the other hand, the cytochrome P-450 molecular weight varied greatly from 100,000 to 600,000 daltons, with the combinations of the two detergents giving the best dissociation (lowest molecular weight) of this very hydrophobic protein. This was very crucial to the purification of transferase since cytochrome P-450 represents a large fraction of the membrane proteins and because of its high hydrophobicity, it is necessary to work with detergent systems which will allow the cytochrome P-450's to be removed. Furthermore, the molecular weight distribution suggests that transferase catalytic unit is much larger than that for cytochrome P-450, although their subunit molecular weights by SDS-polyacrylamide gel electrophoresis are nearly the same.

Microsomes from phenobarbital-induced C57BL/6N mice have been solubilized with a cholate acid derivative, CHAPS, and chromatographed on three different systems. Initially the solubilized microsomes are chromatographed on the hydrophobic phenylsepharose system, followed by DEAE anion exchange chromatography after binding the enzyme at the appropriate ionic strength. Salt eluted protein from the anion exchange system was dialyzed and bound to an affinity column of UDP hexanolamine bound to sepharose. Through much trial and error it became evident that the protein only binds to the ligand at very low ionic strength, and that in order to obtain a homogeneous protein band by SDS-PAGE, the UDP hexanolamine must be bound to the sepharose such that no ionic charges exist in the system as happens with ligand bound to cyanogen

bromide activated sepharose. As the mouse transferase apparently binds weakly to the ligand, one cannot wash with salt to remove nonspecific proteins bound in such an ionic environment. The protein purified by UDP hexanolamine bound to sepharose through an amide linkage is homogeneous and has a molecular weight on SDS-PAGE of approximately 51,000 daltons. The purified transferase preparation catalyzes the conjugation of naphthol, 3-hydroxybenzo[a]pyrene, 4-methylumbelliferone, phenolphthalein, p-nitrophenol, morphine and chloramphenicol. Certain of these substrates require reconstitution with phospholipid.

The Hepa-1 established cell line and four of its mutants have been described for aryl hydrocarbon hydroxylase and 3-hydroxybenzo[a]pyrene-UDP glucuronosyltransferase inducibility by phenobarbital and polycyclic aromatic compounds. The inducibility or the lack of inducibility by either compound in a particular mutant is parallel for the two enzyme systems. The activities for the two enzyme systems are not always induced concomitantly and to the same extent. Phenobarbital is a much better inducer for transferase than for the hydroxylase.

Significance to Biomedical Research and the Program of the Institute: As discussed previously, drug oxygenations and subsequent glucuronide conjugations are catalyzed by a membrane-bound multistep enzyme complex. These enzymes are important in the process of detoxification and elimination of many normal body constituents and many xenobiotics taken in from our environment in the form of food additives, air pollutants, and medication. There are, as well, diseases of humans ascribed to defects (not well understood) in drug-conjugating enzymes. Examples in pediatrics include neonatal hyperbilirubinemia, Gilbert's syndrome, and the Crigler-Najjar syndrome. Therefore, the development of experimental model systems for understanding better the molecular regulation and neonatal development of UDP glucuronosyltransferase is pertinent to the program of the Institute in the following areas: (1) understanding the mechanisms of "immaturity of drug metabolism" in neonates; (2) providing further experimental models for other human pharmacogenetic defects; (3) elucidating the basic enzymology of glucuronidating drug metabolism in order to gain insight into the development of selective inhibitors and stimulators of these drug conjugating enzymes that are useful for beneficial regulation of abnormal metabolism peculiar to various human diseases; and (4) understanding metabolism of various drugs in the fetus, premature and the newborn.

Proposed Course of Project: (1) Extend studies on activation of benzo[a]-pyrene phenols to mutagens and reactive metabolites, (particularly the carcinogenic 2-hydroxybenzo[a]pyrene) and the effect substrates for conjugation has on activation of those phenols; (2) further exploration of certain inbred strains, as well as, a heterogeneous stock of mice to determine substrate preferences for induced transferase by phenobarbital or by 3-methylcholanthrene designed to determine if one or more UDP glucuronosyltransferase is responsible for increased conjugating activities using thirteen different transferase substrates with some associated with the Ah locus; (3) further characterization of the transferase systems in cell culture with respect to number of different transferases, macromolecular syntheses requirements, and activators and general regulation at the molecular

level; (4) determination of bilirubin tolerance in responsive and nonresponsive mouse after hydrocarbon treatment; (5) development of a useful bioassay in cell culture for the quantitation of "breast milk jaundice factor" in human breast milk samples from outpatients and/or inpatients; (6) continued purification of other forms of UDP glucuronosyltransferase after induction by 3-methylcholanthrene as well as from control mice with characterization of purified enzyme; (7) development of antibodies to purified preparations with the ultimate aim of cloning a transferase gene by isolating transferase mRNA identified by antibody precipitation of translatable product; and (8) attempt to develop hybridoma cell lines for monoclonal transferase antibody production.

Publications:

1. Malik, N., and Owens, I.S.: Genetic regulation of bilirubin UDP glucuronosyltransferase induction by polycyclic aromatic compounds and phenobarbital in mice. J. Biol. Chem., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00500-03 DP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hormonal Regulation of Gene Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Howard J. Eisen Medical Officer DP NICHD

COOPERATING UNITS (if any)
Gerald Litwack - Fels Research Institute, Temple University, Philadelphia, PA.
Alan Munck - Dartmouth University, Hanover, New Hampshire
S. Stoney Simons, Jr. - Lab. of Chemistry, NIAMDD, NIH, Bethesda, MD.

LAB/BRANCH
Developmental Pharmacology Branch

SECTION
Unit on Hormonal Regulation of Gene Expression

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6/12	6/12	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with biochemical and molecular aspects of hormone action during fetal development. Adrenal corticosteroids affect many aspects of fetal development and also have teratogenic effects in genetically-susceptible inbred mouse strains. The glucocorticoid receptor is purified by DNA-affinity chromatography and antibodies to the receptor are prepared for use in determining the chemical structure and function of the receptor.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Adrenal corticosteroids regulate many aspects of metabolism and gene expression in the mammalian fetus. The major goal of this project is to understand the mechanism of action of glucocorticoids and to develop a comprehensive understanding of glucocorticoid effects on the developing fetus. The biological effects of glucocorticoids are mediated by a cytosolic receptor protein. This protein has been isolated, and antisera to the receptor have been produced. The antisera provide a unique means for studying glucocorticoid action.

Methods Employed: The cytosolic receptor for glucocorticoids is isolated by DNA-affinity chromatography and polyacrylamide gel electrophoresis. Antisera to the purified receptor are produced in rabbits, and the anti-receptor antibodies are purified. Various immunochemical methods are used to study receptor-antibody reactions including immunoaffinity chromatography, electrophilic affinity labeling, and immunocytochemistry.

Major Findings: An antiserum to the rat liver glucocorticoid receptor was produced initially in a rabbit in 1979. Antisera have been produced in three additional rabbits, and relatively large quantities of serum are now available for immunochemical studies. The initial work with the antisera has dealt with basic characterization of the antibodies and determination of their specificity. Studies with human receptors have been carried out in collaboration with Dr. John Stevens and Dr. Alan Munck. We have shown that human leukemic cells (circulating and cultured lines) contain glucocorticoid receptors that cross react with the antisera.

Using the methods developed for purification of the rat glucocorticoid receptor, we have recently purified glucocorticoid receptors from human-derived tissue culture cells (IM-9). Antibodies to human receptors have been produced in rabbits. These antibodies show much greater reactivity with human receptors and should be extremely useful for analysis and isolation of human receptors from circulating leukemic cells and non-Hodgkins lymphoma (studies in collaboration with Dr. Alan Munck). In collaboration with Dr. S. Stoney Simons, we have applied the techniques of electrophilic affinity labeling and immunochemistry to the study of glucocorticoid receptors. We have been able to label covalently the glucocorticoid receptor and then isolate these receptors by immunochemical methods. These methods can be used to isolate the receptor under denaturing conditions and should provide material of sufficient purity to begin chemical studies (e.g., sequencing) of the glucocorticoid receptor.

Significance to Biomedical Research and the Program of the Institute: The glucocorticoid receptor is present in extremely low concentrations and has been extremely difficult to purify. The anti-receptor antibody provides a new biochemical tool for isolating the receptor and studying its function in regulating gene expression. Immunochemical methods can be used for developmental studies. Teratogenic effects of glucocorticoids such as the development of cleft palate may involve altered receptor concentrations in genetically susceptible inbred mouse strains. Immunocytochemical localization of the receptor in complex tissues of the fetus should provide important new insights into mechanisms of teratogenesis. Mutations involving the

glucocorticoid receptor appear to be responsible for many glucocorticoid resistant leukemias and lymphomas. The antisera are currently being used to evaluate the receptors in circulating human leukemic cells and in cells from human non-Hodgkins lymphomas.

Proposed Course of Project: (1) Molecular biology of the glucocorticoid receptor. The currently available antibodies will be used in conjunction with other affinity techniques for isolation of biologically "active" receptor (i.e. receptor that retains capacity to bind steroids and chromatin) and denatured receptor for physiochemical and protein sequencing studies. (2) Production of anti-receptor antibody in cloned "hybridoma" cultures. Mice will be immunized with receptor, and spleen cells from these animals will be fused with myeloma cells in order to derive monoclonal antibodies to the glucocorticoid receptor. (3) Role of glucocorticoids in genetic susceptibility to birth defects (cleft palate). Immunocytochemical studies will be carried out to determine ontogenesis and distribution of the glucocorticoid receptor in the mammalian fetus. Inbred strains of mice differ in susceptibility to glucocorticoid-induced facial defects. These strains will be used to test the hypothesis that differences in receptor concentration affect the response to glucocorticoids. The antisera currently available cross-react with other mammalian species, and it is possible that developmental studies can be carried out in fetal primates.

Publications:

1. Eisen, H.J., Schleenbaker, R., and Simons, S.S.: Affinity labeling of the rat liver glucocorticoid receptor with dexamethasone 21-mesylate: Identification of covalently labeled receptor by immunochemical methods. J. Biol. Chem., in press, 1981.

2. Eisen, H.J.: Immunochemical approaches to the study of glucocorticoid receptors. In Litwack, G. (Ed.): Biochemical Actions of Hormones. New York, Academic Press, 1981, in press.

3. Hannah, R.R., Nebert, D.W., and Eisen, H.J.: Regulatory gene product of the Ah locus: Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3-methylcholanthrene binding to several moieties in mouse liver cytosol. J. Biol. Chem. 256: 4584-4590, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00032-08 DP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Conformations and Interactions of Proteins and Nucleic Acids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jack S. Cohen	Research Chemist	DP	NICHD
OTHER:	James McGhee	Staff Fellow	LMB	NIAMDD
	Chi-Wan Chen	Staff Fellow	DP	NICHD
	Lou J. Hughes	IPA Guest	DP	NICHD
	Anthony Zador	Summer Student	DP	NICHD
	C.-H. Niu	Staff Fellow	LBP	NIADDK
	Michael Behe	Staff Fellow	LMB	NIADDK

COOPERATING UNITS (if any)

Laboratory of Chemical Physics, NIADDK (for spectrometer maintenance)

LAB/BRANCH

Developmental Pharmacology Branch

SECTION

Unit on Physical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
32/12	32/12	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To elucidate the conformation and interactions of proteins and nucleic acids in solution by nuclear magnetic resonance (NMR) methods. Particularly to investigate the interactions of proteins and drugs with DNA, alone and in nucleoprotein complexes.

PHS-6040

Project Description:

Objectives: 1. To extend the study of protein functional groups (such as Met, Asp, Thr, etc.) which are not amenable to study by methods other than NMR spectroscopy. 2. To study conformations, transitions and binding properties of biologically active peptides and proteins in solution. 3. To investigate the detailed conformations of DNA and their relationships to genetic function, particularly DNA-protein and drug binding.

Methods Employed: High field NMR spectroscopy (^{1}H, ^{13}C, ^{31}P and ^{2}H) and optical spectroscopies (UV, CD). Synthesis of appropriate substances (peptides, oligonucleotides) including use of isotopic enrichment (^{2}H, ^{13}C).

Major Findings:

A. Sequence-Dependent Secondary Structure of DNA in Solution. Nuclease S1 exhibits a marked selectivity for different sequences of DNA. Poly(dGdC).poly(dGdC) is hydrolysed at a rate close to that of calf thymus DNA, while poly(dAdT).poly(dAdT) is hydrolysed at a much faster rate, close to that of single stranded DNA. By comparison with poly(dA).poly(dT) and using different size ranges prepared by sonication we showed that these differences cannot be explained entirely by the amount of single-stranded material present nor by the extent of breathing. We determined the exponential time constants and corrected them for the different size distributions determined from gel electrophoresis. The results indicate that the double stranded conformation of poly(dAdT).poly(dAdT) is susceptible to direct S1 cleavage, which is presumed to arise from an exposed phosphodiester conformation in this polydeoxynucleotide duplex. Further evidence for differences between the structures of the two alternating purine-pyrimidine co-polymer DNAs is provided by differences in their ^{31}P NMR spectra at different salt concentrations. The separation of the two component peaks of the doublet that has been observed for poly(dAdT).poly(dAdT) shows a linear dependence with both NaCl and CsF concentrations, while poly(dGdC).poly(dGdC) exhibits a singlet under the same solution conditions (except for the transition to the Z-form in high NaCl concentrations). Our results do not indicate that the alternating AT polymer forms a "strange double helix" in high salt as concluded by Kypr et al. (Biochem. Biophys. Res. Commun. 99: 1257, 1981), but rather that the separation of the two ^{31}P NMR components represents a salt effect on the alternating form which already exists for this substance in low (0.1 M) salt.

B. Protein Molecular Dynamics. Carbon-13 NMR studies at three frequencies (100, 270 and 400 MHz) have been used to probe molecular dynamics near the active site of three semi-synthetic ribonuclease S' complexes. The (1-15) NH_2-terminal fragments selectively C-13 enriched at Ala-5 (C^{α}, C^{β}, C^{O}), His-12 (C^{ε}), Met-13 (C^{ε}) and Asp-14 (C^{γ}) were complexed with S-protein to form enzymatically-active RNase S' (Niu et al., J. Biol. Chem. 254: 3788, 1979). These complexes were subsequently used for NMR studies which included the determination of relaxation parameters (T_1 and NOE). A major objective is to clarify the contributions of different relaxation mechanisms by using several magnetic field strengths and thereby to determine individual carbon correlation times. Fits and various dynamic (mathematical) models should enable the distinction of local mobility from overall protein rotation and should allow a critical comparison of various views of protein dynamics in solution.

C. Studies of Z-form DNA. 5-Methyl cytosine in place of C in poly(dGdC).poly(dGdC) has been shown using CD spectroscopy to significantly reduce the ionic strength required to convert to the Z-form in solution (Behe and Felsenfeld, Proc. Natl. Acad. Sci. U.S.A. 78: 1619, 1981). At the low ionic strengths needed no aggregation occurs as for the unmethylated polymer and this facilitates spectroscopic solution studies. We have carried out ^{31}P NMR studies on poly(dGm^{5}dC).poly(dGm^{5}dC) prepared by Dr. Behe. A single resonance was observed in very low ionic strength (5 mM Tris), a bifurcated resonance in moderate salt (~0.2 M NaCl) indicating an alternating B-DNA form, and a resolved doublet in high salt (0.8 M NaCl) corresponding to the Z-form. However, the areas of the components were 1:2. When the B to Z transition was effected with Mg^{2+} (5 mM) three peaks of equal area were observed. These results indicate an unusual combination of phosphodiester conformations for the Z form of this DNA in solution.

Significance to Biomedical Research and the Program of the Institute: The results of this work are of fundamental significance in understanding the detailed structure of DNA in solution and its relationship to function. Continuing NMR studies of protein conformations and of protein-DNA complexes and their interactions with drugs in solution should reveal further insights of important physico-chemical processes in developmental molecular biology.

Proposed Course: (1) Studies are planned to relate the conformational variants of DNA to actual functional properties of DNA; particularly DNA-protein and DNA-drug interactions. For this work medium length specific-sequence DNAs will be required, both synthetic and of natural occurrence. (2) Attempts will be made to extend the use of CD_3-labelled Met to study the interaction of cytochrome c with cytochrome oxidase, possibly in a membrane-like environment, or to study cytochrome P-450. (3) Studies of peptide and protein conformation and dynamics will be continued. Proton NMR studies of larger proteins will be re-initiated when our high field (500 MHz) spectrometer is delivered later this year.

Publications:

1. Cohen, J.S. (Ed.): Magnetic Resonance in Biology. New York, John Wiley & Sons, 1980, vol. 1, 309 pages.

2. Cohen, J.S., Niu, C., Matsuura, S., and Shindo, H.: Conformation, mechanism and peptide-exchange of ^{13}C-enriched ribonuclease-S by ^{13}C NMR spectroscopy. In Liu, Q.Y., Mamia, G., and Yasunobu, K.T. (Eds.): Frontiers in Protein Chemistry. New York, Elsevier/North Holland Biomedical Press, 1980, pp. 3-16.

3. Zweier, J., Wooten, J.B., and Cohen, J.S.: Studies of anion binding by transferrin using ^{13}C NMR spectroscopy. Biochemistry 20: 3505-3510, 1981.

4. Cohen, J.S., Wooten, J.B., and Chatterjee, C.L.: Characterization of alternating DNA conformations in solution by ^{31}P NMR spectroscopy. Biochemistry 20: 3049-3055, 1981.

5. Wooten, J.B., Cohen, J.S., and Schejter, A.: Conformational transitions of ferricytochrome c studied by ^{13}C and ^{2}H NMR spectroscopy. In: Interaction Between Iron and Proteins in Oxygen and Electron Transport. New York, Elsevier/North Holland Biomedical Press, 1981, in press.

6. Wooten, J.B., Cohen, J.S., Vig, I., and Schejter, A.: The pH-induced conformational transitions of ferricytochrome c: a ^{13}C and ^{2}H NMR study. Biochemistry, in press, 1981.

7. Cohen, J.S., and Chen, C.-W.: ^{31}P NMR studies of DNA conformation and dynamics. In Levy, G. (Ed.): New Methods and Applications of NMR Spectroscopy. Washington, D.C., American Chemical Society Monograph, 1981, in press.

8. Chen, C.-W., and Cohen, J.S.: Convenient continuous sonication method for preparation of medium-sized polydeoxynucleotides. (Submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00501-04 DP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Non-Invasive Studies of Internal pH and Metabolism of Biological Systems Using Nuclear Magnetic Resonance Methods

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jack S. Cohen	Research Chemist	DP	NICHD
OTHER:	Lev Jacobson	Visiting Fellow	DP	NICHD
	Harvey Pollard		CH	NIADDK
	Snorri Thorgeirsson		LCHPH	NCI

COOPERATING UNITS (if any)

Clinical Hematology, NIAMDD
Laboratory of Chemical Physics, NIADDK (for spectrometer maintenance)

LAB/BRANCH

Developmental Pharmacology Branch

SECTION

Unit on Physical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12/12	12/12	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ^{31}P NMR technique enables signals from phosphates in tissues, cells or granules to be monitored non-invasively. Intracellular pH is measured quantitatively from ATP or Pi signals. Carbon-13 NMR studies of ^{13}C enriched metabolites allow the elucidation of details of metabolism.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: To obtain reliable measurements of internal pH in cells and their components and to understand the processes of membrane transport of ions and protons, and the process of exocytosis. To compare details of metabolism non-invasively in normal, diseased, and cancerous cells.

Methods Employed: High field phosphorus-31 and carbon-13 nuclear magnetic resonance spectroscopy.

Major Findings:

A. Improved Technique for Investigation of Cell Metabolism by ^{31}P NMR Spectroscopy: ^{31}P NMR studies on microorganisms have been carried out with the cells embedded in agarose gel. The novel use of the gel for the NMR studies has advantages over the usual liquid suspensions in terms of improved reproducibility of data and cell viability, with no net loss of spectral quality. To prove this method works polyphosphate formation in E. coli was monitored continuously for up to 24 hours and metabolic changes in yeast for 6 hours. Changes of the intracellular pH during glycolysis in yeast were determined from the chemical shift of the internal Pi. NMR titration curves of Pi in the presence of Mg^{2+} indicate uncertainties in internal pH values estimated by this technique.

B. Studies on Liver Cells: ^{31}P NMR studies of metabolism in a wider range of larger cells at lower effective concentrations become possible with the use of the gel suspension technique (see A). Preliminary experiments on rat liver cells prepared from whole liver by collagenase treatment have been carried out.

C. Studies on Membrane Proton-Translocation: Preliminary results indicate that it is possible to measure the internal pH of empty resealed membrane ghosts quite accurately and non-invasively using trapped indicator substances.

D. Miscellaneous: During the course of the year Dr. Jacobson carried out several other projects: (a) Attempted synthesis of asymmetrical diphosphonates to be used as monitors of internal pH by ^{31}P NMR methods, and also design of lipid soluble phosphorus compounds to be used to measure trans-membrane potential. This resulted in evidence for the formation of a bicyclic hexameric anhydride intermediate in the reaction between DCC and methylene diphosphonate. (b) The ADP/ATP levels in the tent caterpillar (Malcosoma) and the blow fly were monitored through the stages of metamorphosis, providing some unexpected but as yet incomplete results. (c) A study of the paleo-biological inclusions in Amber, particularly well-preserved insects, was initiated by Dr. Jacobson. Of particular interest was the variation of isotopic contents in these organisms of some 50 million years ago, as well as possible amino acid and nucleic acid components.

Significance to Biomedical Research and the Program of the Institute: (1) Prior attempts to measure intracellular pH have been intrusive. ^{31}P NMR has provided a nonintrusive method, so that it should now be possible to obtain accurate values of internal pH, and a greater understanding of ion transport through membranes and of the mechanism of exocytosis. (2) Studies of cell

metabolism using NMR methods should provide insight into subtle aspects of metabolic pathways, and elucidate differences between normal and cancerous states.

Proposed Course: (1) Studies on neurosecretory granules and membrane ghosts will be carried out to elucidate the processes of proton and ion transport. (2) Rat liver cells will be studied non-invasively by ^{31}P NMR to compare pre- and post-cancerous metabolism. (3) Metabolism of sugars will be studied using ^{13}C enriched sugars, particularly to monitor rates of intracellular molecular fragmentation and mixing.

Publications:

1. Jacobson, L., and Cohen, J.S.: Improved technique for the investigation of cell metabolism by ^{31}P NMR spectroscopy. Biosci. Reports 1: 141-150, 1981.

2. Jacobson, L., and Cohen, J.S.: Intracellular pH measurements by NMR methods. In Cohen, J.S. (Ed.): Non-Invasive Probes of Tissue Metabolism. New York, John Wiley & Sons, 1981, in press.

3. Cohen, J.S. (Ed.): Non-Invasive Probes of Tissue Metabolism. New York, John Wiley & Sons, 1981, in press.

The Neonatal and Pediatric Medicine Branch has continued its broad research program in both clinical and basic approaches to the diagnosis, treatment and fundamental mechanisms of diseases of infants and children. Included in the program are genetic diseases, inborn errors of metabolism, disordered nutritional states, congenital malformations, endocrine and metabolic disturbances, as well as the effects of disorders of maternal origin on the neonate. This group also studies the normal biological mechanisms of infant and child development.

Currently, there are three Section and 1 unit actively carrying on these research projects. These include 1) the Section on Developmental Biology and Clinical Nutrition with its unit of Endocrinology; 2) the Section on Human Biochemical and Developmental Genetics; and 3) the Section on Gastroenterology and Nutrition. An overview of each will be presented in brief.

I. The Section on Developmental Biology and Clinical Nutrition has ongoing investigations in three distinct areas: a) the developmental and hormonal mechanisms controlling phospholipid biosynthesis in various fetal and adult organs; b) determining the role of the central nervous system in the pathogenesis of massive obesity with or without the Prader-Willi syndrome, and c) the basic mechanisms involved in the diagnosis, treatment and pathogenesis of a number of metabolic disturbances including glycogen storage disease and hypoglycemia with or without hyperinsulinemia. The Endocrinology Units, (A) is studying the mechanisms of normal and abnormal sexual development in the primate. Other areas of study include the role of LH-RH in the development of the hypothalamic-pituitary-gonad axis. Collaborative studies have also been undertaken to explore the possibility of unusual thyroid hormone dysfunction in patients with cystinosis osteogenesis imperfecta.

a) The developmental studies of phospholipid biosynthesis in fetal lung have concentrated this year in three areas. 1) Insulin stimulates choline kinase activity in fetal lung explants. This effect appears to be mediated by specific insulin receptors, as the amounts of enzyme activation is proportional to the affinities of various insulins to the insulin receptor. Insulin receptors have been detected in lungs of fetal rats as early as 15 days gestational age. When the insulin binding data was subjected to Scatchard analysis, classical curvilinear plots, typical of insulin receptors, were obtained. 2) Morphine sulfate was found to inhibit phosphatidylcholine (PC) biosynthesis in fetal rat lung explants in a dose dependent manner. Naloxone did not reverse this effect. 3) A mixed fetal rat lung cell culture has been established to study biosynthesis and secretion of PC.

b) The studies in childhood obesity have continued. The basic approach has been to ascertain the circadian pattern of the obese child and to repeat these studies when normal weight is attained. In the past year the melatonin rhythm has been added. Attempts to evaluate the level and potential role of β endorphins and bombesin have been unsuccessful for technical reasons. Only preliminary data at this point are available for a limited number of children who have been tested after regaining normal weight. Studies are projected for the determination of responsivity to melatonin for various light intensities in obese vs normal weight. Studies to discern the reason for the differential in the rate of weight loss in males and females will be undertaken. Evaluation of brown fat activities in family members will be pursued when obese and normal

weight members exist. Studies of the difference in handling glucose, fatty acids and/or leucine labeled with stable isotopes will be undertaken.

The ongoing investigation of glycogen storage disease, Type II, has progressed from the purification of human placental lysosomal (1-4) alpha-glucosidase to the production of antibodies to the renal and placental enzyme for urinary assays applicable to family studies. The purified enzyme will also be used to study the role of alpha-glucosidase in normal glycogen metabolism.

In addition, preliminary work has begun to develop techniques to study in vivo glycogen metabolism in obese and glycogen storage disease patients utilizing stable isotopes.

c) Methods to diagnose specific metabolic disorders such as type I glycogen storage disease, receptor defects in hypoglycemia and hyperglycemia and substrate such as ketone bodies, lactate, pyruvate and amino acids have been set up in order to better understand the pathophysiology of these disorders. Insulin receptor sites on red cells in a variety of disorders of carbohydrate metabolism have been measured throughout development and in patients with hypoglycemia both on an off Diazoxide® treatment.

All these studies are designed to determine and understand the basic mechanisms underlying normal growth and development. Animal models are being established to further investigate the mechanisms of the clinical observations.

(A) The unit on Pediatric Endocrinology continues its studies in the following areas: 1) The development of the hypothalamic-pituitary-testicular axis; 2) neuroendocrine secretion in primates; 3) abnormalities in endocrine function in cystinosis.

1) Both bovine and rat Sertoli cell cultures have been studied. We have demonstrated autoregulation of FSH response in rat Sertoli cells including analysis of FSH receptors, FSH stimulation of cyclic AMP and aromatization of testosterone to estradiol. In the bovine Sertoli cells, unlike the rat cells, we have shown FSH stimulates an increase in the enzyme, ornithine decarboxylase. Indirect evidence suggest the Sertoli cell is the source of Mullerian inhibiting substance. Using a high titer antiserum to luteinizing hormone-releasing hormone (LHRH), we have studied effects on androgen binding protein in the developing rat. In addition we have done further studies on the mechanism of the previously shown permanent alterations in the hypothalamic-pituitary-testicular axis. Our laboratory continues its efforts to relate these problems to intersex abnormalities and their relationship to Mullerian inhibiting substance secretion in clinical medicine.

2) A primate model for neuroendocrine evaluation over a short period has been established. We have demonstrated transient alterations in primates treated with different doses of cranial irradiation. Other studies are in progress examining the effect of neuroplasmacologic agents on primate neuroendocrine secretion.

3) Studies in cystinotics have demonstrated pituitary resistance to thyroid hormone. Further in vivo and in vitro studies are in progress in order to better understand the mechanisms involved.

All these studies are designed to better understand developmental endocrine problems in normal and abnormal sexual development.

II. The Section on Human Biochemical and Developmental Genetics continues both its basic science and clinical investigation of cystinosis and other defects in sulphur amino acid metabolism. Other current areas of research include amino acid transport, lysosomal structure and function, role of amino acids and fetal and neonatal nutrition and the biochemistry of early embryonic development. Continuing investigations of glutathione metabolism both in animals and in man have generated new approaches to the therapy of chronic hemolytic anemia in red cell enzyme deficiencies. Ongoing clinical investigations include the evaluation of cysteamine in reducing the storage of cystine in patients with cystinosis, and a collaborative world wide project to study the effectiveness of high doses of vitamin E (alpha tocopherol) in preventing the chronic hemolysis in glucose-6-phosphate-dehydrogenase deficient patients. Both of these investigations are ongoing and definitive results will be forthcoming within the near future.

Major efforts continue on the incorporation of human genes into mammalian cells with the use of liposomes (lipochromosomes). Feasibility of producing lipochromosomes and using them to transfer X-linked genes has been previously reported. Work also continues on the development of a selective system for cystinotic cells in culture and demonstrating differences that exist between normal and cystinotic cells. The section actively pursues study of the effects of various drugs and biochemical pertubations in reducing the cystine content of cystinotic cells as well as studying detailed metabolic pathways and mechanisms in its pathophysiology.

A new contribution has been the demonstration that specific HLA types occur more frequently in patients with the recessive genetic disorder cystinosis than in a comparable normal population. These data represent the first demonstration of this relationship and will be extended to other genetic diseases as well.

In summary, studies will continue to focus in two areas. First the metabolism of amino acids and especially sulphur containing amino acids and peptides and inborn errors of metabolism involving these compounds. The second area will be on amino acids as they relate to nutritional problems and intrauterine and postnatal growth.

III. The Section on Gastroenterology and Nutrition has been studying intestinal epithelial cell migration and differentiation and the nutritional modifications of gene activity and expression in developing and adult mammals. With respect to epithelial cell migration, our efforts have been focused on the establishment of a primary cell culture system which will allow us to define those cell surface components which are responsible for cell attachment to the basement membrane. In addition, an intestinal organ culture system is being developed as an *in vitro* model of cellular locomotion along the crypt-villus axis, so that the roles of protein synthesis and hormonal influences on intestinal cell migration can be investigated. We are also continuing our ongoing research into the control of sucrase/isomaltase synthesis and the mode of incorporation of the complex into the intestinal brush-border membrane. Primary cultures of rat pancreatic exocrine cells are being established to elucidate the role of nutrition in the modulation of gene activity and expression. Initially, studies will focus on the mechanism of adaptation of rat amylase in isolated cells and intact pancreas to carbohydrate in the media or the diet.

In conclusion, the clinical activities of the Neonatal and Pediatric Medicine Branch have expanded significantly over this current fiscal year. The establishment of a pediatric component to the weekly Inter-Institute Endocrine Clinic has attracted patients from around the world. Research protocols have been generated based on these new opportunities. In addition, an Inter-Institute Genetics Clinic spearheaded by this Branch has been initiated and is also generating patient-referrals and protocols.

The short tenure due to fire of the expanded in-patient service of 23 beds was characterized by almost full occupancy at all times. Patients from NICHD accounted for 45 to 50 percent of the in-patient population. With the availability of 25 beds on 9W for the past several months, there has been a significant increase in the patients being studied. As of July 1, 2 medical staff fellows have been assigned full time to the 9W unit.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00126-07 NPMB

PERIOD COVERED October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Developmental Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other Investigators: M. Cornblath, M.D. NPMB, NICHD
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COOPERATING UNITS (if any)

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LAB/BRANCH

Neonatal and Pediatric Medicine Branch

SECTION

Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The control of fetal lung development was investigated by studying the effects of several factors, including insulin, morphine sulfate and prostaglandin production, on the synthesis and secretion of lamellar bodies. A new method of measuring insulin receptors in fetal lung was developed. The number of insulin receptors per cell was found to increase 6-fold during the last trimester in fetal rat lungs. Administration of morphine sulfate to pregnant rats caused a significant acceleration of fetal lung development as assessed by both morphological criteria and lecithin/sphingomylin ratios. The first step in prostaglandin biosynthesis, the hydrolysis of arachidonic acid from phosphatidylcholine was shown to be stimulated by calcium ionophore, suggesting a role for calcium in the production of prostaglandins and the production of disaturated phosphatidylcholine. In conjunction with the prostalandin studies, an imbalance in platelet thromboxane and vascular prostacyclin was demonstrated in vitro in the presence of homocysteine and homocystine, substances elevated in homocystinuria, a disorder of sulfur metabolism.

PHS-6040
(Rev. 2-81)

Project Description:

Objectives: Studies on organ development have focused on fetal and neonatal lung as related to the respiratory distress syndrome (RDS) of human neonates. This disorder occurs in low birth-weight infants, is characterized by progressive pulmonary atelectasis, and accounts for greater mortality and morbidity than any other neonatal disorder or pediatric pulmonary disease. A decrease in the concentration of surfactant, composed primarily of disaturated phosphatidylcholine (DSPC) is closely associated with the development of RDS. Thus, DSPC biosynthesis and secretion are essential to the development of a mature and functional pulmonary system. We have addressed ourselves, therefore, to the following objectives: 1) To investigate the control of phosphatidylcholine biosynthesis and secretion during fetal lung development. 2) To investigate the effects of diabetes and drug addiction during pregnancy on phospholipid synthesis in fetal lung. 3) To investigate the interrelationships between prostaglandin biosynthesis and the production of DSPC.

Methods Employed: DSPC biosynthesis is measured using isotopic substrates, extraction of labelled phospholipids, and separation into individual species utilizing thin layer, paper and HPLC chromatography techniques. Fetal rat lung explants are utilized to study DSPC synthesis in intact cells. Mixed fetal rat lung cell cultures were obtained by trypsinization of fetal lung explants and subsequent plating in cell culture dishes. Type II pneumocyte populations in the cell cultures are assessed by fluorescent staining of lamellar bodies with phosphine dye. Viability of cells is assessed by ^{14}C-Arachidonic acid incorporation into cellular phospholipids. Fetal rat lung homogenates are employed to examine individual PC biosynthetic enzymes. ^{125}I-insulin is utilized to demonstrate specific binding to fetal lung.

Major Findings: Fetal lung membranes were found to specifically bind ^{125}I-insulin. Fetal lung insulin receptor numbers per cell increased almost 6-fold from 16 days gestational age to birth. On the other hand, receptor dissociation constants did not change during this developmental period. The fetal lung membranes were found to contain high amounts of proteolytic activity, which significantly reduced the amount of insulin available for binding. The addition of bacitracin, a protease inhibitor, completely abolished the degradation of insulin, which has previously been a significant problem in accurately measuring insulin receptors in fetal lung.

Daily intraperitoneal morphine sulfate injections of pregnant Sprague Dawley rats caused an acceleration of fetal lung maturation which was most dramatic at 19 days gestational age. Electron microscopy demonstrated that fetal lungs exposed to morphine sulfate possessed significantly higher numbers of lamellar bodies in type II pneumocytes. Biochemically, amniotic fluid from the opiate-exposed fetuses showed significantly higher lecithin-sphingomylin (L/S) ratios at 19 days gestational age when compared to normal fetuses. This is the first demonstration of maternal injection of morphine sulfate causing an increased L/S ratio in amniotic fluid.

A fetal lung organ explant system has been developed to incorporate ^{14}C-arachidonic acid into phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and phosphatidylglycerol. The mixed fetal lung cell cultures established are capable of being stimulated to release ^{14}C-arachidonic acid from phosphatidyl choline.

Normal human platelets and human umbilical artery segments were exposed to homocysteine and homocystine in vitro. Following incubation with ^{14}C-arachidonic acid, platelet thromboxane and 12-hydroxy-5,8,10-heptadecatrienoic acid production increased significantly from control levels in paired samples preincubated with homocysteine or homocystine while vascular protacyclin production was unchanged.

Significance to Biomedical Research and the Program of the Institute: In order to study the metabolic disturbances leading to RDS, basic features of phospholipid metabolism in lung must be identified, particularly those factors which play a role in PC biosynthesis. We have taken the approach of looking into several disorders during pregnancy, including diabetes and drug addiction, that may alter pulmonary maturation in the fetus.

The observation that maternal diabetes is associated with an increased incidence of RDS has led us to look into the relationship between insulin receptor levels and the control of phospholipid synthesis. The observation that receptor numbers increase on a per lung cell basis suggests that the lung becomes increasingly more sensitive to insulin as fetal development progresses. Observing insulin receptor numbers in normal fetal lung development provides a basis for comparisons when examining fetal lung exposed to high insulin levels, such as is the case with maternal diabetes.

The increased L/S ratios in amniotic fluid of fetuses whose mothers had been injected with daily doses of morphine sulfate suggest that this opiate in some way, either directly or indirectly, stimulates phospholipid synthesis in fetal lungs. This could explain why infants of mothers addicted to opiates, although small for gestational age, experience fewer respiratory problems at birth. Furthermore, as maternal endorphan levels increase during normal pregnancy, this natural endogenous opiate may play a role in fetal lung development.

Most studies involving the control of disaturated DSPC levels in fetal lung have been concerned with mechanisms involved with the biosynthesis of DSPC and not its subsequent secretion from Type II pneumocytes. The mixed fetal lung cell culture that has been established will allow for a detailed study of what stimulates disaturated PC secretion from these cells.

A homocyst(e)ine induced increase in platelet thromboxane production which is proaggregartory in the absence of an increase in vascular prostacyclin, which is antiaggregatory, if present in vivo, may contribute to the vascular thromboses characteristic of human homocystinurias.

Proposed Course of Research: The effects of maternal diabetes and malnutrition on the numbers and properties of insulin receptors will be assessed, as well as the effects on phospholipid biosynthesis. The effects of maternal naloxone (a morphine autogonist) injection of normal pregnant rats will be investigated to see if fetal lung development can be retarded. In addition, naloxone treatment of morphine injected rats will be examined to see if the stimulatory effects on fetal lung development by the opiate can be reversed.

The effects of various activators of phospholipase A_2 and arachidonic acid hydrolysis will be investigated. This enzyme may be responsible for the "remodeling" of unsaturated PC to yield disaturated PC, a primary component of sufactant. With the mixed fetal lung cell cultures, the secretion of disaturated

PC will be examined. The role of arachidonic acid and its metabolites in the synthesis and secretion of disaturated PC will also be studied.

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2. Ulane, R.E.: The CDP-choline pathway: choline kinase. In Farrell, P.M. (ed.), The Developmental Biology of the Lung and the Pathobiology of Hyaline Membrane Disease, Academic Press, Inc., New York, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00131-07 NPMB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Human Biochemical Genetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Human Biochemical and Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10	9	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our studies have concerned the metabolic errors of glutathione synthesis and degradation (glutathionuria, glutathione synthase deficiency and gamma glutamyl-cysteine synthase deficiency), glucose-6-phosphate dehydrogenase deficiency in which the capacity for glutathione reduction is decreased, cystinosis, homocystinuria, phenylketonuria, non-ketotic hyperglycinemia, galactosemia and adrenal leukodystrophy. We have been interested in the development of newer forms of treatment and diagnosis for a number of these metabolic disorders and in understanding the mechanisms of disease production and related normal processes. A number of specific treatments are under investigation. Animal models of human genetic diseases are studied. Investigations have also been undertaken to explore the possibility of transferring genetic material into cells with the use of lipochromosomes.

PHS-6040
(Rev. 2-81)

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Project Description:

Objectives: The goals of the project are as follows: (1) to elucidate the basic biochemical defects responsible for selected inborn errors of metabolism in man; (2) to explore the secondary biochemical and physiological derangements caused by the primary meteabolic defect; (3) to develop new or improved methods of treatment; (4) to develop new or improved methods of diagnosis, both antenatal and postnatal; (5) to use genetically abnormal cells and patients to expand understanding of normal human biochemistry, physiology, and development; (6) to see if genes can be introduced into genetically deficient cells using lipid enclosures and to address questions of gene function in this system. We have chosen to focus primarily on derangements of sulphur amino acid metabolism and secondarily on other disorders of amino acid, sugar, and lipid metabolism and on selected derangements of lysosomal function.

Methods Employed: The principal methods employed are: (1) cell culture techniques, (2) spectrophotometric, radiochemical, and fluorometric enzyme assays, (3) differential ultracentrifugation, (4) quantitative ion exchange chromatography of amino acids in physiological fluids, including cellular extracts, (5) radioisotopic measurements, (6) light, phase contrast, and electron microscopy, (7) column and thin-layer chromatography, (8) starch and polyacrylamide gel electrophoresis, (9) osmometry, (10) histochemistry, (11) determinations of red cell and platelet survival after tagging with radioactive chromate, (12) liposome technology and (13) dietetic treatment of man and animals.

Major Findings: Continuing investigations of glutathione metabolism in cultured human cells and in animals have permitted certain observations regarding the role of these enzymes and glutathione itself in biological processes, in addition to the large number of conclusions summarized in earlier project descriptions.

Study of cells from patients deficient in gamma-glutamyl transpeptidase activity, as well as in vivo studies of one such patient, constitute the strongest evidence so far available that this enzyme is not, as previously believed, a significant mediator of amino acid uptake in a variety of human cell types. We are now examining whether such patients have defective capacity to excrete cystine in response to lysine loads, which would be predicted if urinary cystine is derived from renal glutathione catabolism.

Glutathione synthase deficient patients have been shown, in contrast to earlier reports, to have normal intracellular amino acid levels in erythrocytes and leucocytes, and in serum.

Successful correction of reduced intracellular glutathione content and of excessive cells of patients with glutathione synthase deficiency has been oxoproline production in achieved in vitro using a mixture of serine and borate, which inhibits gamma-glutamyl transpeptidase activity.

We have demonstrated that the cells from patients with reduced glutathione synthase activity are abnormally susceptible to oxidative damage as demonstrated by a shortening of red cell survival and a susceptibility to damage and microtubule dissociation in leucocytes during phagocytosis. Both the red cell and leucocyte abnormalities are partially and in some cases wholly correctable after the patients have received large doses of the antioxidant vitamin E for several months.

Since glutathione may be involved in the deiodination of the thyroid hormones we have tested whether liver cells with reduced glutathione content have abnormal metabolism of such compounds. It appears that they do only if the oxidation state, not concentration, of glutathione is reduced. In these investigations glutathione depletion and oxidation using cystine starvation, diamide, and other techniques were utilized.

Since patients with glucose-6-phosphate dehydrogenase deficiency have a reduced capacity to regenerate reduced glutathione after oxidative stress, we have explored the possibility that the hemolytic anemia associated with this disorder might be improved by treatment with high doses of vitamin E. The rate of hemolysis in patients with chronic hemolyzing glucose-6-phosphate dehydrogenase deficiency is not usually reduced with vitamin E tretment. The hemolytic anemia of glutathione synthase deficiency is improved consistently.

We have also demonstrated that the mild chronic hemolysis in Mediterranean type glucose-6-phosphate dehydrogenase deficiency is significantly reduced by high dose vitamin E treatment in a population of Greek subjects. Significant increases in hematocrit and hemoglobin levels, reductions in reticulocyte count and prolongations of red cell survival were documented. It was observed that G6PD deficient individuals have significantly lower vitamin E levels than suitable controls. Further investigations in Israel have confirmed that G6PD deficiency is associated with mild hemolysis and that reduced vitamin E levels are characteristic of such subjects.

In investigations currently underway, we are examining if vitamin E treatment will reduce neonatal hyperbilirubinemia secondary to glucose-6-phosphate dehydrogenase deficiency or whether vitamin E administration will reduce the frequency and severity of the acute hemolytic crises responsible for the major morbidity and mortality in this widely prevalent genetic disease.

The effects of β-carotene, another anti-oxidant, in chronic severe G6PD deficiency are being examined in other clinical trials.

Studies are underway exploring the susceptibility of glucose-6-phosphate dehydrogenase deficient red cells to oxidative stresses in vitro (such as retinol-peroxide or aspirin-peroxide). The phospholipid membrane composition of G6PD and normal cells is also being compared under baseline conditions and during conditions of oxidative stress. The mechanisms of destruction of red cells in G6PD deficiency have also been examined with the use of lectins and an increased agglutinability by Concanavalin A as well as increased binding of the C_3 component of complement has been observed in G6PD deficient red cells.

Alterations in the activity of the enzyme regulating the synthesis and degradation and glutathione have been demonstrated at different time points during fibroblast growth in vitro. These enzyme changes predictably alter intracellular glutathione content during cell growth.

Investigations of cystinosis continue to be a primary interest in our laboratory. A number of significant observations on this disease have been made.

A selective system has been developed for cystinotic cells in vitro based on the remarkable resistance of these cells to high concentrations of extracellular cysteine.

Current clinical trials with the use of cysteamine and phosphocysteamine have demonstrated an in vivo reduction in leucocyte cystine content to near the normal range. Initial clinical observations on renal function are promising but not yet conclusive.

In additional studies we have observed that patients with cystinosis may suffer from frank or compensated hypothyroidism, and also that a regulatory defect involving the pituitary-thyroid axis exists in this disorder. Increased pituitary resistance to thyroid hormone is suggested by the available data. We are presently examining in more detail the response of the pituitary gland to graded doses of exogenous thyroid hormone in cystinosis.

Studies on the enzyme pantetheinase, a cysteamine generating enzyme, demonstrated its presence in human tissues for the first time and documented that pantetheinase deficiency is not responsible for cystinosis; in addition it has been demonstrated that a deficiency in endogenous cysteamine production cannot account for the increased intracellular storage of cystine in this disease.

We are presently examining the permeability of isolated cystinotic and normal lysosomes to a variety of amino acids including cystine in efforts to establish whether the lysosomal storage of cystine is due to altered permeability characteristics of the lysosomal membrane. These studies are being done with labelled amino acid methyl ester substrates as described for rat liver lysosomes by Reed. The studies on cystinotic lysosomes both in vitro and in situ have been a major effort of this laboratory recently. We have demonstrated that cystinotic lysosomes do not have a generalized defect in efflux of multiple amino acids. In addition both cystinotic and normal lysosomes have an extraordinarily low capacity for cystine efflux in the absence of certain additives to the efflux medium. The nature of the factors which enhance cystine efflux from isolated normal and cystinotic lysosomes is currently under intensive investigation. Using methyl esters it has been possible to hyperload normal and cystinotic cells with cystine permitting new types of comparative studies. These studies have demonstrated for the first time that cystinotic cells unequivocally lack a mechanism for cystine clearance present in normal cells. In addition loading leucocytes with methyl ester improves heterozygote detection in cystinosis, which was not previously possible in many individual cases. The kinetics of cystine clearance in heterozygous leucocytes are almost always different than those in normal or cystinotic cells by the methods we have recently developed.

Additional work in our laboratory has also demonstrated that mixed disulfides of cysteine and glutathione can be used to enhance the cystine content of cultured fibroblasts. Additional studies have been performed on the interrelationships between cell cysteine content, various sources of cystine supply, and gamma-glutamyl transpeptidase activity in cultured fibroblasts. Modification of transpeptidase activity with appropriate inhibitors does modify the content of cystine in cystinotic fibroblasts.

We have uncovered some new aspects of the population genetics of cystinosis. The large excess of males over females observed in this disorder is not predicted by classical autosomal recessive inheritance. A striking linkage disequilibrium between cystinosis and the HLA antigen system has been

demonstrated: HLA A3 and B7 are associated with cystinosis substantially more frequently than would occur by chance. Despite these associations of certain HLA types with cystinosis family studies have demonstrated that the cystinosis gene is not closely linked to the HLA locus on chromosome 6.

Our interest in genetic diseases related to cystinosis has led us to the description of a new genetic entity which resembles cystinosis clinically, is more severe, but is not characterized by increased intracellular cystine storage; there is a unique combination of renal pathology and clinical symptomatology in this entity which has been observed in siblings.

Work is also continuing on non-sulphur amino acid inborn errors of metabolism. A new form of phenylketonuria due to decreased intracellular biopterin has been identified. For a long time it has been considered that biopterin is not able to enter the central nervous system to affect neurological function even though exogenous biopterin can be used by the liver and a variety of other organs. We have recently demonstrated however that in our biopterin deficient patient dietary biopterin supplementation can result in a significant clinical improvement accompanied by chemical evidence for biopterin entry into the central nervous system and a tendency to normalize abnormal levels of neuro-transmitters as measured in the cerebrospinal fluid. We believe that this represents an important contribution to improve the therapy of biopterin deficient phenylketonuric variants.

A new in vivo assay for phenylalanine hydroxylase activity has been developed and tested. The assay involves the administration of minute amounts of tritiated phenylalanine to patients under an approved protocol. This approach may lead to elimination of the need for liver biopsy in certain types of hyperphenylalaninemic patients and may improve heterozygote identification.

In laboratory animals, studies related to the disease non-ketotic hyperglycemia have been pursued. We have completed a series of investigations on glycine entry into neonatal and adult rat neural tissue and have examined the effect of benzoate on enzymes which conjugate it with glycine, and on the clearance of glycine from various tissues including brain, in the hyperglycinemic rat. These studies enhance understanding of possible disease mechanisms and approaches to treatment in this very serious disorder, and have confirmed an exceptional sensitivity of the neonatal brain to glycine toxicity.

A major new area of interest has involved the X-linked disorder, adrenal leukodystrophy. We have demonstrated that there is a significant elevation of serum C26 fatty acids in these subjects and have developed an approach to diet therapy based on a restriction of hexacosanoic acid in the food intake of affected patients. Studies are being pursued on determining the contribution of endogenous versus exogenous C26 to the long chain fatty acids stored in patients and in animal models for adrenal leukodystrophy. Fibroblasts from patients, heterozygotes and normal subjects are being investigated using tritium release assays and carbon dioxide evolution from labelled precursors to determine whether there is a defect in long chain fatty acid catabolism in this lethal genetic disease. In collaborative studies we have helped to improve methods for measuring small amounts of C26 and related fatty acids in samples from patients with adrenal leukodystrophy. In addition, studies in tissue culture suggest that clofibrate, which has been advocated as an agent which lowers circulating long chain fatty

acid levels in adrenal leukodystrophy, does not alter tissue content of C26 or C24 in cultured fibroblasts; this suggests that clofibrate therapy may be of limited if any value in patients with adrenal leukodystrophy.

We have also undertaken investigations of the effects of galactose toxicity in the rat on development of the ovary and testis. Amenorrhea with ovarian failure at an early age appears to be characteristic clinically of female galactosemia. We have demonstrated that in rats exposed during fetal life to high concentrations of exogenous galactose, a substantial reduction in oocyte numbers occurs. We have further demonstrated that this toxic effect is most prominent when exposure occurs during the premeiotic phases of oogenesis. This represents to our knowledge the first example of demonstration of prenatal toxicity to germ cells in any animal model for a human genetic disease. Our attempts to demonstrate relationships between gonadal development and toxicity from galactose or its metabolites provides important new areas for investigation of toxic substances affecting germ cells.

In the cytogenetic area, a new chromosomal rearrangement involving the X chromosome has been identified (pericentric X inversion). Furthermore, a unique individual with a 45, X karyotype and the presence of testicular tissue has been identified and studies on the relationship between his pattern of differentiation and the H-Y antigen are under investigation.

We are now using liposome enclosed DNA, chromatin, and chromosomes in studies designed to test whether irreversible alterations in DNA are characteristic of the process of X chromosome inactivation. An improved method for encapsulating DNA into liposomes has been developed in the course of these studies. Studies with viral DNA are also being utilized.

Interest in liposomes has also led us to examine the stability of liposomes as well as natural red cell membranes enriched in C26. This has some relevance to some of the work on adrenal leukodystrophy previously mentioned. We have demonstrated an abnormal stiffening of membranes enriched in C26 in red cells from patients with adrenal leukodystrophy and this work is being further explored in the liposome system.

Because of the importance of prenatal diagnosis in many genetic disorders, we have been interested in improving techniques for growing amniotic cells in culture. A controlled double blind study has now demonstrated that fibroblast growth factor from sheep brain can significantly stimulate the growth of human amniotic cell lines. Other studies involving prenatal diagnosis have included the demonstration that prenatal diagnosis of the adrenogenital syndrome can be achieved by measuring 17-hydroxyprogesterone and androstenedione levels in amniotic fluid.

Significance to Biomedical Research and Programs of the Institute: Cystinosis, homocystinuria, phenylketonuria, and the inborn errors of glutathione metabolism all present clinical pictures of varying degrees of severity, in some cases lethal. These and other heritable disorders studied in our laboratory require better methods of diagnosis, treatment and prevention than are currently available, and our efforts in these directions are essential for the goals of this Institute. In addition, it has been frequently demonstrated that the study of genetic variants in man is a powerful tool for advancing our understanding of

normal and abnormal biochemistry. Our studies on glutathione metabolism illustrate that point. Furthermore, the extension of our work from rare genetic disorders of glutathione metabolism to an extremely common one like glucose-6-phosphate dehydrogenase deficiency demonstrates how the study of rare model defects may open the way to understanding of common disorders with substantial public health significance. An interest in genetic diseases in general should include an interest in more fundamental aspects of human genetics. Our studies related to mechanisms of cell growth and gene integration are consonant with this orientation.

Proposed Course of Project: Future efforts will include: 1) the continuing study of compounds which may be of potential therapeutic benefit in cystinosis and other heritable disorders; 2) further investigation of the mechanisms of lysosomal metabolism of cystine and related compounds in cystinosis; 3) continued investigation of the biochemical abnormalities in G6PD deficiency; 4) continued studies of the enzymes responsible for the synthesis and degradation of glutathione, and their involvement in disorders of its metabolism; 5) the development of an *in vivo* radiochemical assay for phenylalanine hydroxylase activity in patients with PKU and other abnormalities of phenylalanine metabolism; 6) investigation of additional inborn errors of metabolism, especially adrenal leukodystrophy and galactosemia; 7) using gene transfer technology with the hope of understanding and developing approaches to gene therapy of genetic defects.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00132-05 NPMB

PERIOD COVERED October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Differentiation of Intestinal Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator:

N. Kretchmer	Head, DGN	NPMB, NICHD

Other Investigators:

P. Burrill	Staff Fellow	NPMB, NICHD
B. Harrington	Chemist	NPMB, NICHD
I. Bernardini	Chemist	NPMB, NICHD
P. Brannon	Staff Fellow	NPMB, NICHD
J. Riby	Visiting Fellow	NPMB, NICHD

COOPERATING UNITS (if any) J. Bernstein, William Beaumont Hospital, Royal Oak, Michigan; G. Semenza, Lab. Biochem. Tech. Inst., Zurich, Switzerland; J. Roth, NIAMDD, NIH

LAB/BRANCH Neonatal and Pediatric Medicine Branch

SECTION Section on Developmental Gastroenterology and Nutrition

INSTITUTE AND LOCATION NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Section on Developmental Gastroenterology and Nutrition was established to study (1) intestinal epithelial cell migration and differentiation and (2) the nutritional modifications of gene activity and expression in the pancreas of developing and adult mammals. With respect to epithelial cell migration, our efforts have focused on the establishment of a primary cell culture system which will allow us to define those cell surface components which are responsible for cell attachment to the basement membrane. We are also continuing our research into the control of sucrase/isomaltase synthesis and degradation and the mode of incorporation of the complex into the intestinal brush-border membrane. Primary cultures of rat pancreatic exocrine cells were established to elucidate the role of nutrition in the modulation of gene activity and expression. Studies are focusing on the mechanism of adaptation of rat amylase in cultured cells and intact pancreas to carbohydrate in the media or the diet.

PHS-6040

Project Description:

Objectives: Fundamental to the biology of cells and the organization of multicellular systems is the ability of cells to adhere to and migrate along an extracellular matrix. As a model for the study of these related phenomena, we have chosen the intestinal epithelium, where an orderly sequence of cell division and differentiation, concomitant with cellular migration from the crypt to villus tip, results in renewal of the epithelium within two to three days (in the rat). A major effort is being made to (1) identify the cell surface component(s) which anchor the epithelium to its basement membrane, (2) discover the mode of association of this attachment component with both the cytoskeletal structures and the basement membrane and (3) analyze the roles of protein synthesis, cellular metabolism and humoral agents in controlling epithelial locomotion. Furthermore, the regulation of the expression of the enzyme complex, sucrase/isomaltase and its incorporation into the brush-border-membrane remains a major concern of this laboratory. In our attempt to understand the role of nutrition in cellular metabolism, we are investigating the mechanism of adaptation of amylase in primary cultures of pancreatic exocrine cells derived from rats at various stages of development. This adaptation provides a model within which to study nutritional modifications of gene activity and expression throughout the development of the organism. Also, the evolution of this adaptation can be examined by comparing its mechanisms in species that differ in evolutionary age and complexity.

Methods Employed: The principal methods employed are: (1) cell and organ culture techniques, (2) cell dissociation techniques, (3) bright field, phase contrast, immunofluorescent and electron microscopy, (4) column chromatography (affinity, ion exchange, and gel filtration), (5) polyacrylamide slab and disc gel electrophoresis, (6) immunoelectrophoresis, (7) centrifugation and ultracentrifugation, (8) spectrophotometric, fluorometric and radiochemical assays, (9) autoradiography, (10) amino acid analysis, and (11) cDNA hybridization.

Affinity Chromatography - We have continued to investigate the use of the affinity gel, α-glucohydrolase inhibitor - Sepharose 4B (αGHI-Seph), to separate the pancreatic and salivary amylases in serum. Rat serum pancreatic and salivary amylases can be separated by the sequential elution with pH 5.8 buffer (removing the pancreatic isozyme) and 1% glucogen-buffer (removing the salivary isozyme). We are also interested in using this affinity gel to separate amylase isozymes from human serum and are continuing this investigation.

Regulation of Pancreatic Amylase in Cultured Exocrine Cells - We are interested in studying the mechanism of nutrient interactions with regulatory systems and have chosen to focus specifically on the mechanism of adaptation of pancreatic amylase to changes in the composition of the diet. We are establishing primary cultures of rat pancreatic exocrine cells as a model system in which to study amylase synthesis. These cells, when cultured in Ham's F-12 media containing 15% fetal calf serum (FCS), 10^{-5} M carbamyl choline, and 0.1 mg/ml soybean trypsin inhibitor (STI), attached best to culture dishes when plated at a concentration of 6.5 to 13.0 x 10^5 cells/cm^2 of growth area. In cells cultured for 4 days, cellular amylase activity decreased 5-10 fold while cellular protein and DNA remains stable. The rate of secretion of amylase by these cells also decreased 5-10 fold, although the pattern of secretion over 120 min. was similar to that seen in freshly isolated cells. Cells cultured 4 days also retained the ability to synthesize de novo amylase, as demonstrated by the incorporation of ^{3}H-

leu into amylase protein (isolated by αGHI-Seph affinity adsorption).

Utilizing this system of cultured exocrine cells, the effects of various hormones on cellular and secreted amylase activity during 96 hours of culture were examined. None of the hormones or combinations of hormones affected either parameter. The following log doses of hormones and combinations were examined: caerulein (0.01 - 100 ng/ml), insulin (0.001 -50 μg/ml), somatostatin (1 - 100 ng/ml), dexamethasone (10^{-8} - 10^{-6} M), insulin + dexamethasone, insulin + caerulein, insulin + dexamethasone + somatostatin.

Since the presence of FCS in the media might obscure the effects of hormones added to the media, we are establishing conditions for serum-free culture. We found that Waymouth's media supported exocrine cell viability better than Ham's F-12 media. Maintenance of cell viability was improved when Waymouth's media was supplemented with 0.1 mg/ml STI, 50 μg/ml epidermal growth factor (EGF), and 10^{-8} M dexamethasone (dex). Bovine serum albumin (BSA) from 0.1 to 10 mg/ml further improved, in a dose response fashion, cell viability, morphology, and attachment. We are now interested in examining the effects of potential regulating hormones on amylase activity in primary cultures of pancreatic exocrine cells when maintained in this serum free media.

Primary Culture of Intestinal Epithelial Cells - Isolated rabbit intestinal cells have been shown to attach to collagen-coated but not to plain bacteriological plastic dishes. These cells preferentially adhere to type IV collagen and to other collagens tested in the following decreasing order: type III, type I, and type II. Attachment of intestinal cells to collagen is enhanced 30-50% over control when the collagen coated dishes are pretreated with either cell surface or serum fibronectin; the former species is 5-10 times more effective than the latter in promoting cell adhesion. The serum factor, Holmes' α-1 protein enhances cell attachment by 20-50% over control, when added to the incubation medium at 2-300 ng/ml concentration. Pretreatment of type I collagen fiber with 10-30 μg of laminin, a glycoprotein to this substrate by 20-50%, constitutent of basement membranes, also increases cell adhesion 20-50%, but only in the presence of serum. Fibroblasts and bovine corneal endothelial cells lay down extracellular attachment factors in culture. These cells can be lysed under conditions that leave the attachment factors adhering to the tissue culture dishes. Attachment of intestinal cells to the extracellular matrix produced by the endothelial cells (60-80% and above) was twice that observed for the fibroblast matrix or type I collagen (30-40%). Maximal attachment (80-90% of total cells plated) was observed with the endothelial cell matrix when the intestinal cells were incubated in medium with serum. We are continuing thee studies to identify both the serum factor(s) enhancing cell attachment to collagen, and the components of the intestinal cell surface responsible for the adhesion of these cells to the basement membrane.

Cells isolated from the intestine have been maintained in culture for two months. We are trying to determine if these cells are epitheliod using electron-microscopy and immunological techniques.

Sucrase Fall-off - Rat intestinal brush-border enzyme, sucrase/isomaltase, has been purified, and both conventional monospecific and monoclonal antibodies to sucrase/isomaltase are being prepared. These antibodies are to be used to determine the mechanism of lots of sucrase/isomaltase activity in cells on the upper villus.

Significance to Biomedical Research and the Program of the Institute: Definition of the processes involved in differentiation and cell migration may aid in the understanding of malabsorption states such as chronic diarrhea of infancy, short bowel syndrome, coeliac disease and other pathologic states where regeneration of the intestinal mucosa is a major concern. In addition, the understanding of the interaction of cells with the extracellular matrix may add to our knowledge of embryogenesis, organogenesis, tumor metastases, etc.

Changes in the nutritional environment during development and growth may modify differentiation and maturation of cellular processes. To understand this phenomenon better, we need to clarify the process of interaction between nutrients and gene expression. The development of a model system to study the significance of nutritional factors in gene expression is in progress.

Proposed Course of the Project: We are continuing our efforts to define the specific interaction of intestinal epithelial cells with various substrates using primary cell cultures.

Antibodies against α-amylase are being prepared to be used in the development of ELISA and immunoadsorbent techniques for the detection of the enzyme. The proposed roles of dexamethasone and insulin in the regulation of the adaptation of α-amylase will be re-examined in cultured exocrine cells maintained in serum free media. Finally, the development of this regulation will be studied in vitro in exocrine cells isolated from embryonic rats at various stages of development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00135-04 NPMB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Developmental Endocrinology and Neuroendocrinology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Barry B. Bercu

Other Investigators: Teri Brown, Laboratory Technician
Hari Sachs, Student Scientist
Joseph Ober, Student Scientist

COOPERATING UNITS (if any)
SEE ATTACHED SHEETS

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.25	2.0	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying normal and abnormal sexual development in the primate. Specifically, we have examined pulsatile secretion (LH, FSH, LH bioassay and testosterone) in the hypothalamic-pituitary-testicular axis. The primate age range has been from 10 days of age to adult. Our current plans are the expansion of this research to include fetal sexual development.

We have also studied the effects of castration, gonadotropin hormone releasing hormone, and human chorionic gonadotropin at various ages. Other studies conducted in the course of this research include fluorescent labelling of testicular tissue, light and electron microscopy of the testis.

Our previous research with rat and bovine sertoli cell culture systems has been applied to our current primate investigation and will soon be applied to human tissue. Investigation continues in the following areas: 1) pituitary resistance to thyroid hormone in cystinosis, osteogenesis imperfecta and neonatal hypothyroidism, 2) GnRH antiserum studies, 3) cranial irradiation studies, 4) clinical hGH investigations using hGH prepared by recombinant DNA technology.

Cooperating units:

G. August, A. Glasgow, W. Hung, National Children's Hospital

G. Chrousos, NPMB, NICHD

G. Gunsalus, W. Bardin, Rockefeller University

G. Hodgen, PRB, NICHD

Y. Patel, McGill University

D. Poplack, Pediatric Oncology, NCI

J. Schulman, NPMB, NICHD

J. Schwade, NCI

J. Shapiro, CC

B. Weintraub, NIAMDD

Project Descriptions:

Objectives: The goals of the project are as follows: (1) to explore the development of the hypothalamic-pituitary-testicular axis, (2) to elucidate the biochemistry and function of the primate Sertoli cells, (3) to develop a human model for studying Sertoli cell function, (4) to explore intersex problems with relationship to Mullerian Inhibiting Substance and inhibin; both are hormones which are made by the Sertoli cell, (5) to utilize our neuroendocrine primate model (rhesus monkey) to study the effect of cranial radiation and varius drugs on hypothalamic-pituitary secretion, (6) to further study pituitary resistance to thyroid hormone in cystinosis and other metabolic disorders, (7) to evaluate metabolic effects of hGH in growth hormone deficient patients, (8) to further study neuroendocrine abnormalities in patients who have received cranial irradiation.

Methods Employed: The principal methods employed are: 1) cell culture techniques, 2) radioimmunoassay of hormones, 3) radioreceptor assays, 4) hormone bioassays, 5) radioisotopic measures using tritium, I-125, 6) immunization techniques, active and passive, 7) animal surgery, primate and rat, 8) animal cannulation techniques, 9) measurement of cyclic nucleotides, 10) viral cell gransformation, 11) irradiation techiques, 12) administration of recombinant DNA hGH to humans.

Major Findings: There is pulsatile secretion of gonadotropins during sexual development in the male subhuman primate. There is variance in the amplitude and frequency of these pulses which correlates with testicular maturation. Castrated animals demonstrated pulsatile secretion only after advancement to "pubertal age."

Cranial irradiation in primates causes abnormalities in growth hormone secretion. Various functions of rat and bovine Sertoli cell systems have been compared. We have measured several FSH stimulated functions including: cyclic AMP and aromatization of testosterone to estradiol. FSH stimulates ornithine decarboxylase activity in bovine cells. We have demonstrated autoregulation of FSH response in rat Sertoli cells and we have also studied FSH receptors in this tissue culture system.

Using passive immunization of young rats with LHRH antisera we have further studied the development of the hypothalamic-pituitary-testicular axis. Gonadotropin, androgen binding protein, testosterone secretion and hCG/LH receptors were measured.

A useful animal model to study neuroendocrine function in primates was developed. The following drugs and hormones have been studied: somatostatin, melatonin, and bromoergocriptine.

Patients with cystinosis appear to have pituitary resistance to thyroid hormones. TSHα is elevated in most of these patients.

Significance to Biomedical Research and Programs of the Institute: The focus of our laboratory is to better understand human male sexual development and to develop advanced treatment methods of this and other related pediatric endocrine disorders. We have studied neuroendocrine complications of cranial radiation, a technique commonly used in pediatric oncology. Using our primate model, we

should be better able to understand the effects of drugs and hormones on hypothalamic-pituitary function. In addition, we are applying our primate technology to human intersex problems. We will soon obtain the testes of a thirteen year old human female with testicular feminization. Our efforts in these areas are directly related to the goals of this institute because their applications advance our understanding of child development.

Proposed Course of Project: Future efforts will include: 1) the continuing study of the development of the hypothalamic-pituitary-testicular axis in the primate, 2) further investigation of primate Sertoli cells and their functions, 3) continued studies of the abnormalities of the hypothalamic-pituitary axis in humans and monkeys, 5) investigation of intersex problems, 6) continued studies of the hypothalamic-pituitary-thyroid and gonadal axis in cystinosis and other metabolic and endocrine diseases.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00401-03 NPMB

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Disorders of Carbohydrate Metabolism in Infancy and Childhood

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Marvin Cornblath

Other Investigators: George Chrousos
Anthony Adams

COOPERATING UNITS (if any)
See Attached Sheet

LAB/BRANCH
Neonatal and Pediatric Medicine Branch

SECTION
Developmental Biology and Clinical Nutrition

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Our major goal is elucidating the pathophysiology of disorders of carbohydrate metabolism in infants and children. This has included studies of the basic metabolic pathways of glucose, cholesterol and fat in glycogen storage disease (GSD), utilizing studies in patients as well as in fibroblasts. CT technology was used to define glycogen and fat concentrations in liver both in animals and man. The studies of glycosylated hemoglobins in patients with GSD have been expanded. All of these studies have produced important leads and promising results.

Studies of erythrocyte insulin receptors verified their increased numbers in patients with symptomatic hypoglycemia. These specific receptors have also been measured in patients with GSD and with hypopituitarism.

Continuous glucose monitoring has begun to clarify the changes with controlled exercise stress in both children and adults as well as serving as a tool in monitoring induced hypoglycemia in patients with cancer.

A wrist watch alarm which responds to a fall in temperature and sweating has proven useful in the early detection of symptomatic hypoglycemia in young infants and children.

PHS-6040
(Rev. 2-81)

Other Collaborators:

Dr. Robert Schwartz, Brown University

Dr. Noel MacLaren, University of Florida

Dr. Avner Notkin, NDR, NIH

Dr. Judith McLaughlin, University of Maryland

Dr. Avinoam A. Kowarski, University of Maryland

Dr. Herbert Schwartz, Stanford University

Drs. Mary L. Peters and Michael F. Burt, NCI, NIH

Dr. P.T. Ozand, University of Maryland

Dr. Douglas Reed, University of Maryland

Dr. John Doppman, CC/Radiology, NIH

Drs. Ernst Schaefer, NHLBI, NIH

Dr. Zvi Begg, NHLBI, NIH

Dr. Alan Glasgow, Children's Hospital National Medical Center

Dr. Sid Chernick, NIAMDD, NIH

Dr. Murray Brenan, Sloan Kettering Memorial Hospital, N.Y.

Dr. Judith Rapoport, NIMH

Project Description

Objectives: The goals of the project are as follows: (1) to elucidate the pathophysiologic mechanisms involved in various disorders of carbohydrate metabolism in infants and children. (2) To evaluate the currently used diet and therapeutic regimens in the above disorders and modify them as indicated. (3) To study the relationship of hormone effectors, their receptor sites and other parameters relevant to carbohydrate metabolism such as concentrations in blood of glucose, lactate, pyruvate, free fatty acids, ketones, and amino acids. (4) To assess the overall growth and development of these patients by following their growth patterns, the maturation, function and response of their neuroendocrine and peripheral endocrine systems as well as the development of their neurological and psychological behavior. (5) To establish and study appropriate animal models (primate, rodent, etc.) for each of these areas. (6) To establish cell/tissue culture lines for GSD in evaluating metabolic pathways and potential therapeutic modalities.

Methods Employed: The research methodology employed can be separated in (a) clinical and (b) laboratory.

(a) The patients are studied clinically on the 9W pediatric ward of NICHD. Complete physical evaluations, neurological examinations and psychological testings are performed. Diagnostic tests available routinely at the Clinical Center will include EEG, diagnostic x-rays, CT scans, clinical pathology tests, etc. as indicated. A constant glucose monitor developed by Dr. Kowarski is available to study patients with carbohydrate disorders in a more precise and effective manner.

(b) Laboratory investigative tests are performed to study three distinct, but related areas: (1) Insulin receptor sites (number of receptors and affinity to insulin) on circulating erythrocytes, measured by a radioreceptor assay, (2) circulating insulin, growth hormone, cortisol, T_4, and glucagon measured by radioimmunoassay and (3) biochemical determination of substrates and metabolites that either reflect or influence carbohydrate metabolism by micro-automated fluorometric techniques, and (4) liver enzyme studies, glycogen, and fat content determinations are done as indicated.

Major Findings

(1) Projects and studies - Glycosylated hemoglobin functions have been measured in hypoglycemic syndromes and in GSD, Types I, Ia, III and IX in collaboration with Dr. Robert Schwartz and Dr. Herbert Schwartz.

Several minor hemoglobins were measured by high performance liquid chromatography in children with a variety of disorders associated with hypoglycemia. No abnormalities of $HbAI_c$ were found in subjects with glucose-6-phosphatase deficiency or fructose-1,6-diphosphatase deficiency, whereas significant elevations of the negatively charged minor hemoglobins ($HbAI_{a+b}$) were found in all of these patients. The most recent data indicate that there is a lack of correlation between concentrations of hemoglobin AI_{a+b} versus AI_c in the patients with glycogen storage disease in contrast to diabetics where the relationship is linear. This suggests that an entirely different mechanism exists for the increase in these glycosylated hemoglobin fractions in GSD. The

elucidation of the metabolic mechanism involved is currently under study. Chemical characterization of the hemoglobin adduct should contribute to a further understanding of the metabolic alterations in these disorders and may contribute to evaluating modalities of treatment.

(2) Stimulated by an unusual family with onset of insulin dependent diabetes mellitus in 2 of 4 sibs within a two week period, studies of viral antibodies HLA typing and specific endocrine antibodies have been initiated in this family as well as in a group of diabetic patients. To, date the antibody studies have been done in collaboration with Dr. Noel Maclaren at the University of Florida in Gainesville. It would appear that a majority of our patients who have had diabetes for longer than 5 years do not have antibodies against their islet cells. In contrast, a significant number of the diabetics have anti-thyroid and anti-parietal cell antibodies. These findings suggest that these patients are susceptible, in fact, to developing diseases of the thyroid or achlorhydria in the future. The studies of identical twin adults who had circulating anti-alpha cell antibodies by Dr. Maclaren did not reveal any abnormality in these patients ability to secrete glucagon or respond to an arginine tolerance test. Their glucose tolerances as well as all the other parameters of endocrine secretion were within normal limits in both of these adults.

(3) In studies of familial nesidioblastosis, one patient was found to have a relative glucagon deficiency in response to arginine and to induced hypoglycemia. Dramatic improvement occurred with twice daily injections of long acting zinc glycagon. The patient has also demonstrated the effectiveness of the night alarm wrist watch in the early detection of hypoglycemia. She along with two other patients who are post-operative nesidioblastosis but continue to have hypoglycemic episodes have been able to wear their wrist watch which detects a fall in temperature or an increase in perspiration prior to symptomatic hypoglycemia.

(4) The long-term follow-up, management, and erythrocyte insulin receptor data summarizing 9 patients with hyperinsulinemic hypoglycemia since infancy is being prepared for publication.

(5) The observation that CT scans of patients with glycogen storage disease were abnormal led to the investigation of the value of this examination in patients with GSD type I. As a result of a collaborative study with Dr. Doppman in the Division of Radiology, a paper has been prepared for publication describing the CT density of the liver, spleen and kidney of patients with GSD. In order to determine what affect glycogen and fat may have on CT density, a series of curves were obtained utilizing various glycogen concentrations both alone and in combination with various concentrations of fat. In addition to this *in vitro* study, animal studies in rhesus monkeys were initiated to determine if it were possible to increase the liver glycogen concentration by oral feeding of sugar and then reducing the concentration acutely by repeated glucagon injections. Preliminary data in 3 monkeys suggest that this is possible. This has been verified by biopsy analysis and these data are being prepared for publication.

(6) A series of analyses of phospholipids, cholesterol, and triglycerides in 7 patients with GSD suggest that even with continuous nocturnal nasogastric feedings, that gross abnormalities in phospholipid, cholesterol and triglyceride

metabolism persist. These studies are being done in collaboration with Dr. Ernst Schaefer of NHLBI. The patients with glycogen storage disease show patterns of type IV and type V hyperlipidemia. In order to elucidate the mechanisms involved, skin biopsies were obtained for fibroblast cultures. These cultures are now being investigated in collaboration with Dr. Ozand and Dr. Reed at the University of Maryland and Dr. Begg in the HLBI to determine the specific steps in cholesterol synthesis that may be at fault. Preliminary data suggest that the fibroblasts from patients with GSD can readily convert acetoacetate to cholesterol but not glucose in contrast to normal controls. Additional studies are planned to investigate glucose uptake and the glycolytic pathway in the fibroblasts of the patients with GSD versus normal controls.

(7) Patients with hypopituitarism were investigated with two treatment regimens of growth hormone, 1 administered 3 times a week and the other daily for 11 days. Specific insulin receptors on erythrocytes were measured before and after the therapeutic regimens. The preliminary analysis of the data do not show a consistent affect of growth hormone but further analysis is underway. This study was done in collaboration with Dr. Barry Bercu.

(8) Preliminary data on specific insulin receptors in red cells from patients with diabetes mellitus followed at the Children's Hospital National Medical Center are being done in collaboration with Dr. Glasgow. The object of the study was to evaluate specific insulin receptors in diabetic patients receiving 2 or more units per kilo of insulin per day versus those who were requiring 1 or less units per kilo per day. The preliminary analysis would suggest that the insulin receptors are increased in those patients requiring less insulin. Additional patients require study.

(9) In collaboration with Dr. Bob Dons in the Arthritis Institute, we are obtaining experience with the continuous glucose monitor in adult patients during exercise. The data to date are being analyzed and should be ready for publication within the next calendar year. The continuous glucose monitor has also been used in collaboration with a study initiated in the Cancer Institute by Drs. Brennan, Peters and Burt. The principle is to inject picolinic acid into the patient and block gluconeogenesis to induce a generalized hypoglycemia. This is to starve the malignant tumor while glycerol is administered intravenously to support cerebral function. A research protocol has been initiated in collaboration with Dr. Judy Rapaport of the NIMH. The purpose of this protocol is to study the effects of sugar on hyperactivity in children whose parents insist that sugar incites their hyperkinetic behavior. This is being initiated with a double blind study utilizing glucose and sucrose as the test substance in measuring both glucose and fructose response to these two stimuli. In addition, catecholamines, insulin, growth hormone and cortisol will be measured. This protocol is currently undergoing evaluation and approval in the NIMH and should be underway before the end of the fiscal year.

Significance to Biomedical Research and Programs of the Institute: The focus of our research is to elucidate the pathophysiology of disorders of carbohydrate metabolism in infancy and childhood and develop better methods for diagnosis and therapy. Through these efforts the development of the normal child will be better understood. Both objectives are in concert with the goals of the Institute.

Proposed Course of Project: Future efforts will include: (1) the continuing study of the pathophysiology of carbohydrate metabolism disorders in infancy and childhood. (2) A detailed study of the ontogeny of insulin receptors and the interrelationships of insulin and insulin receptors, throughout early development in animal models and man. (3) Evaluation of the efficacy and effects of continuous nocturnal nasogastric feedings in children with glycogen storage disease type I and its effects on the carbohydrate regulatory systems. (4) The effects of Diazoxide® in the treatment of hypoglycemic syndromes in infants and children and elucidation of its mechanism of action especially concerning receptor and postreceptor events. (5) Further study of nesidioblastosis which seems to be a familial disease. (6) The role and interrelationship of glucagon, somatostatin, insulin and growth hormone with insulin receptors and the overall change during growth and development. (7) Continuous glucose monitor evaluation of carbohydrate homeostasis in GSD, in persistent or recurrent hypoglycemia and in reactive hypoglycemia. (8) Basic fat metabolism will continue to be studied in both fibroblasts and in patients with glycogen storage disease as indicated above. (9) The wrist watch alarm to detect hypoglycemia will be utilized in additional patients with hypoglycemia as well as in acute situations for insulin tolerance tests and other hypoglycemia inducing tolerance tests to be done in patients with growth failure or hypopituitarism. (10) Sucrose and glucose tolerance tests will be done in patients who have exhibited hyperkinetic activity and fit into the Rapaport-Cornblath protocol. (11) Studies of both glucose and cholesterol metabolism in fibroblasts from patients with glycogen storage and normal controls will continue.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00402-03 NPMB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mass Spectrometry Facility

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alfred L. Yergey	Research Chemist	NPMB, NICHD
OTHER:	Lawrence Tamarkin	Staff Fellow	NPMB, NICHD
	Nancy Vieira	Biologist	NPMB, NICHD

COOPERATING UNITS (if any) R. Cotter, Johns Hopkins; Peter Klein, Baylor College of Medicine; N. Gershfeld, LPB, NIAMDD; M. Friedman, U.S. Army; J.B. Sidbury, NPMB; NICHD; John Daly, NIADDK; M. Lippman, DCT, NCI; B. Chabner, DCT, NCI; B. Goldman, Worcester Fnd. Exp. Biol., MA; T. Wehr, LSC, NIMH; K. Catt, ERRB, NICHD.

LAB/BRANCH
OSD, NPMB

SECTION

INSTITUTE AND LOCATION
National Institute of Child Health and Human Development, NIH, Bldg. 6, Rm. 140

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Quantitative analysis using solids inlet probe is being done for studies of human calcium metabolism in lactation and metabolic disorders. Thermal desorption mass spectrometry of involatile organic salts has been developed for detection and identification. Mathematical modeling of calcium kinetics and quadrupole mass filters is being done. Multivariate statistical methods are being applied to large mass spectral data sets.

The physiologic role and regulation of the pineal hormone melatonin is being studied in human subjects and animals. Plasma melatonin is being studied in obese children, in precociously pubescent children, and in women with breast cancer. Pineal melatonin is being measured in rodents to investigate the circadian regulation of this hormonal and its ontogeny. Estrogen and progesterone receptors are being measured in hamster uteri to study the effect of acute melatonin treatment on these receptors. The inhibitory effect of melatonin on the induction of mammary tumors in dimethylbenzathracene treated rats is being studied. In vitro production of melatonin by a single chicken pineal gland is being studied.

PHS-6040

Mass Spectrometry Project Description

Objectives: Develop mass spectrometric methods for analysis of substances for which satisfactory analyses do not presently exist, particularly the quantitative measurement of stable isotopically labeled and non-volatile materials. Develop techniques for analysis of data produced in pyrolysis of solid materials within the mass spectrometer source volume.

Develop models to increase understanding of and to improve quadrupole mass filters. Develop models for understanding of calcium metabolism.

Methods Employed: Quantitative analyses are performed by use of specific ion monitoring (SIM) of substances evolving from a heated mass spectrometer solids inlet probe. Using special purpose computers developed for this task, intensity data from particular ions are accumulated in storage registers; ratios of total ion intensities from different registers are obtained after evolutions are complete. Quantitation is obtained by using an internal standard that co-evolves with the analyate. The standard is either a chemical homolog or an isotopically labeled form of the analyate.

The same probe as used in the calcium study has been used to obtain mass spectra of organic salts and sugars. Water solutions of these materials are coated onto rhenium filaments which are then heated inside the ion source.

APL language as implemented on the PDP-10 is used for the multivariate statistical analysis of pyrolysis data sets. This language is selected because of the great convenience it offers for the manipulation of the large data sets produced in pyrolysis in pyrolysis mass spectrometry.

Construction of functioning physical models as well as analytical and numerical solutions of the differential equations of motion that describe ion trajectories in multiple fields are used in the development of models for quadrupole mass filter improvement. The mathematical modeling program SAAM27 in the laboratory of Theoretical Biology, NCI, is being used to develop a multi-compartmental model for calcium metabolic kinetics.

Major Findings: (1) Calcium isotope ratio measurements that are accurate to about 1% of natural abundance and which are reproducible to about 1% have been used to measure tracer quantities of the stable isotoped ^{44}Ca and ^{48}Ca in urine specimens. The methodology has been applied to the study of calcium metabolism in human subjects. The method has been extended to permit the measurement of the low abundance (.0033% of natural abundance) ^{46}Ca isotope. Studies of four subjects with calcium metabolic defects have been completed. The data for the single dystrophic calcification patient indicate that calcium intake is normal, but that there is virtually no excretion of calcium. Data analysis on other studies is incomplete. (2) Mass spectra of a variety of tetralkyl ammonium salts have been obtained. Heating the coated filaments is the only ionization means used. These compounds show quite unusual fragmentation patterns in that the dissociations seem to involve principally even electron species; formation of the dissociation ions can be rationalized in a uniform manner. Similar rationalizations can be used to explain the desorption spectra of mixed alkyl/aryl salts and of choline and several of its derivatives. (3) Programs using APL have been developed to take mass spectal files

into the PDP-10, condition them for analysis and manipulate them so that characteristic patterns in the evolution of thermal degradation products can be recognized. (4) The second-order differential equations for solutions of ion trajectories have been solved by two numerical integration techniques; Taylor-Series Expansion and Predictor-Corrector method. Results appear to be the same for given entrance conditions, but the Predictor-Corrector method may be the less expensive method to implement. (5) Using the SAAM27 program, a 24 compartment model that includes natural abundance calcium as well as intravenous and oral tracers has been developed for a lactating mother. This model has been used to calculate doses of isotopic tracer to be used in our planned study of calcium kinetics in lactating women.

Proposed Course: (1) Mass spectrometry measurement will continue in the areas described while time will be provided for analysis of particular samples as the need arises. (2) Programming will be developed to effect a rapid transfer of large data sets between the mass spectrometer data system and the PDP-10. (3) Numerical integration of the higher order multipole field equations will be undertaken in order to evaluate those geometries for improved mass filter devices. (4) An LC interface will be added to a mass spectrometer to permit studies of peptides and underivatized lipids.

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6. R.J. Cotter, A.L. Yergey, "The Role of Thermally Produced Ions In Mass Spectrometric Desorption Methods," Proceedings of the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN, May 24-29,1981.

Circadian Rhythm Project Description

Objectives: To investigate the role of hormonal and behavioral circadian rhythms in health and disease.

Methods Employed: The techniques employed are: (1) Radioimmunoassay for melatonin and high performance liquid chromatography for validation of the assay; (2) Radioimmunoassays for LH, FSH, prolactin, and cortisol; (3) Radioreceptor assay for hamster prolactin; (4) static incubation of pituitary glands; (5) superfusion of pituitary cells.

Major Findings: In humans: (1) The daily profile of plasma melatonin is essentially unchanged during puberty and in children with precosity; (2) Plasma melatonin does not change as a function of weight loss in either exogenously obese or Prader-Willi syndrome patients; (3) A nocturnal increase in plasma prolactin is absent in both Prader-Willi and exogenously obese subjects; (4) Women (N=10) with Stage I breast cancer whose tumor contains estrogen receptors have lower levels of plasma melatonin than women with estrogen receptor negative breast tumors of normal volunteers.

In rodents: (1) The pineal melatonin rhythm of the Syrian hamster is truly circadian and may be regulated by two oscillators (biological clocks); (2) The daily profile of melatonin may signal changes in daylength by a change in the duration of elevated levels of melatonin during the night; (3) Daily injection of melatonin inhibits mammary tumor development, and pinealectomy enhances mammary tumor development in DMBA treated rats. Those tumor free animals injected with melatonin had lower levels of plasma prolactin than vehicle-injected controls. (4) Treatment of hamsters with clorgyline (an MAO-type A inhibitor) alters pineal melatonin levels and the onset of running activity.

In vitro: (1) Superfused pituitary cells release LH following melatonin (10^{-8}M) treatment; (2) Melatonin (10^{-9}M) treatment for 40 minutes causes an induction of estrogen receptors in an epithelial breast cancer cell line (MCF-F) cells).

Proposed Course: In human subjects: (1) Obese subjects will be monitored by EEG during sleep and blood samples will be taken to correlate prolactin levels with stages of sleep; (2) Normal adult women volunteers will be asked to participate in 24-h blood sampling to define the hormonal profiles of melatonin prolactin, and cortisol.

In rodents: (1) Effects of clorgyline on pineal melatonin synthesis and on running activity will be investigated to determine if this drug selectively alters the neurochemistry of the biological clock.

In Vitro: (1) Superfusion of pituitary cells will be continued at different doses of melatonin and the specificity of the response will be determined; (2) A static organ culture will determine if the effect of melatonin on LH release observed in the superfusion system can be reproduced to allow for a broader investigation of the mechanism of melatonin action.

Publications:

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Project Numbers	Project Title	Principal Investigator
Z01 HD 00021-05	Bioassayable and Radioimmunoassayable ACTH.....	C. Strott
Z01 HD 00022-08	Renin-Angiotensin System and Aldosterone Regulation.....	K. Catt
Z01 HD 00035-09	The Structure and Function of Biologically Active Molecules	E. Gross
Z01 HD 00040-06	Experimental and Theoretical Studies of Hormone Receptor Interaction and Response Coupling	D. Rodbard
Z01 HD 00041-06	Cardiovascular Effects of Thyroid, Hormones, Catecholamines and Cardiotoxic Drugs	D. Rodbard
Z01 HD 00042-06	Mathematical and Statistical Theory of Gel Electrophoresis and Filtration	D. Rodbard
Z01 HD 00043-06	Mathematics and Statistics of Radioligand Assays	D. Rodbard
Z01 HD 00146-06	Structure and Function of Chorionic Gonadotropins	H. Chen
Z01 HD 00147-06	Mechanism of Action of Peptide Hormones in Steroidogenic Cells.....	M. Dufau
Z01 HD 00148-06	Ontogeny of Gonadotropin Receptors and Gonadal Function	K. Catt
Z01 HD 00149-06	Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin	M. Dufau
Z01 HD 00150-06	Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase	M. Dufau
Z01 HD 00151-06	Regulation of Gonadotropin Receptors in Testis and Ovary	K. Catt
Z01 HD 00152-06	Characterization and Radioligand Assay of Gonadotropin Receptors	K. Catt
Z01 HD 00160-06	Biology of the Adrenocortical Cell: Molecular Events in Steroid Biosynthesis and Secretion	C. Strott
Z01 HD 00164-05	Strategy and Mechanisms of Fractionation	N. Nguyen
Z01 HD 00165-06	Isolation and Characterization of Protein Hormones & Other Active Proteins	A. Chrambach
Z01 HD 00170-05	A Randomized Study for the Treatment of Chromophobe Adenomas of the Pituitary Gland with Irradiation	C. Strott
Z01 HD 00171-05	Other PAGE Instrumentation and Procedures	A. Chrambach
Z01 HD 00174-05	Hormonal Control of Ovarian Proteoglycan Synthesis	D. Rodbard
Z01 HD 00176-04	Clinical Evaluation of Adrenocortical Hyper-Function	C. Strott
Z01 HD 00179-03	Inferior Petrosal Sinus Catherization in Diagnosis and Treatment of Pituitary Tumors	C. Strott
Z01 HD 00184-03	Regulation of Pituitary Hormone Secretion	K. Catt

Z01 HD 00185-02	Effect of Temperature and Other Physical Factors on Localization, Natural History and Susceptibility to Disease	D. Rodbard
Z01 HD 00187-02	Hormonal Regulation of Cellular Metabolism	H. Huang
Z01 HD 00188-01	Development of New Analogs of Enkephalin with Increased Receptor Activity and Selectivity	D. Rodbard

ANNUAL REPORT
of the
Endocrinology and Reproduction Research Branch
National Institute of Child Health and Human Development
October 1, 1980 through September 30, 1981

The Endocrinology and Reproduction Research Branch conducts research into the basic and clinical aspects of endocrine regulation and the control of reproductive function. In addition, the Branch provides training for Clinical and Research Associates, and Visiting Fellows and Associates, in laboratory and clinical investigation. The research programs of the Branch are focused on the characterization of protein hormones and their cellular receptors, the structure-function relationships of peptide hormones, the regulation of hormone biosynthesis and secretion, and the study of mechanisms of hormone action. Other areas of interest include the analysis of pituitary-gonadal relationships and the regulation of ovarian activity during the reproductive cycle and pregnancy, with particular emphasis on the participation of hormone receptors in the control of gonadal function. This report describes the recent progress in research on the characteristics and secretion of peptide hormones, their interactions with cellular receptors and mechanisms of target cell action, the control of steroid hormone secretion, and the role of hormones in cellular regulation and clinical disorders.

The Section on Biophysical Endocrinology conducts research on the mathematical and statistical evaluation of endocrine regulatory processes and the properties of protein hormones and other ligands, studied by electrophoretic techniques, mathematical modeling, computer simulation, and biophysical methods, during analysis of ligand-receptor interactions, regulation of the ovarian cycle, and cardiovascular changes in thyroid disease. Current studies on the experimental and theoretical analysis of hormone, neurotransmitters, and drug binding to cellular receptors include the resolution of toad kidney cell receptors for adrenal steroids into two classes of receptors for aldosterone and three for corticosterone. In brain postsynaptic receptors, the interaction of GABA and its antagonist muscimol was analyzed and found to indicate two classes of GABA receptors, both of which are elevated in hepatic encephalopathy. A new β-adrenergic probe, [^{125}I] Iodopindolol, was evaluated by comparison with [1^{25}I]iodohydroxypindolol in skeletal and cardiac muscles by the 'Ligand' and 'Allfit' programs, and found to have significant advantages over the existing ligands employed to assay those sites. Other programs provide curve fitting according to a variety of binding models, either equilibrium or kinetic, and analyze the equilibrium composition of systems containing multiple ligands and binding proteins (e.g., adrenal and gonadal steroids, thyroid hormones, and their binding proteins). Further developments on the mathematical and statistical theories of radioligand assay include on-line calculation of quality control results, and automatic calculation of weighting coefficients in radioimmunoassays. Computer programs for the analysis of physicochemical methods employed to resolve protein hormones were extended to provide derivation of concentration profiles and molecular size, and to

permit optimization of elution patterns from high-performance liquid chromatography.

A new series of dimeric enkephalin analogs was synthesized and evaluated for receptor binding activity in brain membranes and neuroblastoma-glioma cells. These analogs possess high potency and specificty for the "delta" opiate receptor (for enkephalins) as opposed to the "mu" opiate receptor for naloxone and morphine. Such dimeric enkephalin analogs appear to cross-link the "delta" receptor and to have about 10-fold higher affinity than enkephalin, and to show a "sodium shift" characteristic of opiate receptor agonists. The biosynthesis of ovarian proteoglycans by rat granulosa cells was further studied, and the smaller species (MW 10^5) was shown to be stimulated by LH, FSH, and hCG, as well as testosterone and prostaglandins E_1 and E_2. This form was also stimulated by cAMP and a phosphodiesterase inhibitor, suggesting that the cyclic nucleotide system is involved in proteoglycan synthesis.

The pulse wave arrival time (designated QK_d), has been further demonstrated to be useful in evaluating thryroid function under conditions of "diminished thyroid reserve", and in studies on thyroid hormones during hypocaloric feeding in humans. The QK_d has been used to demonstrate that supplementation with oral thyroxin causes improvement at the target organ level, in patients with diminished thyroid reserve as manifested by low or borderline thyroid function with moderate or minimal TSH elevation. In human subjects undergoing prolonged hypocaloric feeding, there is a decrease in serum T4 and T3. Under these circumstances the QK_d is prolonged, suggestive of hypothyroidism at the target organ level. Further, oral supplementation with T3, to maintain a euthyroid level of T3, prevents the changes in QK_d. In contrast, oral supplementation with T4 which results in supra-normal levels of T4 but slightly decreased levels of T3, fails to provide complete return to normal of the QK_d. These findings suggest that QK_d is more closely correlated with T3 than with T4 levels, and that changes in peripheral thyroid hormones during hypocaloric feeding represent an adaptive mechanism which results in a state of hypometabolism.

The use of the thermographic camera has been examined in a variety of disease states. Thermography has been demonstrated to be useful in the detection and diagnosis of varicocele and testicular tumors. The thermographic camera is also useful in evaluation of temperature at the site of infectious disease (e.g. cutaneous and mucocutaneous leishmaniasis) and in a variety of inflammatory diseases. Fever therapy or local heat therapy is being evaluated in a variety of disease states, including sporotrichosis, mucocutaneous leishmanasis, and paracoccidioidomyosis.

The Electrophoresis Unit conducts research on the development and application of electrophoretic techniques for the fractionation and isolation of peptides and proteins of biological interest. Methods such as analytical polyacrylamide gel electrophoresis (PAGE), electrofocusing, and isotachophoresis are employed for the characterization and isolation of hormonal and other regulatory proteins. Recent research has focused on the standardization of procedures for PAGE electrophoresis and focusing, and for re-stacking of protein zones to facilitate the purification of hGH isohormones and hCG. Also, polymers such as Sephadex and agarose were employed in electrofocusing and protein extraction to improve the efficacy of these fractionation and isolation steps.

Human growth hormone isohormone B is needed as a starting material for enzymatic digestion leading to bioactivation, and as a bioactivity control.

A procedure for isolation of hGH-B (28%) from a mixture with isohormones C, D and E (72%) was elaborated, using isotachophoresis at acid pH. Metallothionein, a Zn and Cd binding peptide from liver and brain, was characterized in a crude homogenate as a single charge species, existing in 3 isomeric size forms. The glucocorticoid receptor from chick embryonic retina, purified by sucrose density sedimentation, was found to exhibit protein-protein interactions indistinguishable from the crude solubilized receptor. A monograph on gel electrophoretic methods is in preparation, and methods for electrophoresis in detergent-containing buffers and characterization of membrane proteins were reviewed. Also, a rapid destaining method was developed for SDS-PAGE gels under conditions of protein fixation.

The Section on Molecular Structure conducts research on the analysis, synthesis, and structure-function relationships of biologically active peptides. 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, chemotactic and ion-channel forming peptides, and peptides which inhibit fetal and tumor growth. Synthesis of LHRF analogs was performed to provide purified peptide agonists and antagonists for clinical and basic experimental studies. These include analogs with D-amino acids in positions 2, 4, 6 and 8, p-fluorophenylalanine in position 2, and the alternating pattern of L- and D- amino acids in position 1. Other studies include the analysis and synthesis of molecular assemblies with rare amino acids, such as: synthesis of precursors of rings A and B of nisin-a peptide with α,β-unsaturated and thioether amino acids, and the synthesis of these two cyclic structures; actions of peptides based on nisin and/or linear gramicidins on neonatal and neoplastic tissue; synthesis of analogs of the gramicidins A, B, and C, and correlation of their structures with ion-transport across lipid bilayers; exploration of dehydroalanine resins for synthesis of peptide amides; synthesis of analogs of opioid peptides with agonist and/or antagonist properties; synthesis of analogs of the pentapeptide L-Phe-D-Leu-L-Phe-D-Leu-L-Phe with chemotactic and anti-chemotactic characteristics; structural investigation of the heterodetic pentacyclic peptides cinnamycin and duramycin; development of new techniques for peptide synthesis, amino acid analysis and nonenzymatic cleavage of the peptide bond; and synthesis of partial sequences of cholecystokinin and correlation of structure and function of these peptides.

The Protein Chemistry Unit conducts research on the chemical and biological properties of gonadotropins in tissues and body fluids, with emphasis on the isolation, structure-function relationships, and assay of gonadotropins. Previous studies have demonstrated the presence of an hCG-like hormone (hCG') in the human pituitary gland, with physicochemical, immunological, and biological features typical of hCG. The pituitary gland contains the highest amount of hCG' among non-pregnant tissues, suggesting that the hCG-like hormone could be a precursor of LH in the human pituitary gland. SDS-gel electrophoresis of the hCG' preparation showed dissociation into two subunits of similar MW to the α and β subunits of placental hCG, further confirming the similarity of the pituitary and placental hormones.

A procedure for partial hydrolysis of glycosidic linkages in anhydrous HF was applied to purified pregnancy hCG, with removal of 70% of the carbohydrate groups without significant cleavage of polypeptide bonds or dissociation into

subunits. The modified hCG was cleaved mainly at the reducing ends of mannose and galactose of asparagine- and serine-linked chains, respectively. It retained immunological activity in 3 RIA systems, and showed increased receptor-binding activity in rat homogenates, but was a poor stimulant of testosterone production *in vitro* or uterine weight *in vivo*. Although the modified hCG did not block hLH and hCG effects on the rat uterus *in vivo*, this could be due to the very short plasma half-life of the derivative as compared to intact hCG. Further studies on the *in vitro* activities of the deglycosylated hormone are in progress to evaluate its potency as an antagonist of LH and hCG. Continuing studies on the RIA of urinary hCG employing an antiserum (H93) to the hCG β-subunit carboxyterminal peptide showed increased levels in 5 of 15 male choriocarcinoma patients whose serum hCG levels were considered normal, and who proved to have tumor recurrence. Urinary hCG levels were elevated in all of 95 female patients with trophoblastic disease, and will be correlated with their serum hCG concentrations.

The Metabolism Unit studies the regulation and hormonal control of glycogen metabolism in normal and diabetic tissues, and the mechanisms by which protein kinase and phosphoprotein phosphatase regulate the activity of glycogen synthase. In recent studies, a second cAMP-independent casein kinase from rabbit skeletal muscle has been purified to homogeneity. This enzyme consists of two different subunits having molecular weights of 42,000 and 27,000, and phosphorylation of glycogen synthase by this kinase results in the incorporation of ^{32}P into two major tryptic peptides. The sites in glycogen synthase phosphorylated by this kinase are different from those phosphorylated by cAMP-dependent protein kinase, phosphorylase kinase, and cAMP-independent casein kinase-1. Based on the structural similarities between cAMP-independent kinases which regulate glycogen synthase activity and those kinases which regulate protein synthesis, the cAMP-independent casein kinases are probably multifunctional enzymes. The cAMP-independent casein kinase-1, which was found to phosphorylate and inactivate glycogen synthase, has been shown to phosphorylate and activate phosphorylase kinase. This phosphorylation results in the incorporation of phosphate into a subunit of phosphorylase kinase. The finding that the activity of phosphorylase kinase can be regulated by cAMP-dependent and -independent protein kinases will provide further insight into the hormonal regulation of phosphorylase kinase. Phosphorylase kinase from rabbit skeletal muscle was found to be activated by Mg^{2+} in a manner synergistic with that caused by calmodulin and Ca^{2+}. This regulatory mechanism can provide a means of activation of phosphorylase kinase without covalent modification as previously observed under electrical stimulation. Liver glycogen synthase from normal and diabetic rats was extensively purified, and the enzyme from diabetic animals, unlike that from normal animals, could not be activated by protein phosphatase. The glycogen synthases from these two sources also differ in their molecular weights and kinetic properties. These results indicate that a less active form of glycogen synthase is present in diabetic rats, and could provide an explanation for the presence of levels of liver glycogen in diabetic animals.

The Section on Hormonal Regulation 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, lactogens, angiotensin, and gonadotropin-releasing hormone (GnRH).

Current studies on the characteristics of gonadotropin receptors in the rat testis revealed marked differences in the binding affinities of the LH/hCG receptors for various luteotropins. Analysis of the interactions of hCG, hLH and oLH with testis receptors showed that each hormone bound to a common set of receptors with similar binding capacity. Although the association rate constants of the three hormones were similar, dissociation rates were much slower for hCG and hLH than for oLH. The high binding affinity and slow dissociation of hCG contribute to its high bioactivity and its tendency to occupy and stimulate LH receptors for prolonged periods. The hCG:receptor complexes of the rat testis were also found to undergo a process of progressively decreasing reversibility, which was temperature-dependent and led to increasing resistance to washing and dissociation by chaotropic agents. This process may be important in determining the life of the hormone:receptor complexes and the extent to which they are subjected to internalization and degradation in the Leydig cell.

Regulation of gonadotropin-receptor sites was studied in testicular and ovarian target cells of immature and adult rats. The role of prolactin in maintaining LH receptors was examined in adult rats during metoclopramide infusion to elevate serum PRL and treatment with bromergocryptine to suppress PRL secretion. In adult rats, injection of oLH caused an initial rise in both LH and PRL receptors, followed by a transient loss of PRL sites and a more protracted decrease in LH receptors. Metoclopramide infusion increased basal LH receptors, but did not alter the loss of sites caused by gonadotropin treatment, indicating that elevation of prolactin levels does not influence the short-term aspects of LH receptor regulation. The early transient increase in testis PRL receptors after LH treatment and adrenal PRL sites after ACTH was mimicked by treatment with 8-bromo cyclic AMP, and was blocked by aminoglutethimide, indicating the dependence of the early up-regulation process on steroid secretion. In contrast, 8-bromo cyclic AMP treatment was not followed by the later loss of PRL receptors caused by LH and ACTH, suggesting that hormone-induced depletion of PRL receptors is attributable to processing at the membrane level rather than to cyclic AMP-mediated actions within the target cells. The positive regulation of testicular LH receptors after oLH treatment was also shown to be associated with the phase of steroid secretion, and to be blocked by inhibitors of steroidogenesis (aminoglutethimide), and microfilament function (cytocholasin B). Suppression of PRL secretion by bromocryptine delayed the recovery of LH receptors and steroid responses in testes of GnRH agonist-treated adult rats, but had no effect on PRL receptors. In developing rats, bromocryptine reduced the normal rise in testicular LH receptors, but had no effects on pubertal development and androgen secretion. Thus, testicular maturation proceeds normally despite the presence of marked hypoprolactinemia and moderately decreased LH receptors throughout development, and testicular prolactin receptors appear to be largely independent of the circulating PRL concentration.

Regulation of ovarian receptors and responses by FSH and GnRH was studied in cultured rat granulosa cells. GnRH agonists were shown to block the rises in cyclic AMP and cyclic GMP induced by FSH treatment, suggesting that the inhibitory effects of GnRH in ovarian function are caused by impairment of cyclic nucleotide production. The actions of FSH on cultured granulosa cells were shown to include prominent morphological changes, with aggregation into multicellular clusters and formation of gap junctions and microvilli, as well as the expression of LH and PRL receptors and progesterone secretion. These structural and biochemical effects of FSH were completely blocked by GnRH

agonists, demonstrating the ability of the releasing peptide to inhibit all aspects of FSH-induced granulosa cell differentiation.

Studies on the ontogeny of pituitary-gonadal function were performed to define the profile of GnRH receptor development in the pituitary gland, and to examine the regulation of LH receptors and steroidogenesis in the neonatal rat testis. In male and female rats, total pituitary GnRH receptors increased progressively with age, with early peaks in tissue concentration concomitant with elevated serum gonadotropin levels during neonatal development. The correlations between pituitary GnRH receptors and LH content, serum gonadotropins, and gonadal steroids, are consistent with the physiological role of GnRH receptors in regulating the activity of the pituitary-gonadal axis during development and sexual maturation. When testicular LH receptors were studied in immature rats, receptor-binding affinity and content per Leydig cell were similar to values observed in the adult. However, the testosterone responses of neonatal Leydig cells were about 4-fold more sensitive than those of adult cells to hCG, and low-dose hCG treatment caused an increase in LH receptors that did not occur in the adult. High-dose hCG treatment increased Leydig cell numbers and testosterone responses to gonadotropin, and caused transient receptor loss due to occupancy by the injected hCG. In contrast, adults showed the usual marked and prolonged loss of LH sites and desensitization of of steroid responses. There is a marked difference in the response of the fetal-neonatal and adult populations of Leydig cells to hormonal stimulation, the former showing mainly trophic changes, and the latter displaying prominent receptor loss and impaired steroid responses after the initial stimulation by hCG.

In a related area, studies were performed on the mechanisms by which gonadotropin-releasing hormone (GnRH) controls of secretion LH and FSH by anterior pituitary cells. This includes characterization and analysis of the GnRH receptors in anterior pituitary cells and membranes, and the roles of calcium, cyclic nucelotides and other intracellular regulators in the mechanism of action of GnRH. Receptors for GnRH have been shown to be regulated by the releasing hormone, which causes an increase in its own pituitary binding sites, and by gonadal steroid hormones. The mechanism by which GnRH stimulates gonadotropin release has been shown to be unrelated to cyclic nucleotides, but is highly dependent on calcium, and current studies suggest that phospholipid metabolites, especially arachidonic acid and its derivatives, may have an important function as mediators of GnRH action. Also, the fate of the GnRH receptor complexes was studied by video intensification microscopy after binding of a fluorescent GnRH analog to cultured pituitary cells. The initially diffuse distribution of the bound peptide was followed by the formation of polar aggregates that were subsequently internalized, indicating that GnRH receptors undergo redistribution during stimulation of LH release, followed by endocytosis and subsequent degradation and/or intracellular actions.

Previous studies on the actions and metabolism of angiotensin II were extended by the demonstration that circulating angiotensin II remains detectable in rat plasma for up to two days, due to tissue generation of the octapeptide. The rise in adrenal angiotensin II receptors that occurs after nephrectomy cannot be attributed to the withdrawal of circulating AII, but is more likely to result from the marked hyperkalemia that develops in nephrectomized rats. The basis for the decreased pressor activity of des-Asp^1-AII was analyzed by measurementof its binding affinity for smooth muscle receptors, and of its local and systemic degradation. Reduced receptor binding

affinity for muscle receptors was observed in rat, though not in dog and rabbit, but in all cases, the heptapeptide was degraded more rapidly than AII by muscle membranes, and was cleared more rapidly from the circulation. These findings have shown that increased metabolism is an important determinant of the weaker pressor activity of the heptapeptide. The mechanism by which prolonged ACTH treatment inhibits aldosterone production was studied in the rat, and was found to be associated with a decrease in AII receptors and impaired 18-hydroxylase activity in the zona glomerulosa. These changes are secondary to suppression of the renin-angiotensin system by adrenal steroids produced during protracted administration of ACTH. In normal rats, the action of angiotensin II on aldosterone secretion was further shown to be highly calcium-dependent and to be mimicked by increases in cytosolic calcium during treatment with the calcium ionophore A-23187. Increases in phospholipid metabolism were also observed in angiotensin-stimulated glomerulosa cells, with consistent changes in the turnover of phosphatidic acid and phosphatidylinositol.

The actions of angiotensin II on aldosterone production in adrenal glomerulosa cells was found to be specifically inhibited by somatostatin. This effect of somatostatin is mediated by high-affinity receptors for the tetradecapeptide that are highly concentrated in the zona glomerulosa. The presence of immunoreactive somatostatin in the adrenal glomerulosa zone indicates that local production of the peptide could act as a regulator of the adrenal response to angiotensin II.

Vascular receptors for angiotensin II were analyzed in the rat mesenteric artery during altered sodium balance, and found to be decreased by salt restriction and increased by salt loading. These changes are the reciprocal of those which occur in the adrenal gland, and contribute to the altered pressor responses during changes in sodium balance, and to the reduced vascular reactivity associated with high blood angiotensin II concentration. Brain receptors for AII were also analyzed and found to be present in several regions, including OVLT, hypothalamus, subfornical organ, area postrema, and median eminence. Increases in AII receptors during dehydration were observed in OVLT and subfornical organ, and will be evaluated in relationship to drinking responses during changes in water balance.

The Section on Molecular Endocrinology investigates the molecular basis of peptide hormone action, with particular emphasis on the characterization of gonadotropin receptors, activation of steroid biosynthesis in gonads and adrenal, and analysis of the biological activity of circulating gonadotropins. In further studies of receptor-cyclase interaction, gonadotropin-induced binding of Gpp(NH)p (a non-metabolizable analog of GTP) in Leydig cell membrane showed dose correlation with activation of adenylate cyclase, consistent with the role of GTP binding in controlling the activity of the catalytic enzyme subunit during hormone stimulation. The amount of Gpp(NH)p bound to Leydig cell membranes was considerably greater than the number of LH/hCG binding sites (50 pmol/mg of protein vs. 0.8 pmol/mg of protein), suggesting that interaction of each hormone receptor with LH/hCG could activate a large number of nucelotide regulatory units. The occurrence of such an amplification factor between hormone-receptor complex formation and nucleotide binding is relevant to recent observations on the existence of oligomers of the receptor-nucleotide protein that could allow maximal production of the activating nucleotide-GTP complex with minimal occupancy of hormone receptors. The

Leydig cell membrane could possess a more highly developed oligomeric receptor-nucleotide protein complex than that attributed to the liver, adipocyte, or erythrocyte adenylate cyclase systems.

Luteinized rat ovaries are a rich source of LH receptors, and were analyzed for lactogenic binding sites as a potential source of receptors for purification and for investigation of receptor assembly and turnover. Parallel studies were carried out in lactating rabbit mammary preparations for comparison of binding capacities in the two target tissues. These studies have demonstrated that luteinized rat ovaries are a richer source of lactogenic receptors than lactating rabbit mammary gland (4-fold per mg protein) and are thus of value for receptor purification and related studies. The 7- to 8-fold increase in binding capacity seen with detergent treatment in both of these tissues may represent nascent receptors unavailable for hormone binding.

Studies on the role of cyclic nucleotides in human sources have demonstrated that human seminal plasma yields considerably higher values for cyclic nucleotides when compared with spermatozoa. These values vary markedly both between different individuals and in specimens from the same individual at different times. Cyclic AMP levels were unrelated to those of fructose (from the seminal vesicles) or zinc and citric acid (from the prostate). Washed sperm suspensions contained 13 to 27 pmol cyclic AMP/100 million spermatozoa. Adenylate cyclase activity of human semen was confined to the spermatozoa; it was several times higher in the presence of Mn^{2+} than Mg^{2+} and was unresponsive to sodium fluoride. The latter findings are consistent with enzyme activity derived from a dissociated catalytic component of adenylate cyclase.

The phosphorylation of Leydig cell substrates by cAMP-dependent protein kinase during hormone action was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Stimulation of isolated Leydig cells with hCG for 10 min caused dose-related increases in phosphorylation in ten bands of Mr 22,00-90,000 with ED_{50} for hCG ranging 0.10-0.3 pM. Incubation of Leydig cells with dibutyryl cyclic AMP caused phosphorylation of bands identical with those stimulated by hCG. Further resolution of hCG-induced phosphorylation products by two-dimensional gel electrophoresis revealed ten discrete components with M_r range 22,000 to 105,000 and pIs 5.4 to 6.6, seven of which corresponded with bands that were increased during hormone action or nucleotide stimulation in the one-dimensional system. These studies have demonstrated that both hCG and dibutyryl cyclic AMP induce phosphorylation of multiple proteins in the Leydig cell. These events were correlated with hCG-stimulated increases in cyclic AMP and testosterone production, indicating that the steroidogenic response to LH receptor occupancy is mediated by activation of the cyclic AMP/protein kinase/phosphorylation sequence during hormonal stimulation.

The mechanisms responsible for gonadotropin-induced testicular desensitization were analyzed by direct assay of androgen biosynthetic enzymes and cytochrome P-450 in microsomes and cytosol from testes of adult male rats. Animals were treated with hCG (2 and 10 μg) to induce a post-stimulation decrease in testosterone production, previously shown to result from reduced conversion of progesterone to androgen. The larger dose of hCG also caused an 'early' biosynthetic defect due to impaired formation of pregnenolone from endogenous precursors. Dose-dependent inhibition of both 17 α-hydroxylase and 17,-20-desmolase activities was observed after treatment with hCG with no change in 3 β-hydroxysteroid dehydrogenase and 17 β-hydroxysteroid dehydrogenase. The

reductions in microsomal enzyme activity were accompanied by a comparable decrease in cytochrome P-450 levels. Treatment of hypophysectomized rats with 17β-estradiol (0.1-20 μg) reducted testosterone responses to hCG in a dose-dependent manner (by 20 to 60%), and caused further decreases of microsomal enzymatic activities and of the levels of cytochrome P-450, but not of 3β-hydroxysteroid dehydrogenase. The similarity of estrogen-dependent lesions to those produced by hCG treatment further indicates the involvement of endogenous estrogen in the development of microsomal enzymatic lesions in gonadotropin-induced desensitization of testicular and androgen production. The decreased pregnenolone synthesis in response to hCG stimulation in cells with the early biosynthetic defect was partly restored by addition of lipoproteins, either HDL or LDL, to the incubation medium. A decrease in HMG CoA reductase activity observed 24 to 72 hrs after hCG treatment, and the restoration of steroid responses to in vitro hormone stimulation by addition of lipoproteins, have localized a site for the early biosynthetic lesion observed during desensitization by gonadotropin. The control of 3-hydroxy, 3methylglutaryl coenzyme A reductase (HMG CoA reductase) in testicular Leydig cells was studied during cholesterol depletion in 4-aminopyrazolopyrimidine (4-APP) treated rats. These studies have demonstrated that testicular HMG CoA reductase, in contrast to the adrenal enzyme, is not regulated by changes in circulating cholesterol. The ability of Leydig cells from 4-APP-treated animals to continue androgen biosynthesis in vitro indicated that these cells possess an active steroidogenic pathway from precursors prior to cholesterol. The marked fall in circulating testosterone in 4-APP-treated animals is attributable to decreased LH secereton, rather than to a change in HMG CoA reductase activity.

The effects of gonadal steroids on LH bioactivity was analyzed in female rats with 4-day estrous cycles, by radioimmunoassay and RICT assay of serum and pituitary LH throughout the estrous cycle. A marked increase in bio- and immunoactive serum LH occurred at 20 hr of proestrous; serum LH bioactivity rose higher than immunoactive LH levels at the time of the LH surge, with a consequent increase in B:I ratio. The rise in serum estrogen preceded the peak levels of LH. After gonadectomy, a peak of serum LH occurred at the same time as in controls, but no increase of LH bioactivity occurred at the time of the LH peak. These studies indicate that the acute rise in estrogen could be responsible for the release of LH with high bioactivity from the pituitary into the circulation at the time of the surge, since castration at a time prior to the maximal increase in estrogen prevented the preovulatory rise in the bio:immuno ratio. In studies performed to define changes in the properties of pituitary LH in castrated male rats, isoelectrofocusing of pituitary extracts showed consistent profiles with six discrete peaks of LH. After electrofocusing, the radioimmunoassay pattern of control pituitaries showed two main LH peaks of almost equal magnitude and four minor peaks. Assay of the individual peaks for biological activity showed that the B:I ratio of LH becomes progressively higher with increasing isoelectric point, and that orchidectomy decreases the B:I ratio of all LH components by 100%. These decreases are maximal at 45 days and return to near-control values at 60 days. This homogenous decrease in bio:immuno ratio in all peaks could indicate that the carbohydrates which are concerned with the charge of the molecule do not contribute to the biological activity of LH. In human studies, assays of bio- and immuno-activities of serum LH were performed in samples taken at 20-minute intervals in men, cycling women, and post-menopausal women. Initial results in men and post-menopausal women indicate that bioactive LH

shows pulsatile release coincident with radioimmunoassay values, but of 2 to 4-fold greater magnitude, with a consequent increase in B:I ratio. In contrast, only minor pulsatile release of either bioactive or immuno-active LH was detected during the follicular and luteal phases of the menstrual cycle. The pulsatile release of LH observed in many human and animal studies probably reflects coordinated granule release from pituitary gonadotrophs, and could serve to minimize end-organ desensitization that could be produced by exposure to constantly elevated levels of the trophic hormone.

The Adrenal Research Unit investigates the physiology and regulation of adrenal steroidogenesis, by characterization of cellular steroid-binding proteins, and soluble factors which mediate steroidogenic responses to ACTH, and analysis of cellular mechanisms of cholesterol utilization in steroid biosynthesis. Current areas of research involve the identification, characterization, and physiology of noncatalytic proteins (membrane bound and/or soluble) of the adrenal cortex that interact with specific steroid ligands; examination of the role of microtubules and contractile proteins in steroid synthesis; identification, characterization and physiology of adreno-cortical secretory proteins; and examination of soluble stimulatory and inhibitory factors. These studies have led to the identification of six specific binding proteins for cholesterol/cholesterol sulfate, 20 α-hydroxycholesterol, pregnenolone, pregnenolone sulfate, and 21-hydroxypregnenolone. Research is also continuing in the role of cytoskeletal elements in steroid secretion, and the development of assays for circulating ACTH. Clinical studies include the evaluation of adrenocortical hyperfunction, petrosal sinus catheterization for diagnosis of pituitary tumors, and the response of chromophobe to irradiation therapy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00021-05 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Bioassayable and Radioimmunoassayable ACTH

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
	Kevin Catt	Chief	ERRB, NICHD

COOPERATING UNITS (if any)
None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25		0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two independent methods of characterizing and quantitating ACTH from clinical samples are being developed, a classical radioimmunoassay with materials supplied by the NIAMDD anmd a bioassay, using as an endpoint the corticosterone production of isolated rat adrenal fasciculata cells. Both assays have been found to be sensitive enough for human use and to have specificity which is suitable. An extraction procedure is being developed and is the final step in completing both assays.

In addition gel chromatography and ion exchange chromatography are being used to characterize the ACTH. Also, the columns are being used to prepare biologically active ^{125}I-labelled ACTH for receptor studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00022-08 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Renin-Angiotensin System and Aldosterone Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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4.0	3.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to investigate physiological and pathological aspects of the renin-angiotensin system, with emphasis on the regulation of aldosterone secretion by the zona glomerulosa of the adrenal gland. Specific areas of research include the characterization and regulation of angiotensin II receptors, the actions of angiotensin II and other regulators of aldosterone secretion, and the production and metabolism of angiotensin II. Enzyme-dispersed adrenal cells are employed for analysis of the relations between angiotensin II receptors and aldosterone production, and the interactions between angiotensin II and other factors such as ACTH, sodium, and potassium. The effects of altered electrolyte balance on angiotensin II receptors and adrenal steroid responses are studied, in particular the effects of sodium intake on the concentration of angiotensin II receptors in adrenal and smooth muscle cells. These studies are designed to clarify the control of adrenal and vascular sensitivity to angiotensin II during altered sodium balance, the mechanisms by which angiotensin II regulates the concentration of its receptors in adrenal and smooth muscle, and the activation pathways that are initiated by occupancy of angiotensin II receptors.

Objectives:

This project aims to clarify the role of the renin-angiotensin system in the regulation of aldosterone secretion and blood pressure in physiological and pathological conditions. This includes characterization of the properties and regulation of angiotensin II receptors, and their role in the activation of adrenal steroidogenesis; elucidation of the mechanisms of action of angiotensin II and other regulators of aldosterone secretion in the zona glomerulosa; comparison of the angiotensin II receptors of adrenal and smooth muscle, and their regulation by electrolyte changes and angiotensin II; and evaluation of the renin-angiotensin system and aldosterone secretion in certain forms of clinical and experimental hypertension.

Methods Employed:

Radioimmunoassays of renin activity, angiotensin II, somatostatin, aldosterone, corticosterone, cortisol, and cyclic nucleotides. Binding studies with ^{125}I- and ^{3}H-angiotensin II in adrenal cells and membranes, and uterine, vascular, and brain membranes; binding studies with ^{125}I-somatostatin analogs in adrenal and pituitary membranes; enzymatic dispersion of adrenal glomerulosa and fasciculata cells. In vivo and in vitro stimulation of aldosterone responses in rat and dog adrenal cells. Measurement of agonist and antagonist activities of angiotensin II and somatostatin analogues upon receptor binding and aldosterone production in vitro.

Major Findings:

1. Angiotensin II and adrenal receptors after nephrectomy

It is now generally accepted that mineralocorticoid secretion is predominantly controlled by angiotensin II, which exerts trophic actions on the adrenal glomerulosa and acute regulatory effects on aldosterone biosynthesis. The trophic actions of angiotensin II include stimulation of angiotensin II receptors and enzymes of the aldosterone biosynthetic pathway, with corresponding enhancement of the aldosterone secretory capacity of the adrenal gland. We have previously shown that the positive regulatory action of angiotensin II on its adrenal receptors occurs with elevations of the circulating peptide concentration within the physiological range, and probably contributes to the increased sensitivity of the adrenal during sodium deficiency. In this action, angiotensin II differs from most other peptide hormones, which frequently decrease their homologous target-cell receptors. However, the adrenal angiotensin II receptors increase after nephrectomy, and this has been interpreted as evidence for a tonic down-regulating effect of angiotensin II on its adrenal receptors. To clarify these conflicting views, we evaluated the effects of nephrectomy on adrenal angiotensin II receptors in relation to blood angiotensin II and plasma electrolyte levels. This study revealed that hyperkalaemia contributes markedly to the post-nephrectomy increase in adrenal angiotensin II receptors, and that circulating angiotensin II levels persist for an unexpectedly long period after nephrectomy, presumably due to tissue generation of the octapeptide.

2. Mechanisms of inhibition of aldosterone secretion by adrenocorticotropin.

The mechanisms by which prolonged administration of ACTH causes a decrease in aldosterone secretion were studied in the rat. After 6 days of treatment with ACTH, blood corticosterone was elevated and plasma aldosterone was decreased in rats maintained on either a normal or low sodium diet. Plasma renin activity (PRA) was also decreased, probably secondary to increased sodium and/or fluid retention. In collagenase-dispersed glomerulosa cells from adrenals of ACTH-treated rats, angiotensin II receptors were markedly decreased, as were the in vitro aldosterone responses of angiotensin II, ACTH, 8-bromo-cAMP, and potassium. However, the production of deoxycorticosterone and precursor steroids was increased, indicating the presence of a block in the late aldosterone biosynthetic pathway. Measurement of the activity of biosynthetic enzymes of the steroidogenic pathway in isolated mitochondria revealed an 80% increase in side-chain cleavage enzyme in both glomerulosa and fasciculata mitochondria from ACTH-treated rats. Although ACTH injection also increased 11-hydroxylase activity in the fasciculata zone, this enzyme was reduced by 50% in capsular mitochondria. The 18-hydroxylase activity in adrenal capsular mitochondria was markedly decreased by ACTH treatment in both normal and sodium-restricted animals. The importance of ACTH-induced steroidogenesis in the development of altered glomerulosa cell function was indicated by the ability of aminoglutethimide to prevent the inhibitory effects of ACTH on angiotensin II receptors and PRA. It is likely that the observed inhibition of the renin-angiotensin system is responsible for the decrease in angiotensin II receptors and 18-hydroxylase, since both are highly dependent on the trophic effect of angiotensin II. The specific lesions produced in adrenal glomerulosa cells by long-term ACTH treatment include decreased levels of angiotensin II receptors, 11-hydroxylase, and 18-hydroxylase. These changes are secondary to the suppression of renin-angiotensin activity and are responsible for the impaired aldosterone secretion that results from prolonged treatment with ACTH.

3. Photoaffinity labeling and solubilization of angiotensin II receptors.

The physical characteristics of the receptors for angiotensin II in dog adrenal cortex and uterus were determined after affinity labeling. ^{125}I-2-nitro-5-azidobenzoyl-angiotensin II, a photosensitive angiotensin II analogue which retained aldosterone-stimulating activity, was used to couple the octapeptide specifically and irreversibly to its membrane receptors. After solubilization with Triton X-100, the covalent hormone: receptor complex was analyzed by gel filtration and sucrose density gradient centrifugation. Two radioactive species were consistently observed, with calculated M_r values of 126,000 ± 10,000 and 64,500 ± 11,000. The elution profiles of solubilized adrenal and uterine particles were almost identical. When the solubilized complexes were subjected to sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis, a single radio-active band was detected upon autoradiography, with M_r = 65,000 ± 6,000 for adrenal cortex and 68,000 ± 7,000 for myometrium. These results indicate that the receptors for angiotensin II in adrenal cortex and uterus are composed of two subunits of similar molecular weight, and that the common functional properties of the receptors from both tissues are probably related to their similar physicochemical characteristics.

4. Regulation of vascular angiotensin II receptors by sodium intake.

Changes in sodium intake exert well-defined and opposite effects on adrenal and vascular responsiveness to angiotensin II (AII). Whereas the adrenal glomerulosa zone becomes more sensitive to AII during sodium restriction, vascular sensitivity to AII is decreased during sodium restriction and increased by sodium loading. The extent to which regulation of smooth muscle AII receptors is involved in such altered vascular responsiveness was examined by assay of ^{125}I-AII binding in the mesenteric artery and urinary bladder of rats during low and high sodium intake. The AII receptors of vascular smooth muscle were found to be similar to those of the mesenteric artery in terms of their binding properties and regulation by altered sodium intake. During sodium restriction, blood AII was elevated and AII receptor concentration was significantly decreased (by 40%) in both tissues. Conversely, sodium loading was accompanied by decreased blood AII and an increase in smooth muscle AII receptors. The changes in AII binding during sodium restriction were not attributable to occupancy of receptors by endogenous AII, and no effect on receptor affinity was observed at either extreme of sodium intake. Elevation of the circulating AII concentration within the physiological range by infusion of the octapeptide for 2-4 days decreased AII receptor concentration in urinary bladder particles. These findings demonstrate that smooth muscle AII receptors are regulated during altered sodium intake, at least partially via changes in the circulating AII concentration, in a manner reciprocal to the adrenal glomerulosa receptors. Such modulation of vascular AII receptors by the renin-angiotensin system could be responsible for the altered pressor responses that accompany changes in sodium balance, and for the reduced vascular reactivity that occurs in patients with high levels of circulating AII.

5. Modulation of aldosterone secretion by somatostatin.

The octapeptide angiotensin II is a major regulator of the adrenal glomerulosa zone, acting both as an acute stimulus of aldosterone secretion and as a trophic hormone which increases steroidogenic enzymes and angiotensin II receptors in glomerulosa cells. Angiotensin II also mediates the adrenal effects of altered sodium balance, and is essential for the aldosterone response to sodium restriction. However, the adrenal effects of angiotensin II are often attenuated during sodium loading, suggesting that other local or humoral factors modulate its actions on adrenal glomerulosa function. Somatostatin, the somatotropin release inhibiting factor of the hypothalamus, has been shown to inhibit the secretion and action of several pituitary and non-pituitary hormones. Because somatostatin has been found in several non-neural tissues, and seems to act as a local regulator of endocrine function, we examined the possibility that it may also modulate the effects of angiotensin II in the adrenal glomerulosa cell. These studies have shown that low concentrations of somatostatin specifically inhibit angiotensin II-stimulated production of aldosterone, and that this action is mediated by specific, high-affinity receptors for somatostatin in the zona glomerulosa.

6. Role of calcium and phospholipids in aldosterone secretion.

Previous results have demonstrated that angiotensin II and potassium regulate aldosterone production in the adrenal via a cyclic nucleotide-independent mechanism which requires calcium. Our earlier studies utitizing calcium uptake inhibitors (lanthanum and verapamil) indicated that increases in intracellular calcium were important in the stimulation of aldosterone production. To examine the effect of increased cytosolic calcium on aldosterone production, we analyzed the effects of the calcium ionophore A-23187 on aldosterone synthesis in isolated rat adrenal glomerulosa cells. Concentrations from 0.1 to 1.0 μM A-23187 increased aldosterone production in a dose-dependent manner; this effect of A-23187 was dependent on the extracellular calcium concentration, and was not accompanied by detectable increases in cyclic AMP production. In contrast to the stimulation of steroidogenesis elicited by A-23187 alone, the ionophore partially inhibited the aldosterone responses to angiotensin II and potassium. In angiotensin II-stimulated cells, 0.2 μM A-23187 caused half-maximal inhibition of aldosterone production and 2 μM A-23187 inhibited aldosterone production by about 70%. The ionophore did not affect the binding of angiotensin II to isolated glomerulosa cells, and did not change the sensitivity of the cells to either angiotensin II or potassium. However, A-23187 reduced the total amount of steroid produced in response to these stimuli, most markedly when the extracellular calcium concentration was less than 0.4 μM. These results indicate that although A-23187 partially inhibits the steroidogenic responses to angiotensin II and potassium, increases in cytosolic calcium caused by submicromolar ionophore concentrations result in stimulation of aldosterone production by adrenal zona glomerulosa cells.

It has been suggested that phospholipid metabolism may play an essential role in mediating the action of hormones which mobilize calcium within the cell. In the adrenal, it has been shown that ACTH increases phospholipid synthesis in the zona fasciculata by a cyclic-AMP dependent mechanism. Angiotensin II and potassium have also been reported to stimulate the synthesis of phosphatidic acid, phosphatidyl inositol, and polyphosphoinositides in adrenal capsular tissue. To investigate the role of phospholipids in the actions of angiotensin II and potassium, we employed isotopic labeling of isolated adrenal glomerulosa cells. Angiotensin II was found to stimulate the turnover and synthesis of both phosphatidic acid and phosphatidylinositol, while physiological concentrations of potassium mainly stimulated the turnover of phosphatidylinositol. It will next be determined if the stimulation of phospholipid metabolism requires calcium, and whether a particular phospholipid class is responsible for the increased mitochondrial pregnenolone synthesis which results in increased aldosterone production.

7. Receptor binding, metabolism, and pressor effects of angiotensin II and des-Asp^1-angiotensin II.

The heptapeptide, des-Asp^1-angiotensin II, is present in the circulation and is biologically active in terms of stimulation of aldosterone production and pressor responses. However, the heptapeptide displays only 10 to 30% of the pressor activity of angiotensin II. The reduced activity of the hepta-

peptide was evaluated in relation to its binding affinity for smooth muscle receptors, local degradation, and metabolic clearance rate, in rat, dog and rabbit. In the rat, the heptapeptide showed reduced affinity for smooth muscle receptors, whereas in the dog and rabbit, angiotensin II and des-Asp^1-angiotensin II had identical binding properties. Des-Asp^1-angiotensin II was degraded more rapidly than angiotensin II by smooth muscle membranes. Such preferential degradation was more marked in the rat, and contributes to the lower binding affinity of the heptapeptide in this species. A common feature in all three species studied was the faster clearance of des-Asp^1-angiotensin II from the circulation, indicating that increased metabolism is an important determinant of the weaker pressor activity of the heptapeptide.

8. Angiotensin II binding in the brain.

In addition to the peripheral actions of angiotensin II on the adrenal glomerulosa zone and smooth muscle, the peptide has well-defined actions in the central nervous system, including increased drinking behavior and elevation of blood pressure. The study of angiotensin II receptors in the brain provides a system to investigate the central actions of the peptide. Specific and high affinity receptors for angiotensin II, with similar characteristics of those in the adrenal gland and smooth muscle, were found in several areas of the brain, including OVLT, periventricular area, hypothalamus, subfornical organ, area postrema, and median eminence. The increased drinking response after 24 hrs of dehydration in rats was accompanied by increases in angiotensin II receptors in OVLT and subfornical organ, without changes in receptors in other zones of the brain. Studies to determine the mechanism of such local receptor regulation are currently in progress.

Significance:

These studies have provided further information about the humoral control of aldosterone secretion, and the role of the renin-angiotensin system and angiotensin receptors in the regulation of adrenal and smooth muscle function. The characterization of solubilized AII receptors will permit more detailed analysis of receptor stucture and function, and the finding that somatostatin modulates aldosterone secretion reveals a new aspect of the regulation of adrenal function.

Proposed Course:

Further studies will be performed on cellular mechanisms of action of angiotensin II, and the steroid biosynthetic changes induced by stimuli of aldosterone secretion. The relations between changing sodium balance and angiotensin II receptors will be further analyzed in adrenal, vascular smooth muscle, and brain. The contribution of both systemic and locally-formed angiotensin II to circulating angiotensin levels and adrenal regulation will be examined in more detail. Further characterization of angiotensin II receptors will be performed, with attempts to purify the sites and to raise antibodies against the receptor protein. The role of calcium and phospholipid metabolites in aldosterone responses will be pursued by

analysis of calcium fluxes, and of the calcium-dependence of altered phospholipid metabolism during hormone action. It will also be determined whether particular types of phospholipids are involved in the stimulation of pregnenolone synthesis, and ultimately of aldosterone production, in hormone-stimulated glomerulosa cells. These studies will contribute to the definition of the series of biochemical steps that are initiated by hormone-receptor interaction and which lead to altered cellular function and aldosterone secretion.

Publications

Aguilera, G., Cappponi, A., and Catt, K.J.: Regulation and Properties of Adrenal and Vascular AII Receptors. In Laragh, J.H., Buhler, F.R., and Seldin, D.W. (Eds.): Frontiers in Hypertension Research. New York, Springer-Verlag, 1981, pp. 598-601.

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Capponi, A.M., Aguilera, G., Fakunding, J.L., and Catt, K.J.: Angiotensin II: Receptors and mechanisms of action. In R.L. Soffer (Ed.): Biochemical Regulation of Blood Pressure, New York, John Wiley & Sons, 1981, pp. 205-262.

Capponi, A.M., and Catt, K.J.: Solubilization and characterization of adrenal and uterine angiotensin II receptors after photoaffinity labeling. J. Biol. Chem., 255: 12081-12086, 1980.

Fakunding, J.L., and Catt, K.J.: Dependence of aldosterone stimulation in adrenal glomerulosa cells on calcium uptake: Effects of Lanthanum and Verapamil. Endocrinology, 107: 1345-1353, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00035-09 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Structure and Function of Biologically Active Molecules

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are directed at the analysis and synthesis of molecular assemblies with rare amino acids, for instance (a)synthesis of precursors of rings A and B of nisin-a peptide with α,β-unsaturated and thioether amino acids-and the synthesis of these two cyclic structures; (b)actions of peptides based on nisin and/or linear gramicidins on neonatal and neoplastic tissue; (c)synthesis of analogs of the gramicidins A,B, and C, and correlation of their structures with ion-transport across lipid bilayers; (d)synthesis of peptides with agonistic and antagonistic effects on fertility regulating hormones, including large-scale preparation of luteinizing hormone releasing factor (LRF) for clinical purposes; (e)exploration of dehydroalanine resins for synthesis of peptide amides; (f)synthesis of analogs of opioid peptides with agonist and/or antagonist properties; (g)synthesis of analogs of the pentapeptide L-Phe-D-Leu-L-Phe-D-Leu-L-Phe with chemotactic and anti-chemotactic characteristics; (h)structural investigation of the heterodetic pentacyclic peptides CINNAMYCIN and DURAMYCIN: (i)development of new techniques for peptide synthesis, amino acid analysis and nonenzymatic cleavage of the peptide bond; (j)synthesis of partial sequences of cholecystokinin and the correlation of structure and function of these peptides.

Project Description

Objectives:

Structural elucidation, chemical modification, and partial and/or total synthesis of biologically important peptides which: (a) incorporate unique structural features brought about by the presence of α,β-unsaturated and thioether amino acids; (b) represent portions of the sequence of hormones of the reproductive endocrine system and are useful for immunological investigations and applications; (c) display hormone releasing or hormone release inhibiting properties; (d) affect neonatal and neoplastic tissue; (e) show ionophoric characteristics and mediate the transport of ions across lipid bilayers and biological membranes; (f) are chemotactic or antichemotactic for neutrophils; (g) are structural variants of the naturally found amino acid assemblies with opiate like activities. Chemical, physical, and biological studies with membrane active peptides. Chemical modification of amino acids and development of nonproteolytic methods for the selective cleavage of the peptide bond. Development of new approaches to the synthesis of peptides of physiological interest. Design, development, and implementation of new techniques and instrumentation in amino acid analysis and peptide synthesis. Development of amino acid analytical approaches for the detection of uncommon constitutents in physiological fluids with the aim of providing a tool for the early diagnosis of pathological states.

Methods Employed:

Highly selective and thermostable proteases (e.g. thermolysin) in combination with newly developed nonproteolytic techniques for the structural elucidation of peptides with infrequently seen amino acids. Dehydroalanine linkage of amino acids to carriers in the solid phase synthesis of peptides and peptide amides. Alkylamidated residues of dehydroalanine to incorporate ω-amide functions in endo-positions of the peptide chain. Novel-type protecting groups meeting the requirement of highly selective removal in peptide synthesis. Techniques for the synthesis of amino acids with multiple centers of chirality under the conditions of stereochemical control. Advanced techniques for the separation of closely related molecular species and their analysis, e.g. specifically developed sample application and data acquisition in amino acid analysis; counter current distribution, conventional and high pressure liquid chromatography; electrophoresis; circular dichroism; polarimetry; mass spectrometry; nuclear magnetic resonance; X-ray analysis.

Major Findings:

Peptide Synthesis and Biology: (a) Luteinizing Hormone Releasing Factor (LRF) and analogs of the same synthesized by various techniques of peptide bond formation. The highly purified products meet all applicable chemical and physical criteria. Biologically, they are fully active and ready for clinical studies aimed at the induction of ovulation and the correction of precocious puberty. The large scale synthesis of the decapeptides called for the development and application of highly refined techniques of peptide synthesis to incorporate side chain functions, such as the guanido group of arginine and terminal amides. Activation and methods of protection had to be modified for the incorporation of sensitive amino acid residues, such as tryptophan,

tyrosine, and serine. Considerable experience has been gained with the improvements made in the approaches to the synthesis of these delicate peptide structures. It will bear its fruit now that amino acids are being assembled to give molecular species that are hyperactive or display antagonistic properties. Analogs provided for biological screening and clinical exploration contain D-amino acids in positions 2,4,6, and 8, p-fluorophenylalanine in position 2, the alternating pattern of L- and D- amino acids, α,β- and γ,δ-unsaturated amino acids in position 1. (b) Analogs of the Linear Gramicidins: i. HCO $(L\text{-Ala-Gly})_7$-L-Ala-$NHCH_2CH_2OH$, a distinct departure from the amino acid compositions of the naturally occurring analogues, is a novel pentadecapeptide in which all amino acids with aromatic side chains have been replaced. The strictly observed pattern of alternating configurations of L- and D-amino acids has been broken for the first time and it has been demonstrated that it is possible to replace all D-amino acids by GLYCINE without adverse effect on the conformation of the β-helix, the prerequisite for channel formation and ion transport across lipid bilayers. The nonchiral amino acid glycine adequately and functionally substitutes for the D-amino acid residues of the naturally occurring analogs of the linear gramicidins. Present data are in support of the formation of a β-helical structure by HCO-$(L\text{-Ala-Gly})_7$-L-Ala-$NHCO_2CH_2OH$ as indicated by the detection of single channel characteristics for the molecule once embedded in the lipid bilayer. ii. HCO-$(-L\text{-Val-D-Val})_7$-L-Val-$NHCH_2CH_2OH$ and shorter chain segments of the molecule synthesized for studies on lipid bilayers and in experiments with neonatal and neoplastic tissue to probe for fetal resorption inducing and tumor growth inhibiting activity. Rationale: the naturally occurring linear gramicidins display both activities indicated, however, not without side effects presumably due to the presence of the aromatic side chains. Such groups are not present in the newly designed molecule. Other analogs of the linear gramicidins prepared and investigated: formyl-$(L\text{-tryptophyl-glycyl})$-$(L\text{-tryptophyl-D-leucyl})_6$-L-tryptophylethanolamine, formyl-$(L\text{-tryptophyl-D-leucyl})_7$-tryptophyl ethanolamide, and fragments, e.g. $L\text{-Phe-D-Leu})_2$-L-Phe, for the synthesis of formyl-$(L\text{-phenylalanyl-D-leucyl})_7$-L-phenylalanyl-ethanolamine. Valine Gramicidin A has been prepared via deformylation of natural Valine-Gramicidin A, attachment of formyl-D-methionine, removal of the methionine residue by treatment with cyanogen bromide and reformylation of the resulting product. The peptide extended by one amino acid residue gives ion-conducting channels, the reconstituted product - structurally identical with the naturally occurring molecule - matches all channel properties of Gramicidin A. iii. Continued Synthesis of Thyrotropin Releasing Hormone (TRH) - pyro-Gly-His-Pro-amide - for clinical studies.iv. Synthesis of Heterodetic Multicycle Peptides: 1. Nisin. Approaches to the synthesis of the biologically active (induced fetal resorption; inhibition of the growth of tumor cells) H_2N-terminal fragment of nisin call for the individual structures. First requirements for the synthesis of the various rings were the successful access to the unusual structural feature seen in Ring A of nisin. The α.β-unsaturated amino acid DEHYDROALANINE is incorporated into suitable peptide structures via β-elimination of the appropriate precursors properly incorporated in the correct position in the form of an O- or S-substituted derivative of serine and/or cysteine, respectively. Great difficulty is associated with the preservation of the chirality of meso-lanthionine, to be placed into Ring A, and β-methyllanthionine required for Ring B. The latter has been assembled successfully for the first time. β-Methyl-cystine, a precursor of β-methyllanthionine, displays the interesting property of turning Ninhydrin-negative upon storage. v. Synthesis of radio-labelled formyl- and tert.-butyloxycarbonyl-$(L\text{-phenylanalyl-D-leucyl})_2$

-L-Phenylalanine (vide supra, the segments for the synthesis of analogs of the linear gramicidins) for studies in chemotaxis. The formyl derivative is an agonist, the tert.-butyl derivative an antagonist of chemotaxis (neutrophils). To establish the mechanism of action of the agonist and antagonist, p-halophenylalanine has been incorporated in different positions of the peptides for catalytic dehalogenation to provide tritium-labelled derivatives. vi. Synthesis of enkephalin analogs for the exploration of agonist and antagonist properties for physicochemical investigations; vii. Synthesis of cholecystokinin partial sequences of the 26-33 region of the molecule were performed via solid-phase techniques. The highly acclaimed phenacyl ester protection of the β-carboxyl group of aspartic acid led to α,β- peptide bond rearrangement during deprotection with sodium thiophenoxide. A new useful deprotecting agent-in its use free of the side reactions- has been found in SELENOPHENOL.- viii. Synthesis of peptides via dehydroalanine resins: the amide-functions in OXYTOCIN have been incorporated simultaneously via preformed and suitably protected DEHYDROALANINE residues. The peptide so synthesized shows the full amount of oxytocic activity. Analysis: Peptides - Cinnamycin and Duramycin. Hydroxyaspartic Acid-Dehydroaspartic acid. Hydrolysates of cinnamycin and duramycin contain the erythro-and threo- forms of hydroxyaspartic acid. An unknown component in the hydrolysate was likely to be derived from hydroxyaspartic acid, and possibly its dehydration product, namely dehydroaspartic acid. Comparison with an authentic sample confirmed the unknown constituent to be dehydroaspartic acid. The relationship between hydroxyaspartic acid and dehydroaspartic acid (reversible formation via hydration and dehydration) may well be of physiological significance. Amino acids found in cinnamycin and duramycin and linked in other ways via β-elimination and addition reactions are lanthionine, β-methyllanthionine, and lysinoalanine.

Significance to Biomedical Research and the Program of the Institute:

The synthesis of peptides with unique structural features is a prerequisite for the understanding of the effects of these molecules on endocrine function. Amino acid assemblies capable of controlling the release of hormones are of foremost concern. The execution of well-controlled procedures of peptide synthesis provides molecular species for clinical applications aimed at the correction of impairments seen in events of reproductive physiology and at fertility regulation.

Nisin, the H_2N-terminal fragment of nisin, and the linear gramicidins act on neonatal and neoplastic tissue by inducing fetal resorption in rodents and inhibiting the growth of tumor cells. The modes of action responsible for these effects remain to be established. Working hypotheses call for:(a) the addition of essential nucleophilic groups in the physiological environment across the double bond of unsaturated amino acids, such as dehydroalanine (cf. nisin and its fragments); (b) the disturbance of vital ion equilibria existing on either side of the biomembrane (cf. the ionophoric and transmembrane-channel forming properties of the gramicidins A, B, and C, and their synthetic analogs). α,β-Unsaturated amino acids may be vital to many physiological processes. The structural features seen in these unique amino acids are related to the amide and keto-function - both very well established structural elements of physiological consequence - and to the peptide bond - a linking feature of no lesser biological significance.

The amide function is seen at the carboxyl terminus of all structurally established hormone releasing factors. It is also found in the same position of numerous other biologically active peptides, for instance all neurohypophyseal hormones (vide supra the synthesis of OXYTOCIN with the simultaneous incorporation of amides in the COOH-terminal and in endo-positions). The use of dehydroalanine as vehicle for the attachment of peptides to carriers in solid phase peptide synthesis and the direct conversion of this amino acid to the amide function in exo- and endo-positions of the peptide chain are a development that benefited from the chemistry developed in this laboratory subsequent to the discovery of dehydroalanine and dehydrobutyrine in nisin and subtilin.

The linear gramicidins are distinguished from all other known peptide structures by the regularly repeating pattern of L- and D-configurations. This gives rise to a unique hydrogen bond stabilized helix capable of embedding in lipid bilayers and biomembranes and the mediation of ion transport through the transmembrane channels formed. The effects of the native linear gramicidins and their synthetic analogs on neonatal and neoplastic tissue (vide supra) are believed to be the consequence of the physical properties indicated and brought about by the structural features intrinsic to these molecules.

The peptides for which chemotactic and anti-chemotactic properties have been demonstrated (vide supra) are partial sequences of analogs of the linear gramicidins. Assuming a mode of action different from that involving ion transport only, more subtle details of the relationship between structure and chemotaxis are to be investigated subsequent to the incorporation of radiolabel (dehalotritiation of phenylalanine).

The synthesis of partial sequences of hormones (and other physiologically important amino acids assemblies) that are responsible for antigenic activity provides important tools for the purpose of early diagnosis (radioimmunoassay) under normal and/or pathological circumstances.

Proposed Course of Project:

Improvements in the gram-scale synthesis of hormone releasing and hormone release inhibiting factors for fundamental and clinical investigations. Synthesis of H_2N-terminal protected peptide amides with properties antagonistic to the release of luteinizing and/or follicle stimulating hormone. Synthesis of analogs of the linear gramicidins with and without radiolable for autoradiographic investigations and studies to elucidate the modes of action of these peptides in the various physiological effects they cause (action on neonatal and neoplastic tissue; embedding in membranes; exploration as chemotactic and/or anti-chemotactic agents.) Synthesis of partial sequences and individual ring systems of nisin; exchange of amino acids in and incorporation of radiolable into these peptides.

Synthesis of analogs of: (a) opioid peptides (enkephalin and endorphin) with the prime objective of providing antagonists; (b) chemotactically and anti-chemotactically active peptides with alignments of the amino acids that are patterned on partial sequences of the linear gramicidins; (c) synthesis of peptide with the simultaneous incorporation of amides in the COOH- terminal

and in endo-positions, e.g. Substance P. Incorporation of radiolabel (tritium via dehalotritiation) for the purpose of exploring proposed mechanisms of chemotaxis.

Development and improvement of: (a) methods for the synthesis of peptides; (b) the protection of the functional groups of amino acids (the large scale synthesis of the luteinizing hormone releasing factor is one of cases that made evident the inadequacies of existing protecting groups; in this particular situation none of the protections for the guanido groups of arginine proved satisfactory; ornithine, suitably protected at the δ-amino group was employed as precursor; a guanidination reaction was executed subsequent to deprotection; several of the protecting groups under consideration are based on the chemistry of thioether and α,β-unsaturated amino acids; (c) Non-proteolytic techniques that are either applicable in general to the fragmentation of peptides and proteins or are conceived for the specific needs of establishing rare structural features, such as those seen in cinnamycin and duramycin. Correlation of identical structural components (e.g. α,β-unsaturated amino acids lanthionines, lysinoalanine, and hydroxyaspartic acid) in peptides (nisin, subtilin, cinnamycin and duramycin) of strikingly different origin by the pursuit of phylogenetic questions.

Improvements in deprotection techniques applicable to the removal of protecting groups from ω-carboxyl groups (cf. the use of SELENOPHENOL). This and related agents are to be explored for their applicability in the solid-phase synthesis of peptides.

Isolation, purification, and characterization of peptides of natural origin and chemical synthesis of peptides for the study of their inhibitory potential on intrauterine growth. Systematic screening of compounds of different chemical structure for activities on neonatal and neoplastic tissue. Spectroscopic investigation (nuclear magnetic resonance with different nuclei and circular dichroism) of the linear gramicidins and fragments of nisin in their interaction with lipid bilayers of known composition and adequately analyzed biomembranes. Conductance measurements and detailed investigations of the ion transport brought about by the gramicidins A, B, and C and synthetic analogs with amino acids exchanges. Isolation and characterization of subcellular constituents from developing tissue. Improvements in the design and construction of amino acid analyzers and peptide synthesizers.

Publications:

Schiffmann, E., Aswanikumar, S., Venkatasubramanian, K., Corcoran, B.A., Pert, C.B., Brown, J.H., Gross, E., Day, A.R., Freer, R.J., Showell, A.H., and Becker, E.L. (1980) Some Characteristics of the Neutrophil Receptor for Chemotactic Peptides. FEBS Letters 117: 1-7.

Gross, E. (1980) Amino acid assemblies with rare structural components: Chemical, biological, and biosynthetic aspects, in Nucleic Acids and Proteins, Proceedings of Symposium on Nucleic Acids and Proteins, Shanghai, China, October 1979, Shen Zhao-wen, Ed., Science Press, Beijing, China, pp. 70-86.

Gross, E. and Meienhofer, J., Eds., (1981) The Peptides - Analysis, Synthesis, Biology. Volume 3, Protection of Functional Groups in Peptide Synthesis. Academic Press, New York, NY.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00040-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Experimental and Theoretical Studies of Hormone Receptor Interaction and Response Coupling

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A correlated series of experimental and theoretical studies regarding hormone receptor interactions has been undertaken. A variety of model systems have been utilized, including the receptors for aldosterone, corticosterone glucocorticoids, enkephalins, endorphins and opiates, benzodiazepines, LHRH, insulin and beta-adrenergic agonists and antagonists. By development of appropriate mathematical models, computer programs for curve-fitting and data analysis, and of improved experimental designs, we have been able to characterize several of these ligand-receptor systems. Special attention has been given to the cases of 1) multiple ligands reacting simultaneously with multiple classes of sites; 2) the presence of multiple sub-sites on receptor areas; 3) the interaction of ligand-binding with an ionophore; 4) the interaction of receptors for different ligands on the same cell membrane; 5) the interaction of divalent ligands with the cell surface membrane.

PHS-6040
(Rev. 2-81)

Objectives:

This project is intended to develop both experimental and theoretical methods for analysis of hormone, neurotransmitter, and drug binding to receptors. Methods are under study for characterization of the ligand-binding system in terms of several physico-chemical parameters, and the coupling to response of the target cell. Special emphasis is now placed on the examination of properties of divalent ligands.

Methods Employed:

Laboratory techniques are employed for study of the kinetics of binding of peptide hormones, steroid hormones, drugs, and neurotransmitters to their naturally occurring receptors, using appropriately labeled ligands. The techniques of mathematical modeling, computer simulation, curve-fitting, and several methods of statistical analysis are also employed.

Major Findings:

By the use of computerized curve-fitting of data collected in a series of carefully controlled experiments, we have determined that toad kidney A-6 cells contain two classes of receptors for aldosterone and at least three receptors for corticosterone. Two of these receptors are shared by corticosterone and aldosterone. Furthermore, the low-affinity receptor occupancy correlates best with activity data, as measured by Na^+ transport across the epithelium, suggesting that this is the physiological receptor.

A new probe of the beta-adrenergic receptor, [^{125}I]Iodopindolol has been validated by comparison with Iodo-hydroxybenzylpindolol, which has a high non-specific binding component, in rat skeletal and dog cardiac muscle plasma membranes. Both equilibrium binding studies and mutual displacement experiments were performed and data analyzed using programs LIGAND and ALLFIT which were developed in this laboratory.

Studies leading to more efficient design of ligand binding experiments are under way. The outcome of these studies may allow experimentalists to use significantly less tissue in the performance of a ligand-receptor experiment, or may allow more incisive conclusions to be drawn from the data generated using the usual amount of experimental material, animals, tumors, etc. Furthermore, these theoretical studies and resulting computer programs may allow the experimentalist to determine that certain hypotheses cannot be tested using a ligand binding experiment, no matter how efficiently designed. For instance, if the K's for two classes of receptors differ by only a factor of two or less, no ligand binding experiment, no matter how well controlled, will distinguish them. Therefore, different experimental approaches must be tried, such as finding a more selective ligand.

Another study has been undertaken to analyze the binding of gamma amino butyric acid (GABA) and its antagonist, muscimol, to brain post-synaptic receptors, in normals and subjects with hepatic encephalopathy. By careful control of experimental error and by computer modeling of the resulting data, it has been shown that GABA has at least two binding sites, both of which are elevated in hepatic encephalopathy. Muscimol can displace GABA completely,

yet seems to enhance GABA binding when present in only small amounts. Possible mechanism for this effect (e.g. positive cooperativity) are being investigated.

A series of new computer programs have been developed and released for general distribution. This includes a program called "SCATPACK" which is a collection of several programs written in BASIC for the DEC-10 computer, for analysis of ligand-binding experiments. A preliminary program converts observed counts bound into bound over total (B/T), bound over free (B/F), concentrations of bound and free ligand, and makes corrections for variable cell number, non-specific binding, and related considerations. A second program, "SCAT02", provides nonlinear least squares curve-fitting (with weighting) to describe a variety of models, including any number of independent classes of sites, an Adair "Stepwise" model, and a "discrete affinity distribution" with fixed values for the affinity constant. Program "SCAT03" performs similar functions, using the "DeMeyts" model involving positive or negative cooperativity. Additional programs are provided for fitting the association and dissociation curves, either in the noncooperative case, or in cases involving K_d as a linear function of occupancy. Associated programs permit graphical display of observed data and fitted curves. This program, along with several others, is now being distributed through the Biomedical Computing Technology Information Center.

Another new program, designated "TRANSPORT", has been developed. This program describes the equilibrium composition of a system involving multiple ligands (e.g. 21 endogenous steriods) binding to multiple binding proteins (e.g. testosterone estradiol binding globulin, corticosteroid binding globulin, albumin). The program solves a large family of nonlinear algebraic equations in an efficient manner, and provides detailed tabular and graphical inscription of the system. The use of this program to predict free testosterone levels and or free cortisol levels in human subjects has been verified, by comparison with new experimental techniques developed by Dr. P. Siiteri of the University of California, San Francisco. The program has been implemented on the DEC-10 computer and on the HP9845. The program also permits simulation of the effects of several drugs (e.g. danazol) on the binding of steroids to the naturally occurring transport proteins. Further, the program permits calculation of the free cortisol concentration under special circumstances, such as in the adrenal vein, or the free testosterone level in the spermatic vein. This same program is useful for calculation of the equilibrium between thyroxine, triiodothyronine, and other thyroid hormone metabolites, and thyroxine binding globulin, thyroxine binding pre-albumin, and albumin.

Significance to Biomedical Research and the Program of the Institute:

Much current research interest centers around the binding of hormones, drugs and neurotransmitters to their naturally occuring receptors and/or transport proteins. The models which have been developed here should be generally applicable to endocrinology and to neurobiology. Further, these models are now sufficiently well advanced, so that they can be utilized to make predictions in specific systems, and to assist significantly in the design of experiments to discriminate among the possible mechanisms of action.

Proposed Course:

We expect that the models currently under study will continue to evolve

in collaboration with several experimentalists at the National Institutes of Health and elsewhere. The models will be progressivly refined and used as guides to experimentation. As new experimental results become available, the models will be reexamined and modified further. In this process, a series of computer programs will be developed for routine use in analysis, and for optimization of design of experiments. The insights gained by these studies are likely to lead to new experimental approaches, with suggestions for development of new ligands with greater affinity or selectivity. This has already occurred in the case of the analysis of the opiate receptor.

Publications:

Munson, P. D. and Rodbard, D.: LIGAND: A Versatile Computerized Approach for Characterization of Ligand-Binding Systems. Anal. Biochem. 107: 220-239, 1980.

Thakur, A. K., Jaffe, M. L., and Rodbard, D.: Graphical analysis of ligand-binding systems: evaluation by Monte Carlo studies. Anal. Biochem. 107: 279-295, 1980.

Rodbard, D., Thakur, A.J., and Munson, P.J.,: Quantitative Characterization of Hormone Receptors. Cancer. 46: 2907-2918, 1981.

Dunn, J. F., Nisula, B. C., and Rodbard, D.: Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone binding globulin and corticosteroid binding globulin in human plasma. J. Clinical Endocrinology and Metabolism. 53: 58-68, 1981.

Pugeat, M. M., Dunn, J. F., Rodbard, D., and Nisula, B. C.: The significance of drug interactions with human TeBG and CBG under physiological conditions: a new approach. J. Steroid Biochem. in press.

Ezrailson, E.G., Garber, A.J., Munson, P.J., Swartz, T.L., Birnbaumer, L., Entman, M.L.:[^{125}I] Iodopindolol: a new Beta-Adrenergic Receptor Probe. J. Cyc. Nucl. Res. 7: 13-26, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00041-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cardiovascular Effects of Thyroid, Hormones, Catecholamines and Cardiotoxic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
Other	R. Osburn		NNMC
	E. C. Ridgway	Professor, Thyroid Unit	Massachusetts General Hospital

COOPERATING UNITS (if any)
National Naval Medical Center, Bethesda, Maryland
Massachusetts General Hospital, Boston, Massachusetts

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

The pulse wave arrival time (designated QK_d), has been further demonstrated to be useful in evaluating thyroid function under conditions of "diminished thyroid reserve", and in the evaluation of the changes in thyroid hormones which occur during hypocaloric feeding in humans. The QK_d has been used to demonstrate that supplementation with oral thyroxin results in improvement at the target organ level, in patients with diminished thyroid reserve as manifested by low or borderline low thyroid function with moderate or minimal TSH elevation. In human subjects undergoing prolonged hypocaloric feeding, normally there is a decrease in serum T4 and T3. Under this set of circumstances, the QK_d is prolonged, suggestive of hypothyroidism at the target organ level. Further, oral supplementation with T3, to maintain a euthyroid level of T3, prevents the changes in QK_d. In contrast, oral supplementation with T4 which results in supra-normal levels of T4 but slightly decreased levels of T3, fails to provide complete return to normal of the QK_d. These findings suggest that QK_d is more closely correlated with T3 than with T4 levels, and further suggests that the changes observed in peripheral thyroid hormones levels during hypocaloric feeding is indeed an adaptive mechanism which results in a state of hypometabolism.

Objectives:

Develop and evaluate methods for monitoring of thyroid function, and utilize these in the context of ongoing clinical studies of various disorders including hypocaloric feeding (starvation) in man and the problem of "diminished thyroid reserve".

Methods Employed:

The use of "Sphygmo-Recording", i.e., measurement of the QK_d interval, is the principal method employed. An automated device measures the interval between the onset of the QRS complex of the electrocardiogram and the arrival of the pulse wave at the brachial artery, as manifested by the onset of the Korotkoff sound at diastolic pressure. This technique is utilized in association with the measurement of systolic time intervals (pre-ejection period, left ventricular ejection time, PEP/LVET), and techniques for clinical evaluation of patients with thyroid disorders.

Major Findings:

During hypochloric feeding in man, there is a fall in serum T3 and a rise in reverse T3. The question has arisen, whether this is a chemical aberration, or whether it represents an adaptive mechanism, indicating an effective state of hypothyroidism. The present study showed that during hypocaloric feeding, there is a statistically significant increase in the QK_d interval, and that this increase was seen reproducibly when hypocaloric feeding was instituted on two or three occasions for the same individual. There was a concomitant, statistically significant drop in pulse rate, also consistent with a relatively hypothyroid state. When T3 was administered orally during hypocaloric feeding, to maintain the T3 level essentially constant, the rise in QK_d and fall in pulse rate were both prevented. In contrast, when T4 oral supplementation was given, so that T4 levels rose above the basal levels, but T3 levels remained slightly below the control levels, a significant change was still seen in QK_d and pulse rate. This suggests that the QK_d correlates better with serum T3 than with serum T4. Further, this suggests that the chemical changes observed during hypocaloric feeding are part of an adaptive mechanism conserve energy metabolism.

Patients with diminished thyroid reserve, as clinically evidenced by low normal values for T4 and T3 with an elevated baseline TSH, were evaluated using the OKd machine. These patients showed a statistically significantly prolonged QK_d interval and also aberrations in the PEP/LVET ratio, consistent with hypothyroidism. When these individuals were given a small oral supplementation with thyroxine, the QK_d interval returned to normal and there was concomitant change in pulse and PEP/LVET. These observations suggest that there is indeed an entity of "diminished thyroid reserve".

Significance:

At present the QK_d interval is the best available measure of the effect of thyroid on peripheral metabolism and on "target tissues" in general. In the recent studies, it has been shown to be as good or superior to the use of systolic time intervals. As shown previously, the QK_d interval is superior to the use of cholesterol, creatine phosphokinase (CPK), and other metabolic parameters. As such, it is useful in evaluating patients with suspected thyroid and/or pituitary disease.

Proposed Course:

The QK_d is to be utilized selectively in special cases where a reliable measure of end-organ function is necessary, in addition to the measurement of plasma hormone levels.

Publications:

Ridgway, E. C., Cooper, D. S., Walker, H., Rodbard, D., and Maloof, F.: Peripheral Responses to Thyroid Hormone Before and After L-thyroxine Therapy in Patients with Decreased Thyroid reserve. J. Clin. Endocrinol. Metab. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00042-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mathematical and Statistical Theory of Gel Electrophoresis and Filtration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
	P. J. Munson	Mathematician-Statistician	ERRB, NICHD
Other	Y. Shimohigashi	Visiting Fellow	ERRB, NICHD
	A. Chrambach	Senior Investigator	ERRB, NICHD
	D. Bunow	Staff Fellow	DCRT

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25		

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mathematical and statistical considerations involved in the estimation of molecular weight, molecular radius, Stokes radius, frictional coefficient and other physical-chemical parameters have been evaluated. The theory of high-pressure liquid chromatography and thin-layer chromatography has been analyzed with a view toward utilizing thin-layer chromatography to predict elution patterns on high-pressure chromatography.

PHS-6040

Objectives:

Improvement of the performance of physico-chemical techniques employed for the analysis and purification of protein and polypeptide hormones.

Methods Emplyed:

Polyacrylamide gel electrophoresis, gel filtration, sucrose density gradient sediment, sucrose density electrophoresis of cells and proteins, isoelectric focusing, high-pressure liquid chromatography, thin-layer chromatography. Mathematical modeling and simulations are employed in attemps to predict the properties and optimized conditions of these various analytical separation procedures.

Major Findings:

A model has been developed to permit optimization and prediction of elution patterns for high-pressure liquid chromatography, employing data readily obtainable from reverse-phase thin-layer chromatography on a pilot basis. This should permit conservation of time by experimentalists, and should save precious material in several applications. The problem of estimating the precision of molecular weights, frictional coefficient, and actual ratio has remained under evaluation. The computer programs employed for routine analysis of polyacrylamide gel electrophoresis and related procedures have been improved and updated.

Significance to Biomedical Research and the Program of the Institute:

These physico-chemical techniques are utilized extensively in the purification and analysis of protein and polypeptide hormone, receptors, and other macromolecules. The theoretical, mathematical and statistical analyses of these techniques often results in improved interpretation of results by providing the experimentalists with an indication of the standard error and/or 95% confidence limits for various physical-chemical parameters derived. Many experimentalists have assumed that these measurements are $\pm$ 1 or 2 percent or $\pm$ 5 percent, when in many situations these parameters can only be measured $\pm$ 10, $\pm$ 25 or sometimes even $\pm$ 35 percent. That has led to unwarrented conclusions and controversies which were unnecessary, because the precision of the data did not warrent the claims that were being made.

Proposed Course:

We anticipate a major effort to analyze the time course of development of pH gradients, conductivity gradients, and the complete chemical composition of complex mixtures of buffer constituents or ampholytes, to permit a more rigorous definition of the theory of isoelectric focusing. The computer programs for routine use need to be updated, to take advantage of new developments in graphical techniques, and to permit use of recently developed methods of "extended nonlinear least squares curve-fitting". These techniques should permit automated estimation of the precision of the measurements, and thus simplify the procedures for the experimentalist.

Publications:

Rodbard, D., Cole, B. R., Murakami, T., Computer Analysis of Concentration Profiles: Automated Peak Detection, Characterization, and Estimation of Molecular Size. Steroids. 34: 1-14, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00043-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Mathematics and Statistics of Radioligand Assays

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
	Peter Munson	Mathematical-Statistician	ERRB, NICHD
	A. K. Thakur	Visiting Associate	ERRB, NICHD
Other	J. Huston	Programmer	DMB, DCRT

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

The mathematical and statistical theories of radioimmunoassay and related techniques have been under development. We have been interested in the enzyme multiplied immunological technique (EMIT), the enzyme linked immuno adsorbent assay (ELISA), and the chemiluminescent assays. The mathematical theory of optimization of RIA, and appropriate computer programs have been further developed.

PHS-6040
(Rev. 2-81)

Objectives:

Development of mathematical and statistical methods for evaluation of radioimmunoassay systems, for optimization of radioligand assays, and for statistical quality control of RIA laboratories.

Methods Employed:

Mathematical modeling, statistical analysis, computer stimulation and analysis of laboratory data for a wide variety of hormones.

Major Findings:

Improved methods have been developed for quality control of radioimmunoassays. Currently we are working on the development of computer programs for on-line, automatic calculation of quality control results. Previously developed programs for analysis of radioimmunoassay results have been improved, particularly with regard to the automatic calculation of weighting coefficients. We are also working on techniques related to "extended least squares", in which the parameters of the weighting program are estimated simultaneously with the parameters of the underlying model, to reduce the two step procedure to a one step procedure.

Significance to Biomedical Research and the Program of the Institute:

The computer programs developed for analysis of radioimmunoassay results and for quality control have been used extensively by numerous members of the Branch, of the Institute, and of numerous laboratories throughout the world. These programs require continual update, modification, or revision and improvement.

Publications:

Rodbard, D.: In Langan, J., Clapp, H. (Eds.): Mathematics and Statistics of Ligand Assays: An Illustrated Guide, Masson, Chap. 3: 45-101, 1981.

Munson, P.J. Rodbard, D.: Statistical Methods for comparison of two Assay methods (weighted linear regression with errors in both variables). In Wilson, D.W. (Ed.): Quality Control of Radioimmunoassays, Tenovus Research Institute Symposium, 1981, in press.

Rodbard, D., Cole, B., and Munson, P.J.: The Need for Innovative Approaches to Radioimmunoassay Quality Control. In Wilson, D.W. (Ed.): Quality Control of Radioimmunoassays, Tenovus Research Institute Symposium, 1981, in press.

Rodbard, D.: Mathematics and Statistics of Radioimmunoassay and Radio-Ligand Assays. In, Syllabus, The Endocrine Society Training Program on Immunoassay, Bethesda, Md. 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00146-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Structure and Function of Chorionic Gonadotropins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	H. C. Chen	Senior Investigator	ERRB, NICHD
	S. C. Huang	Visiting Associate	ERRB, NICHD
	Y. Shimohigashi	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)
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P.C. Ouyang, National Taiwan University Hospital, Taipei, Taiwan

LAB/BRANCH
Endocrinology and Reproduction Research Branch

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves studies on the chemical, immunological and biological properties of gonadotropins in tissues and biological fluids with emphasis on structure-function relationships and application of sensitive and specific detection methods for monitoring the secretion of gonadotropins in normal subjects and patients with neoplasms.

PHS-6040
(Rev. 2-81)

Objectives

To demonstrate the presence of hCG or an hCG-like substance in tissues and fluids of normal subjects and patients with neoplasms, with an attempt to understand its biological role in reproduction and development.

To generate hCG-specific antisera using well-characterized synthetic peptides as antigens and to prepare an immunoabsorbent that permits the development of improved immunoassay methods in monitoring a low level of hCG activity.

To elucidate the role of carbohydrate moieties of hCG in the mechanism of hormonal action.

Methods Employed

Peptide synthesis, elementary analysis, polarimetry, amino acid analysis, sugar analysis, chromatographic techniques (i.e., gel, ion exchange, partition and bioaffinity), high performance liquid chromatography, electrophoresis, rat uterine weight assay, immunization of rabbits, radioimmunoassay, radio-ligand receptor assay, interstitial cell testosterone production assay, high speed digital computer analysis, spectrophotometry and spectrofluorometry.

Major Findings

1. The hCG-like substance in human pituitary glands

Previously, data have indicated that a gonadotropic substance in human pituitary extracts resembles hCG but differs from LH in its molecular size, isoelectric points and immunological characteristics. Although the hCG-like substance (hCG') is more ubiquitous than generally believed, the pituitary gland contains the highest amount among the tissues of non-pregnant normal subjects.

A recent SDS-gel electrophoresis study has revealed that the partially purified hCG' was dissociated into two components which migrated in a manner identical to that of the α- and β- subunits of hCG. This result further demonstrates the resemblance between the hCG-like substance in human pituitary glands and hCG.

2. The role of carbohydrate moieties in the biological and immunological activities of hCG

A procedure employing anhydrous hydrogen fluoride, which permits partial hydrolysis of glycosidic linkages, has been applied to the treatment of hCG. Under controlled conditions (0°C, 60 minutes), 70% of carbohydrate moieties were removed from hCG without either cleavage of a polypeptide bond or significant dissociation into subunits (less than 3%). Unlike its precursor molecule, HF-treated hCG no longer binds to concanavalin A. As revealed by SDS-polyacrylamide gel electrophoresis studies, the molecular sizes of its two subunits have reduced to 70% of their original sizes.

Based on the monosaccharide composition of HF-hCG, cleavages occurred predominantly at the reducing ends of mannose and galactose of asparagine-

linked and serine-linked chains, respectively. Differences in susceptibility to hydrolysis among the carbohydrate chains were noted.

The HF-hCG showed a slight reduction in immunological potencies in three hCG/^{125}I-hCG radioimmunoassay systems, namely, H80 anti-hCG (10%), Sb6 anti-hCGβ (20%) and H93 anti-hCGβ-COOH peptide (30%). However, HF-hCG was 2.5 times more effective than hCG in replacing ^{125}I-hCG in a testis homogenate receptor binding assay. In spite of the high receptor binding activity, the HF-hCG at a dose level two orders of magnitude above the effective dose of hCG failed to stimulate Leydig cell testosterone production or to increase uterine weight of immature rats in an in vivo assay. It also failed to antagonize hCG and hLH activities in a rat uterine weight assay. Studies of plasma half-life have indicated that the HF-hCG exhibits extremely short plasma half-life as compared to that of hCG.

3. Generation of hCG-specific antisera

Antisera were produced in rabbits immunized against a synthetic peptide, N^{α}-pentaglycylpentatriacontapeptide (hCGβ, res. 111-145), conjugated to bovine thyroglobulin. Similar to antisera previously raised against conjugates of analogous synthetic peptides, these new antisera have the capacity only to bind the end portions of carboxyl-terminal peptide, not the preceding peptide sequence.

In order to investigate the importance of terminal Pro-Gln as a primary antigenic site for hCG-specific antisera, a series of peptides elongated from res. 131 of hCGβ toward the carboxyl-terminus were synthesized.

4. HCG in urine of patients with trophoblastic diseases and testicular tumors

Kaolin-acetone extracts of 24-hour urine specimens from 95 female and 15 male patients were assayed by the H93-radioimmunoassay. Among 32 urine specimens from the 15 patients whose serum hCG levels were considered normal, 5 patients showed elevated levels of urinary hCG and were found to have recurrence. All 744 specimens from the 95 female patients showed elevated levels of urinary hCG. Their serum hCG levels are not yet available.

Significance to Biomedical Research

1. The evidence that an hCG-like substance is present in the human pituitary gland of normal subjects is enhanced by detailed chromatographic and electrophoretic analyses in conjunction with immunological and biological assays.

2. The carbohydrate moieties in hCG are essential not only for the maintenance of a normal plasma half-life but also for eliciting stimulation of steroidogenesis. These findings may provide an explanation of why some patients who secrete high levels of "hCG" do not always result in hypergonadism. The "hCG" in those cases may not have intact carbohydrate structure.

3. Sufficient amounts of hCG-specific antisera have been accumulated for distribution to the biomedical community.

4. The availability of highly specific RIA for measuring urinary hCG has made significant improvement in detecting, staging and monitoring trophoblastic diseases.

Proposed Course

1. To devise simple and practical separation methods for hCG or the hCG-like substance in tissues or biological fluids that permit studies of chemical, biological and immunological properties of hCG from various origins or at various stages of development.

2. To continue investigation of the effect of carbohydrate structure of hCG on the mode of hormonal action in vivo and in vitro with emphasis on the HF-treatment after the complete removal of sialic acid and galactose residues from two major types of carbohydrate chains.

3. To conduct antigenic characterization of hCG-specific antisera using peptides elongated from the amino terminal portion of the unique carboxyl-terminal peptide of hCGβ.

Publications

1. Matsuura, S., and Chen, H.C.: Synthetic peptide strategy for the determination of antigenic sites in the unique COOH-terminal portion of hCG β-subunit. In Liu, T.Y. and Schechter, A.N. (Eds.): The Chemical Synthesis and Sequencing of Peptides and Proteins. Amsterdam, Elsevier, In press, 1981.

2. Javadpour, N., and Chen, H.C.: Improved hCG detection utilizing the β-subunit carboxyl-terminal radioimmunoassay of concentrated 24 hour urine in patient with testicular cancer. Urology. In press, 1981.

3. Gulyas, B.J., Matsuura, S., Chen, H.C., Yuan, L.C., and Hodgen, G.D.: Visualization of binding and internalization of a horseradish peroxidase-hCG conjugate by monkey luteal cells. Biology of Reproduction, In press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00147-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Peptide Hormones in Steroidogenic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Maria L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	Kevin J. Catt	Chief	ERRB, NICHD
Other	K. Nozu	Visiting Fellow	ERRB, NICHD
	A. Dehejia	Student Scientist	ERRB, NICHD
	E. H. Charreau	Visiting Scientist	ERRB, NICHD
	J. Calvo	Guest Worker	ERRB, NICHD
	S. Matsuura	Visiting Scientist	ERRB, NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Sections on Molecular Endocrinology and Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad goal of this project is to understand the steps involved in the hormonal control of steroidogenesis. This includes the metabolic events that follow stimulation of hormone receptors in steroidogenic cells by trophic hormones. Studies were focused on the interaction of hormones with their receptors, and on analysis of coupling mechanism between receptor occupancy and androgen, progesterone or corticosteroid synthesis. The major topics examined were (1) the relationship of receptor occupancy to cyclic AMP and protein kinase activation and phosphorylation of proteins involved in steroidogenic events during acute dose-related increases in steroid production; (2) characterization of cyclic AMP-dependent protein kinases of steroidogenic tissues; (3) the sequence of events which leads to stimulation or inhibition of enzymes of the steroidogenic cells.

PHS-6040
(Rev. 2-81)

Objectives:

To define the mechanisms of action of trophic hormones upon steroidogenesis and growth in steroidogenic cells.

Methods Employed:

Purification of Leydig cells by Metrizamide gradients. Characterization of LH, HDL, LDL and prolactin receptors by binding analysis in cell membranes and intact Leydig cells. Measurements of extracellular, intracellular and receptor bound cyclic AMP by radioimmunoassay. Protein kinase assay. Gel filtration, sucrose gradient centrifugation, ion exchange chromatography, DEAE cellulose and polyacrylamide gel electrophoresis for characterization of protein kinase holoenzyme and regulatory subunits. Thin-layer chromatography on alumina and silica gel for characterization of steroids. Culture of Leydig cell tumors. Electron microscopy and light autoradiography. Polyacrylamide and SDS gel electrophoresis, isoelectric focusing and two-dimensional gel electrophoresis.

Major Findings:

1. Hormone-induced phosphorylation of endogenous proteins.

Hormonal stimulation of dispersed Leydig cells by gonadotropin concentrations in the range that produces a graded testosterone response is accompanied by a simultaneous increase in endogenous cyclic AMP bound to the intracellular receptor protein. The steroidogenic response to trophic hormone appears to be highly compartmentalized, and minute increases in hormone-stimulated cAMP cause activation of protein kinase and subsequent regulation of key steroidogenic enzymes. Over the steroidogenic dose-response range in the Leydig cell, cAMP was bound to the cytosolic type I holoenzyme. Following occupancy of cyclic AMP receptors, a dose-related increase in protein kinase activity was observed over the range of steroidogenic response in Leydig and adrenal cells, and was detectable from early times of 15 sec to 1 min. These changes were accompanied by corresponding increases in binding of cAMP to the regulatory subunit.

The phosphorylation of Leydig cell substrates by cAMP-dependent protein kinase during hormone action was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and autoradiography. During stimulation of isolated Leydig cells with hCG for 10 min. dose-related increases in phosphorylation were observed in ten bands of Mr 22,000-90,000 with ED_{50} for hCG ranging 0.10-0.3 pM. These hCG-induced increases in phosphorylation correlated with the stimulation of cyclic AMP and testosterone production in parallel experiments. The maximum hCG-stimulated increases in phosphorylation were from 50 to 600 per cent over control levels. Incubation of Leydig cells with dibutyryl cyclic AMP caused phosphorylation of bands identical with those stimulated by hCG. Five of the hormone-stimulated bands observed in intact Leydig cells corresponded with cyclic AMP-dependent phosphorylated bands evoked by this nucleotide in Leydig cell extracts. Further resolution of hCG-induced phosphorylation products by two-dimensional gel electrophoresis revealed ten discrete components with Mr range 22,000 to 105,000 and pIs 5.4 to 6.6. Seven of these proteins corresponded with bands that were increased during hormone action or nucleotide stimulation in the one-dimensional system, and the hormone-induced increases ranged from 90 to 700

per cent. Thus, these studies have demonstrated that both hCG and dibutyryl cyclic AMP induce phosphorylation of multiple proteins in the Leydig cell. These events are correlated with hCG-stimulated increases in cyclic AMP and testosterone production, indicating that the steroidogenic response to LH receptor occupancy is mediated by activation of the cyclic AMP/protein kinase/phosphorylation sequence during hormonal stimulation.

2. Trophic hormone control of receptors and steroidogenic responses in the Leydig cell.

a) Modulation of Leydig cell during estrogen treatment and hCG-induced desensitization of androgen biosynthesis and cytochrome P-450 levels.

The mechanisms responsible for gonadotropin-induced testicular desensitization were analyzed by direct assay of androgen biosynthetic enzymes and cytochrome P-450 in microsomes and cytosol from testes of adult male rats. Animals were treated with single subcutaneous doses of hCG (2 and 10 μg) to induce a post-stimulation decrease in testosterone production, previously shown to result from reduced conversion of progesterone to androgen. In addition, the larger dose of hCG caused an earlier biosynthetic defect due to impaired formation of pregnenolone from endogenous precursors. Dose-dependent inhibition of both 17α-hydroxylase and 17-20 desmolase activities by 30% and 90% was observed after treatment with 2 and 10 μg hCG, respectively. In contrast, hCG treatment caused no change in the activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. The reductions in microsomal enzyme activity were accompanied by a comparable decrease in cytochrome P-450 levels. Similar correlations between the microsomal cytochrome P-450 content and the same enzymatic activities were observed after hypophysectomy. Treatment of hypophysectomized rats with 17β-estradiol (0.1-20 μg) reduced testosterone responses to hCG in a dose-dependent manner (by 20 to 60%), and caused further decreases of microsomal enzymatic activities and of the levels of cytochrome P-450, but not of 3β-hydroxy and 17β-hydroxysteroid dehydrogenase. The dependence of 17α-hydroxylase and 17-20 desmolase on cytochrome P-450 levels was indicated by the constancy of specific activity of the microsomal enzymes when expressed in terms of cytochrome P-450. The similarity of estrogen-dependent lesions to those produced by hCG treatment further indicates the involvement of endogenous estrogen in the development of the microsomal enzymatic lesions in gonadotropin induced-desensitization of testicular androgen production.

To extend the analysis of receptor down-regulation and mechanisms responsible for the inhibitory actions of estrogen upon Leydig cell function, we initiated studies on the in vitro control of LH receptors and steroidogenic responses by gonadotropin and estradiol in acutely cultured Leydig cells. Since rat Leydig cells exhibit marked reduction of both LH receptors and microsomal enzymes after three days in culture, we have commenced studies employing short-term (24-48 hr) Leydig cell cultures as in vitro system to study the regulatory actions of gonadotropins upon cellular receptors and steroidogenesis. Afte 24 hr in culture, adult rat Leydig cells retained 60% of their LH receptors and showed no change in maximal testosterone response to hCG. During initial studies we have optimized an in vitro system suitable for the investigation of gonadotropin-dependent regulatory mechanisms.

b) Regulation of early enzymatic steroidogenic step in the Leydig cell in gonadotropin-induced desensitization.

The control of 3-hydroxy, 3-methylglutaryl coenzyme A reductase (HMG CoA reductase) in testicular Leydig cells was studied in Leydig cells with gonadotropin-induced steroidogenic enzyme lesions of the early biosynthetic pathway (prior to pregnenolone) and of the late pathway (17α-hydroxylase, 17-20 desmolase) of androgen biosynthesis. After treatment with a single dose of 10 μg hCG (to produce the early and late steroidogenic lesions, a marked increase in HMG CoA reductase activity was observed at 4 hr, followed by a significant reduction of 50 to 70% below the control value for up to 72 hrs. In contrast, cells from animals treated with 2 μg hCG (to produce only the late steroidogenic lesion) showed no changes in enzyme activity. The decreased pregnenolone synthesis in response to hCG stimulation in cells with the early biosynthetic defect was partly restored by addition of lipoproteins, either HDL or LDL, to the incubation medium. The decrease in enzyme activity observed at 24 to 72 hrs after hCG treatment, and the restoration of steroid responses to in vitro hormone stimulation by addition of lipoproteins, have localized a site for the early biosynthetic lesion observed during desensitization by gonadotropin.

c) HMG CoA reductase in the Leydig cell.

The control of 3-hydroxy, 3-methylglutaryl coenzyme A reductase (HMG CoA reductase) in testicular Leydig cells was studied during cholesterol depletion in 4-aminopyrazolopymiridine (4-APP) treated rats. Following 4-APP treatment, plasma cholesterol, LH, and testosterone levels were markedly decreased and pituitary LH content was normal or increased. However, there was no change in testicular HMG CoA reductase activity, whereas the adrenal enzyme was stimulated 15-fold. The testosterone responses of Leydig cells to hCG stimulation in vitro were similar in normal and 4-APP-treated rats, though the latter group showed a significant reduction of both LH and prolactin receptors. These studies have demonstrated that testicular HMG CoA reductase, in contrast to the adrenal enzyme, is not regulated by changes in circulating cholesterol. The ability of Leydig cells from 4-APP-treated animals to continue androgen biosynthesis in vitro indicates that these cells possess an active steroidogenic pathway from precursors prior to cholesterol. The marked fall in circulating testosterone in 4-APP-treated animals is attributable to decreased LH secretion, rather than to a change in HMG CoA reductase activity.

Significance:

The hormonal control of Leydig cell function and androgen production is an essential component of male sexual development and reproductive capacity. In addition to its significance to the Endocrinology and Reproduction Research program, this project has the potential to provide knowledge about the general actions of peptide and protein hormones upon target cells in tissues other than the gonads.

Proposed Course:

1. (a) Investigation of the characteristics of protein kinase holoenzymes in adrenal and Leydig cell will be continued.

(b) The role of protein kinase in gonadotropin action will be further analyzed by measurement of endogenous protein kinase activity with localization and identification of substrate phosphorylation in dispersed Leydig cells during LH stimulation in vitro.

(c) Investigation of the functional compartmentalization of cyclic AMP responses in the Leydig cell will be continued by localization of cyclic AMP bound to receptor protein and immunofluorescence microscopy of cyclic AMP in intact Leydig cells.

2. Extend analysis on the mechanism of LH-induced receptor regulation and steroidogenic lesions in Leydig cells in culture.

3. Activation of cholesterol side-chain cleavage and cholesterol esterase by LH in purified Leydig cells and of early enzymes prior to cholesterol formation.

4. The role of lipoproteins in Leydig cell steroidogenesis, and their participation during LH action, will be analyzed.

5. Studies on the effects of trophic hormones and steroid metabolites upon steroidogenic enzymes will be pursued.

6. Studies on the action of hCG on gene expression will be commenced.

Publications:

Nozu, K., Dufau, M.L., and Catt, K.J.: Estradiol receptor-mediated regulation of steroidogenesis in gonadotropin-desensitized Leydig cells. J. Biol. Chem. 256: 1915-1922, 1981.

Nozu, K., Matsuura, S., Catt, K.J., and Dufau, M.L.: Modulation of Leydig-cell androgen biosynthesis and cytochrome P-450 levels during estrogen treatment and hCG-induced desensitization. J. Biol. Chem., in press, 1981.

Dufau, M.L., Sorrell, S., and Catt, K.J.: Gonadotropin-induced phosphorylation of endogenous proteins in the Leydig cell. FEBS Letters, in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00148-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Ontogeny of Gonadotropin Receptors and Gonadal Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other	R. Clayton	Guest Worker	ERRB, NICHD
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	M. Katikineni	Staff Fellow	ERRB, NICHD
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COOPERATING UNITS (if any)

None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with analysis of developmental characteristics of gonadotropin receptors during fetal life and sexual maturation. Studies are performed on the emergence of receptors and biochemical responses of the pituitary-gonadal system during development, on the maturation of testicular and ovarian endocrine function, and on the control of gonadal sensitivity during sexual maturation.

Objectives:

To characterize the receptor-mediated control of pituitary and gonadal hormone secretion during fetal development and sexual maturation.

Methods employed:

Radioligand assay of pituitary GnRH receptors and gonadal receptors for LH, FSH, and prolactin. Radioimmunoassay of pituitary and gonadal hormones.

Major Findings:

1. Ontogeny of pituitary GnRH receptors in the rat.

Developmental changes in pituitary GnRh receptors were evaluated by longitudinal analysis of GnRH binding sites, and compared with pituitary and serum gonadotropins and gonadal steroid hormones from birth to sexual maturity. In male rats, the pituitary concentration of GnRH receptors increased in early life to a maximum of 260 fmol/mg protein between 15 and 30 days of age. However, the total number of pituitary GnRH receptors increased progressively with age and pituitary weight, reaching a plateau of 110 fmol/gland at 40 days of age. These changes in GnRH binding reflect the number of receptor sites rather than altered binding affinity, which remained constant during development ($^{Ka}=3.3\pm0.4\times10^{9}M^{-1}$ in males and $2.6\pm0.6\times10^{9}$ M^{-1} in females). The initially high GnRH receptor concentrations in the male were associated with elevated serum LH levels (60 ng/ml) and a peak in serum FSH on day 30. During the pubertal rise in serum testosterone, GnRH receptor concentrations showed a reciprocal decline to adult values. In female rats, the highest concentration of GnRH receptors (265 fmol/mg protein) occurred between 15 and 20 days, while the total pituitary GnRH receptor content plateaued earlier than in males at about 25 days of age. The decline in pituitary GNRH receptor concentration occured 5 days earlier in females than males, corresponding to the earlier fall in serum LH and FSH (about 15-20 days) in females. In both male and female rats, GnRH receptors correlated closely with intrapituitary LH. These changes in pituitary GnRH receptors, pituitary LH content, serum gonadotropins, and sex steroids, are consistent with a physiological role for GnRH receptors in regulating the activity of the pituitary-gonadal axis during development and sexual maturation.

2. Regulation of LH receptors and steroidogenesis in the neonatal rat testis.

Testicular LH receptor regulation and steroidogenesis were studied in 5-day-old rats, in which Leydig cells are derived from the fetal population of interstitial cells. The affinity of hCG binding was similar in the neonatal and adult testis; in neonatal testis, the hCG binding capacity per unit weight was 5-10% of the adult level, but was similar to the adult value when expressed per Leydig cell. The sensitivity of the testosterone response to hCG was 4-fold higher in neonatal Leydig cells, whereas the basal and maximal rates of testosterone production were similar in neonatal and adult cells. No clear differences were seen in cAMP production. Treatment with a low dose of hCG (20 IU/kg BW sc) caused a 40-60% increase in LH receptors after 1 to 3 days, without affecting the Leydig cell number; such an effect was not

observed in the adult. A higher dose of hCG (600 IU/kg BW sc) increased the number of Leydig cells per testis about 2-fold in 2 days, and a significant increase in basal and hCG-stimulated testosterone production occurred. In adult animals, similar treatment caused marked receptor loss and desensitization of testosterone production. The high dose of hCG totally abolished available neonatal testis LH receptors, but complete recovery of binding occurred between 48 and 72 h. In contrast, more than 10 days were needed for complete recovery of LH receptors in adult testes. Measurements of tissue-bound hCG indicated that the decrease in neonatal testis LH binding was due to receptor occupancy without net loss of binding sites.

These findings demonstrate that in comparison to the adult, the following features are characteristic of the fetal population of rat testis Leydig cells: 1) Testosterone production is more sensitive to gonadotropic stimulation. 2) No hCG-induced desensitization of androgen biosynthesis is seen. 3) Low-dose hCG treatment increases the number of LH receptors. 4) High-dose hCG treatment decreases LH binding for a shorter period than in the adult. The loss of binding is explained by receptor occupancy, without net loss of LH receptor sites. 5) High-dose hCG treatment also causes a rapid increase in the number of Leydig cells.

Significance:

The characterization of pituitary and gonadal receptors and androgen regulation during fetal and neonatal life, and in immature animals, will assist in the clarification of mechanisms determining male phenotypic sexual development, and those controlling gonadal function during sexual maturation.

Proposed Course.

The role of protein hormones and their receptors in mediating aspects of fetal testis maturation will be examined, and the influence of pituitary hormones on the development of gonadal receptors for LH/FSH and prolactin, and gonadal steroidogenesis, will be further analyzed during maturation in the rat.

Publications.

Chan, V., Clayton, R.N., Knox, G. and Catt, K.J.: Ontogeny of Pituitary GnRH Receptors in the Rat. Endocrinology 108: 2086-2092, 1981.

Huhtaniemi, I.T., Katikineni, M. and Catt, K.J.: Regulation of Luteinizing-Hormone-Receptors and Steroidogenesis in the Neonatal Rat Testis. Endocrinology 109: 588-595, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00149-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Maria L. Dufau	Chief, Section on Molecular Endocrinology	ERRB, NICHD
	Kevin J. Catt	Chief	ERRB, NICHD
Other	Angela Solano	Research Associate	ERRB, NICHD
	Alfonso Garcia-Vela	Research Associate	ERRB, NICHD

COOPERATING UNITS (if any)
Dept. of Gynecology, Mass. General Hospital, Boston, Mass.; Primate Research Center, Atlanta, Georgia; Department of Medicine, University of Rome, Italy; Dept. of Pathology, University of New Mexico; Utah State University.

LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Long range studies on the in vitro bioactivity of luteinizing hormone and chorionic gonadotropin in man and other species were continued. The recently developed in vitro bioassay for the measurement of LH and hCG in serum has been more extensively applied to studies on gonadotropin regulation in man and other species.

PHS-6040
(Rev. 2-81)

Methods Employed:

1. In vitro bioassay for serum gonadotropin based on testosterone production in collagenase-dispersed rat interstitial cells (RICT assay).

2. Radioimmunoassays for human LH. Radioimmunoassay for rat LH and for rLH subunits. Radioimmunoassay for rat prolactin, FSH and estradiol.

3. Radioreceptor assay for LH, prolactin and FSH using iodinated tracers of hCG, hGH and hFSH, respectively, to determine number of ovarian gonadotropin and prolactin receptor sites. Evaluation of hormone receptor occupancy in ovarian tissue was performed by elution of bound endogenous LH and subsequent measurement of the released hormone by RIA.

4. Gel filtration on Sephadex G-100 of rat pituitary extract, and measurement of eluted fractions by radioimmunoassay to detect intact rLH and subunits. Isoelectricfocusing of pituitary LH.

Major Findings:

1. Modulation of serum and pituitary luteinizing hormone bioactivity by gonadal steroids.

Previous studies on the analysis of circulating LH on human serum have shown marked sex differences between the bio:immuno ratios of the secreted hormone. In men, B:I ratios are usually 2-4, whereas those in normal women at all stages of the cycle are close to unity. Post-menopausal women and patients with Turner's syndrome also have higher B:I ratio than cycling women. The elevated B:I ratio in Turner's syndrome can be reduced to the normal cycling values by prolonged estrogen replacement therapy. In addition, the plasma LH of prepubertal boys has a B:I ratio close to unity, and shows an increased ratio after the onset of testicular androgen secretion.

This concept is also supported by recent observations during physiological studies, castration and hormonal replacement in the male rats. To further the understanding on the role of gonadal steroids, LH bioactivity was analyzed in female rats with 4-day estrous cycles, to determine the radioimmunoassay and RICT profiles of serum and pituitary LH throughout the estrous cycle; a marked increase in bio- and immunoactive serum LH occurred at 20 hr of proestrus. The serum LH B:I ratio was relatively constant (0.92 ± 0.08) throughout the cycle except at the time of the LH surge, when it rose to 1.5 ± 0.2 ($p < 0.01$). Pituitary LH content increased to a maximum 6 hr before the LH surge, then rapidly decreased to a nadir at the time of the surge. A decrease in the B:I ratio of pituitary LH, from 1.36 ± 0.07 at all other times of the cycle to 1.16 ± 0.16 (p 0.01) at the time of the surge, accompanied the increase in serum B:I ratio observed during the LH surge. We have previously observed an increase in plasma B:I ratio at the time of the mid-cycle LH peak in the rhesus monkey, and in the human during GnRH stimulation in the late follicular phase, near the time of the mid-cycle LH peak. These findings suggest that the preovulatory surge of LH is released from a pituitary pool of high biological activity. Since the preovulatory rise in estrogen secretion could be responsible for release of LH with high bioactivity, we analyzed serum LH bio- and immunoactivity in rats castrated at 12 hr of proestrus, prior to

the major increase in circulating estradiol. In this study, as observed previously in control animals, serum LH bioactivity rose higher than immunoactive LH levels at the time of the LH surge, with a consequent increase in B:I ratio at the time of the surge. The rise in serum estrogen preceded the peak levels of LH. After gonadectomy, a peak of serum LH occurred at the same time as in controls, but the usual increase of LH bioactivity did not occur at the time of the LH peak and no differences in the B:I ratio was observed. These studies indicate that the acute rise in estrogen could be responsible for the release of LH with high bioactivity from the pituitary into the circulation at the time of the surge, since castration at a time prior to the maximal increase in estrogen prevented the preovulatory rise in the bio:immuno ratio. In addition, castration at other times of the cycle did not cause changes in the bio:immuno ratio.

The bioactivity of circulating LH appears to be rapidly modulated by gonadal steroids, possibly via changes in glycosylation of the hormone and consequent changes in biological activity. Also, we have attempted to define changes in the properties of pituitary LH in castrated male rats that would explain the observed changes in bio:immuno ratio. Isoelectrofocusing of pituitary extracts showed consistent profiles with six discrete peaks of LH, without variation in the isoelectric point (pI) of the peaks. After electrofocusing, the radioimmunoassay pattern of control pituitaries showed two main LH peaks of almost equal magnitude (peaks II and III) with pI's of 9.2 and 9.02, respectively, and four minor peaks, I, IV, V, VI, with pI's of 9.44, 8.76, 8.64, and 8.46, respectively. A similar isoelectric pattern has been reported using column separation of pituitary LH with increased B:I ratios toward the alkaline pH. Five days after castration, there is a reduction of peak VI and an increase of peaks I and II; at 20 days all the peaks double with the exception of peak II, which is three times control levels; at 45 days, there is a pronounced increase in all the peaks, especially the minor ones with the most dramatic rise in peaks IV and V. Sixty days after castration, the profile of pituitary LH is twice the magnitude of control, but is closer to the relative profile of control pituitary LH than at any other time after castration. When the peak tubes of the individual peaks were assayed for biological activity and the bio:immuno ratios calculated, it was clearly apparent that the B:I ratio of LH becomes progressively higher with increasing isoelectric points of components, and that orchidectomy decreases the B:I ratio of all LH components by 100%. These decreases are maximal at 45 days and return to near control values at 60 days. This homogeneous decrease in bio:immuno ratio in all peaks could indicate that the carbohydrates which are concerned with the charge of the molecule do not contribute to the biological activity of LH. If they were involved, one would expect the individual peaks to maintain their B:I ratios throughout castration, with reductions in the absolute size of the more basic peaks of high B:I ratio and increases in those with the lowest B:I ratio on the less alkaline range.

2. Studies on the pulsatile release of LH.

We have now extended our evaluation on the bioactivity and radioimmunoactivity of serum samples from normal males and postmenopausal females, taken every 10 min for four hours. Bioactive LH in men and postmenopausal females showed a pulsatile release coincident with the radioimmunoactive measurements. However, the bioactivity of LH at the peak of the pulse is 2-4-fold larger than the corresponding immunoactive levels with consequent increase in bio:immuno ratio. In addition, it can be seen that the maximum B:I ratios are coincident with the peak of the LH

pulses, the highest values being 4 to 6. It is of interest that the physiological mode of LH secretion is such as to avoid the end-organ desensitization that could occur if similar concentrations of highly bioactive hormone were secreted at the peaks in a constant fashion. These studies were performed in collaboration with Dr. Franco Fraioli, University of Rome, and Drs. Janet McArthur and I. Beitins, Massachusetts General Hospital.

Significance to Biomedical Research:

The application of the RICT assay to the measurement of LH in human serum during LHRH stimulation has provided information about the qualitative and quantitative changes in the LH molecule at different stages of the menstrual cycle. Also, the application of the RICT assay to the assay of LH in monkey serum has provided the scientific community with a convenient method for the measurement of this hormone in primates. The recent development in the more sensitive RICT assay (5- to 10-fold permits measurement of LH in one hundredth of a milliliter of male samples, allowing multiple and frequent sampling) is of great value for the measurement of bioactive LH levels in children and in adults during suppression therapy. Also, this modification permitted measurement of the low circulating levels of LH in rats.

The RICT assay can be applied to different laboratories throughout the world, and is expected to give similar results when identical standards are employed. This is an improvement over certain aspects of RIA, which are subjected to the individual characteristics of each antibody employed for assay. The high sensitivity of the RICT assay will permit measurement of the levels of LH in serum from birth to puberty with good precision. This will also offer advantages over the RIA, which at these early times frequently measures levels just slightly above the lower limit of the sensitivity of the assay. This methodological approach is at present the preferred in a number of animal species including some classical animal models, since radioimmunoassays in several species is not readily available or not yet developed. This technique has clearly demonstrated that the biological activity of LH secreted in man, rhesus monkey and rat is modulated by gonadal steroids (estrogen and androgen).

Proposed Course:

1. Studies on the modulation of rat LH bioactivity by steroids, and on the biosynthesis of LH in the pituitary, will be continued.

2. Studies on rat chorionic gonadotropin. We will extend our previous observations by following serum rCG biological activity throughout pregnancy from the time of fertilization. We will also evaluate rCG in extracts of blastocyst, implantation sites and placenta and will assay the bio-immunoactivity of the pituitary throughout pregnancy. Antibodies to rat LH, hCG, oLH, rabbit LH and subunits will be screened for cross-reactivity with rCG, for the purpose of establishing an immunofluorescence method to study CG distribution and secretion. Ultimately, when the site of secretion is identified, the hormone will be isolated, chemically characterized, and employed for production of antibodies to rCG.

3. Validation of RICT assay on mink plasma for understanding of hormonal involvement during development of secondary infertility in this animal.

4. Further investigation to gain insight into the nature of the B:I ratio differences observed between the different groups of subjects will include: a) examination of different LH pools released from the pituitary during stimulation studies; b) physical characterization of serum LH of the different groups of subjects.

5. Evaluation of LH values from birth through puberty.

6. Studies on the evaluation of bioactivity of LH during pulsatile release of the hormone will be continued.

Publications:

Solano, A.R., Garcia-Vela, A., Catt, K.J., and Dufau, M.L.: Regulation of ovarian gonadotropin receptors and LH bioactivity during the estrous cycle. FEBS Letters 122: 184-188, 1980.

Solano, A.R., Garcia-Vela, A., Catt, K.J., and Dufau, M.L.: LH Bioactivity and Regulation of Ovarian Gonadotropin Receptors during the Estrous Cycle. In Schwartz, N.B., and Hunzicker-Dunn, M. (Eds.): Dynamics of Ovarian Function. New York, Raven Press, 1981, pp. 123-128.

Dufau, M.L., Nozu, K., Dehejia, A., Garcia-Vela, A., Solano, A.R., Fraioli, F., and Catt, K.J.: Biological Activity and Target Cell Actions of Luteinizing Hormone. In Motta, M. (Ed.): Pituitary Hormones and Related Peptides from Cell Biology to Clinical Applications. New York, Academic Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00150-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization and Purification of LH/hCG Receptors and Adenylate Cyclase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	K. J. Catt	Chief	ERRB, NICHD
Other	J. Wimalasena	Visiting Fellow	ERRB, NICHD
	M. Koppelman	Clinical Associate	ERRB, NICHD
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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with solubilization, characterization and purification of gonadotropin and prolactin receptors and adenylate cyclase of testis and ovary. In addition to analyzing the properties of gonadotropin receptors and adenylate cyclase, these studies will lead to an analysis of the physical and functional relationships of the binding site and the enzyme complex in the cell membrane and in solution.

Objectives:

(1) Solubilization of LH, FSH, and prolactin receptors from gonadal target cells; (2) Binding analysis of the solubilized preparations, comparative studies with particulate receptors; (3) Characterization of gonadotropin receptors; (4) Purification of LH/hCG receptors for structural analysis and antibody formation; (5) Characterization of particulate testicular and ovarian adenylate cyclase; (6) Solubilization of cyclase from testicular and ovarian membranes; (7) Relationship between receptor sites and adenylate cyclase enzyme complex; and (8) Comparative studies of adenylate cyclase complex of ovarian and testicular tissue with other cyclases (i.e., catalytic unit, water-soluble cyclase in seminal fluid).

Methodology:

Assay of particulate and soluble receptors by ligand-binding and centrifugation, millipore filtration and polyethylene glycol precipitation; adenylate cyclase assay; cyclic AMP radioimmunoassay; sucrose density gradient centrifugation; gel filtration and affinity chromatography; SDS gel electrophoresis.

Major Findings:

1. Characterization of prolactin receptors in the rat ovary. Unmasking of specific binding sites with detergent treatment.

Luteinized rat ovaries, previously shown to be a rich source of LH receptors, were analyzed for lactogenic binding sites as a potential source of receptors for purification and for investigation of receptor assembly and turnover. Parallel studies were carried out in lactating rabbit mammary preparations for comparison of binding capacities in these two target tissues. Particulate receptors (P) from the 25,000 x g pellet of homogenate, and Triton X-100 treated 240,000 x g particulate (PD) and solubilized (S) fractions of luteinized ovary and lactating mammary tissue were incubated for 16 hrs at 22°C with ^{125}I-hGH and increasing amounts of unlabeled hormones. Separation of bound and free tracer was performed by filtration for the P and PD fractions; the S receptors were precipitated twice with polyethylene glycol. In displacement studies, human growth hormone (hGH) and ovine prolactin (oPRL) were equipotent, while human placental lactogen (hPL) was 20% and ovine growth hormone (oGH) 1-3% as effective as oPRL in both ovary and mammary P and PD preparations. hGH was 6-fold more effective than oPRL in displacing ^{125}I-hGH from the S ovarian receptors, but only 16% as effective in the S mammary receptors. hPL and oGH retained the same potency relative to oPRL in both S receptors as that seen in the P fractions. The 1-5% potency of oGH relative to oPRL can be accounted for by oPRL contamination rather than invoking somatogenic binding sites. Scatchard analysis revealed a high affinity binding site with K_a (M^{-1}) = 5×10^9 in the ovary P, PD, and S preparations and a second low affinity site (about 9×10^7 M^{-1}) with high error in the P and PD preparations. The binding capacity of the high affinity site (fmol/mg protein) was 1853 ± 589(P); 3372 ± 2998(PD); and 11420 ± 1564(S), $P < 0.01$, S vs P. Mammary receptors showed a high affinity site with K_a (M^{-1}) = 7×10^9 (P); 15×10^9 (PD); and 4×10^9 (S), (differences not significant) and a low affinity site (about 10^7) with high error in P, PD, and S. The binding capacity of the high affinity site (fmol/mg protein) was 458 ± 174(P); 480 ± 162(PD); and 2801 ± 775(S), $P < 0.05$, S vs P. Luteinized rat ovaries are a richer source of lactogenic receptors per mg protein than lactating rabbit mammary gland, and are thus of value for receptor purification and

related studies. The 7- to 8-fold increase in binding capacity seen with detergent treatment in both of these tissues may represent nascent receptors unavailable for hormone binding.

2. LH-induced nucleotide binding and activation of adenylate cyclase.

Our previous studies have indicated that gonadotropin-induced binding of Gpp(NH)p in Leydig cells may represent interaction of the hormone-receptor complex with the guanyl nucleotide regulatory site. This increase in binding sites could be attributed to exposure of hindered sites or, as recently proposed, to hormone-induced exchange of guanyl nucleotides with the nucleotide species (probably GDP) that occupies the regulatory site. The latter effect would favor the binding of GTP or analogs such as Gpp(NH)p and would lead to concomitant activation of the catalytic subunit. Recent studies showed dose correlation between hormone-stimulated nucleotide binding and activation of adenylate cyclase in the Leydig cell membranes, consistent with the direct role of GTP binding in controlling the activity of the catalytic enzyme subunit during hormone stimulation.

The amount of Gpp(NH)p bound to Leydig cell membranes regulation by LH was considerably greater than the number of LH/hCG binding sites (50 pmol/mg of protein vs. 0.8 pmol/mg of protein). This difference suggests that interaction of each hormone receptor with LH/hCG could activate a large number of nucleotide regulatory units, resulting in a 50-fold magnification of the gonadotropin-binding reaction. This is in contrast with the rather small fraction of guanyl nucleotide sites involved in catecholamine action, in which a close relationship between hormone-receptor complexes and nucleotide units has been processed. The latter studies are based on indirect evidence of nucleotide release from regulatory sites under conditions in which hormone-induced dissociation of bound nucleotide can be evaluated.

Based on direct assay of nucleotide binding, our results indicate that each hormone-receptor complex could interact with a relatively large number of nucleotide regulatory units in the Leydig cell. The occurrence of such an amplification factor between hormone-receptor complex formation and nucleotide binding is relevant to recent observations on the existence of oligomers of the receptor-nucleotide protein that could allow maximal production of the activating nucleotide-GTP complex with minimal occupancy of hormone receptors. In relation to this model, the Leydig cell membrane could possess a more highly developed oligomeric receptor-nucleotide protein complex than that attributed to the liver, adipocyte, or erythrocyte adenylate cyclase system. The presence of a hormone-stimulated nucleotide binding process in Leydig cell membranes should permit more detailed clarification of the interactions between hormone-receptor complexes, guanyl nucleotides, and adenylate cyclase during the regulation of testicular function of gonadotropic hormones.

3. Cyclic nucleotides in human semen.

In agreement with earlier studies, we have found that the human seminal plasma yields considerably higher values for cyclic nucleotides when compared with spermatozoa. However, we have also shown that these values vary markedly both between different individuals and in specimens from the same individual at different times. Levels recorded for seminal plasma were 25 to 18,937 nM of cyclic AMP and 0.54 to

3.57 nM of cyclic GMP. These levels were unrelated to those of either fructose (from the seminal vesicles) or zinc and citric acid (from the prostate). We have demonstrated also that in most specimens of seminal plasma the level of cyclic AMP remained stable during a 1-hour incubation at 37°C or during prolonged storage at -40°C. Washed sperm suspensions contained 13.2 to 27.2 pmol cyclic AMP/100 million spermatozoa. Adenylate cyclase activity of human semen was confined to the spermatozoa; it was several times higher in the presence of Mn^{2+} than Mg^{2+} and was unresponsive to sodium fluoride. The latter findings are consistent with enzyme activity derived from a dissociated catalytic component of adenylate cyclase.

Proposed Course:

1. Further purification and characterization of prolactin and LH/hCG receptors from rat ovarian homogenates.

2. Development of antibodies to purified receptor proteins, and their use in analyzing receptor function and turnover.

3. Further characterization of gonadotropin-induced binding of GTP and GTP analog to the guanyl nucleotide regulatory protein.

Significance:

Characterization of LH receptors and their relationships to adenylate cyclase will assist in understanding the basic biochemical mechanisms which control the reproductive functions of the testis and ovary. Also, further elucidation of the receptor-cyclase interaction will be of general value in understanding the mechanisms of action of protein hormones.

Publications:

Dufau, M.L., Hayashi, K., Wimalasena, J., Sorrell, S., Baukal, A., and Catt, K.J.: Peptide Hormone Receptors and Control of Steroidogenesis. In Funder, J. (Ed.): "Endocrinology 1980," Proceedings of the Intl. Congress of Endocrinology, Australian Academy of Sciences, Melbourne, Australia, 1980.

Dufau, M.L., Baukal, A.B., and Catt, K.J.: Hormone-induced guanyl nucleotide binding and activation of adenylate cyclase in the Leydig cell. Proc. Natl. Acad. Sci. USA 77: 5837-5841, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00151-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Gonadotropin Receptors in Testis and Ovary

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program is directed at the analysis of peptide hormone receptors in testis and ovary, with particular reference to the control of receptor concentration by changes in homologous and heterologous hormones. Desensitization of gonadal adenylate cyclase and steroidogenic responses by gonadotropic hormones is followed by receptor loss and turnover or processing of the hormone-receptor complex. These processes are investigated in Leydig cells and ovarian luteal cells to determine the effects of hormone-induced receptor regulation on gonadal responsiveness, and to analyze the mechanism and consequences of target-cell desensitization. The receptors and direct actions of gonadotropin-releasing hormone (GnRH) in the ovary and testis are also analyzed, in relation to the recently discovered anti-gonadal effects of GnRH agonists.

Objectives:

To analyze the regulation of gonadotropin receptor biosynthesis, expression and turnover in gonadal target cells.

Methods Employed:

Receptor concentration and affinity are measured by binding studies with ^{125}I-labeled LH, hCG, hFSH, hGH (for prolactin receptors) and GnRH agonists, and [^{3}H]dihydroalprenolol for β-adrenergic receptors. Analysis of receptor binding data is performed by computer methods applied to saturation curves and Scatchard plots. Isolated luteal and Leydig cells are prepared from ovary and testis by collagenase dispersion and purification by density gradient centrifugation. Receptor binding (to LH, FSH, PRL, GnRH and β-receptors is measured in tissue homogenates and whole cells. Adenylate cyclase is measured in particulate ovarian fractions, and cyclic AMP and steroid production in dispersed luteal and Leydig cells are determined by radioimmunoassay.

(a) Hormonal regulation of testicular LH and prolactin (PRL) receptors

The effects of gonadotropic stimulation on testicular LH and PRL receptors were examined in the absence and presence of elevated serum PRL levels. In adult male rats, administration of ovine LH (oLH) caused an increase in both PRL and LH receptors within 40-120 min. This initial rise in testicular receptors was followed by transient loss and recovery of PRL sites, and a more prolonged loss of LH receptors. In contrast, the administration of bovine PRL or the elevation of circulating PRL levels during metoclopramide infusion caused no change in the lactogenic sites. A significant positive effect of increased serum PRL levels on testicular LH receptors was observed during metoclopramide infusion, but this did not prevent the loss of LH receptors caused by the administration of oLH. Similarly, prior treatment with bovine PRL failed to prevent the LH-induced loss of testicular LH receptors. These findings demonstrate that PRL and LH receptors exhibit concomitant but distinct forms of regulation during Leydig cell stimulation by oLH. Testicular receptor sites for both LH and PRL undergo a common process of increased availability during the early phase of Leydig cell activation by gonadotropins. This transient increase in both binding sites is followed by a subsequent period of depletion that is short-lived for the PRL sites and prolonged for the LH receptors. The long-term action of PRL on the maintenance of testicular LH receptors does not influence the acute increase and subsequent loss of sites that follows gonadotropic stimulation of the Leydig cells.

(b) Regulation of adrenal and testicular prolactin receptors

Receptors for PRL in the adrenal, ovary, and testis undergo marked and rapid depletion after trophic stimulation by ACTH and LH, respectively. The mechanism of such heterologous regulation of PRL receptors in the testis and adrenal cortex was further analyzed in hormone-treated male rats. Hormone-stimulated turnover of PRL receptors was not prevented by treatment with cycloheximide (75 and 300 μg), which caused an 80% fall in testicular and adrenal sites within 1 h, followed by a return to normal in 24 h. When

steroid secretion was inhibited by treatment with 4-aminopyrazolopyrimidine for 4 days, with a 90% decrease in serum cholesterol, ACTH and LH retained the ability to reduce PRL receptors by 60-70% 12 and 24 h after injection. Similarly, during blockade of steroidogenesis by aminoglutethimide, treatment with 10 U ACTH or 100 µg ovine LH still reduced PRL receptors by about 70%. When adrenal and testicular steroidogeneses were stimulated by the administration of 8-bromo cAMP, serum corticosterone and testosterone were increased by 10-fold after 1 h, but there was no decrease in PRL receptors at the later times (12 and 24 h) when ACTH and LH caused marked receptor depletion. However, an early increase of 20-40% in PRL receptors was observed 1 h after treatment with 8-bromo cAMP, corresponding to the phase of increased steroid secretion. Similar increases in PRL receptors were evident in the adrenal and testis 1 h after treatment with the respective trophic hormones, again when steroid secretion was elevated. These early changes in adrenal and testicular PRL receptors after hormonal stimulation were completely inhibited in aminoglutethimide-treated rats, indicating their dependence on steroid secretion. These findings demonstrate that PRL receptors undergo rapid depletion in rat adrenal cortex and testis after trophic hormone stimulation. The mechanism by which ACTH and LH cause depletion of PRL receptors does not appear to depend upon cAMP-mediated responses, and may represent a form of concomitant receptor processing at the cell membrane level. The early increase in adrenal and testicular PRL receptors caused by trophic hormones and 8-bromo cAMP occurs during the phase of increased steroid secretion, and probably reflects changes in membrane conformation that accompany the steroid secretory response.

(c) Positive regulation of testicular LH receptors

Subcutaneous injections of ovine LH (oLH) caused an acute increase of rat testis LH receptor sites, as measured by in vitro binding of ^{125}I-labeled hCG. There was a concomitant increase in testicular lactogen receptors, and both sets of sites reached a maximum 1 h after hormone injection. The increase was transient, and binding returned to the control level in 1.5-2 h. With increasing doses of oLH, hCG binding increased gradually and was significantly elevated with doses of 30-1000 µg. The maximum increase in hCG binding averaged 44% when measured in testicular membrane preparations, and 93% in collagenase-dispersed Leydig cells. The enhanced binding was due to a true increase in the number of binding sites, with no change in the equilibrium association constant (Ka). The increased binding coincided with the rapid increase in plasma oLH and testosterone levels, as well as with the oLH-induced stimulation of testicular testosterone and cAMP formation measured in vitro. During the maximum increase in hCG binding, the testosterone response of Leydig cell suspensions to hCG stimulation in vitro was slightly reduced, but the dose-response curve for cAMP production was unchanged. The maximum production rates of testosterone and cAMP were unchanged during the phase of increased receptor binding. Treatment with cytochalasin B, an inhibitor of microfilament function, and with aminoglutethimide, an inhibitor of steroid hormone biosynthesis, abolished the positive effect of oLH on subsequent in vitro [^{125}I]iodo-hCG binding. However, colchicine, a microtubule inhibitor, had no effect on the positive receptor regulation by oLH. These findings demonstrate that the transient increase in LH binding that accompanies the testosterone and cAMP responses to LH is dependent on intact

microfilament function and steroidogenesis. The short time course of the increased binding and the simultaneous increase of both LH and lactogen receptors suggest that the phenomenon may be related to LH-induced changes in cell membrane conformation. Such changes could lead to exposure of cryptic receptor sites on the surface of hormone-stimulated Leydig cells.

(d) Effects of hypoprolactinemia on gonadotropin and lactogen receptors.

The effect of 2-bromo-α-ergocriptine (BR)-induced hypoprolactinemia on the induction and maintenance of testicular gonadotropin and lactogen receptors was studied in 60-day-old rats after receptor regulation by a gonadotropin-releasing hormone agonist analog [D-Ser(tBu)^{6}]des-Gly^{10}-GnRH N-ethylamide (GnRH-A)] and in young animals during sexual maturation. In adult animals, BR treatment delayed the reappearance of LH binding in the testis after GnRH-A injection, but had no effect on the recovery of lactogen binding. BR treatment also inhibited the increase of LH binding that occurred in control animals during the experimental period, but did not affect lactogen binding. Furthermore, BR potentiated the effect of GnRH-A on the decrease of Leydig cell testosterone synthesis observed 2 days later in vitro. Eight days after GnRH-A injection, concomitant BR treatment significantly inhibited the recovery of Leydig cell cAMP production. In peripubertal (25- to 47-day old) animals, BR diminished the normal rise of testicular LH receptors, but did not affect the increase in lactogen receptors. Serum testosterone levels and other features of pubertal development, such as balano-preputial separation and spermatogenesis, were unaffected by hypoprolactinemia. In neonatal female animals, significant lactogen binding was detected at 3 days of age, whereas hCG binding was not demonstrable until 9 days after birth. These findings indicate that the expression of lactogen receptors precedes that of LH receptors in the developing gonad, and that the increase of LH binding in the testis during pubertal development requires normal circulating PRL levels. In adult animals, hypoprolactinemia potentiates GnRH-A-induced desensitization of steroidogenesis and cAMP formation, as well as LH receptor down-regulation, delaying the recovery of these phenomena. Although decreased serum PRL levels were associated with a marked reduction in testicular LH receptors both during development and in adult life, the absence of changes in lactogen receptors indicates that the latter sites are largely independent of the circulation PRL concentration.

2. Regulation of ovarian receptors and responses

(a) Direct effects of GnRH upon cyclic nucleotide production in rat granulosa cells.

GnRH mediates the release of FSH and LH from the anterior pituitary and is thus responsible for the hypothalamic regulation of gonadal function under physiological conditions. An additional and unexpected action of GnRH in the ovary has been indicated by recent reports that GnRH also exerts direct, antigonadal effects in vivo and in isolated ovarian cells. These extra-pituitary actions of GnRH include inhibition of FSH-induced steroidogenesis and LH receptor formation in granulosa cells, and blockade of ovulation and ovarian weight gain in intact animals stimulated with gonadotropins. The mechanism of these inhibitory responses in the gonads has not been identified.

The hormonal activation of steroidogenesis in granulosa cells is mediated through the adenylate cyclase system, and exogenous FSH influences the formation of both cAMP and cGMP in granulosa cells in vitro. To determine whether the antigonadal actions of GnRH in granulosa cells are related to alterations in either of the cyclic nucleotides during hormonal stimulation, production of cyclic nucleotides and progesterone was analyzed during culture of rat granulosa cells with FSH and GnRH agonists. FSH stimulated the production and accumulation of both cAMP and cGMP, as well as progesterone, during a 48-h incubation period. Addition of GnRH or an agonist analog, [D-Ala^6]des-Gly^{10}-GnRH N-ethylamide (GnRHa), did not influence the cyclic nucleotide response to FSH in the first 6 h of incubation, but caused dose-dependent inhibition of the FSH-induced rise in cyclic nucleotide production from 24 to 48 h of incubation. Cellular production of both cyclic nucleotides and progesterone was decreased by GnRHa concentrations as low as 10^{-12} M, with maximum inhibition at 10^{-9} M GnRHa. These results suggest that the in vitro antigonadal actions of GnRH and related peptides are expressed through inhibition of cyclic nucleotide production.

(b) Hormonal regulation of cytodifferentiation in cultured granulosa cells.

Differentiation of granulosa cells of the ovarian follicle in vivo is accompanied by the sequential development of plasma membrane receptors for FSH, LH and prolactin. Other morphological and functional changes that occur during granulosa cell differentiation include follicular antrum formation and development of intercellular junctions, as well as progesterone secretion. We have recently shown that ovarian tissue contains receptors for gonadotropin-releasing hormone (GnRH). Also, GnRH agonists block gonadotropin-induced formation of LH receptors and progesterone production in both granulosa cells and luteal cells. These findings indicate that hypothalamic peptide hormones can exert a direct effect on ovarian function, though the mechanisms of LH receptor induction and differentiation of granulosa cells by FSH and its inhibition by GnRH remain to be defined. Recently, induction of LH receptors by FSH has been described in cultured granulosa cells obtained from hypophysectomized diethylstilbestrol (DES)-treated immature rats. We have employed this system to analyze the morphological and biochemical changes associated with the process of granulosa cell differentiation in vitro. Such cultured granulosa cells displayed pronounced intracellular and intercellular changes after 48 hr of exposure to follicle-stimulating hormone (FSH) in vitro. As determined by light and electron microscopy, most of the FSH-treated cells became highly aggregated and grew in multilayered clusters. Numerous gap junctions were seen between cells, indicating the presence of significant intercellular communication. Microvilli densely covered the surface of the hormone-stimulated cells, which contained enlarged mitochondria with convoluted cristae, characteristic of steroidogenic cells. Luteinizing hormone receptors, identified by autoradiography with ^{125}I-labeled human chorionic gonadotropin, were mainly associated with aggregated cells, whereas single cells were usually free of the labeled hormone. Addition of a gonadotropin-releasing hormone agonist prevented the appearance of luteinizing hormone receptors and markedly impaired cyclic AMP and progesterone production, as well as the morphological changes induced by FSH. The majority of the granulosa cells grown in the absence of either hormone assumed a flattened,

smooth shape and grew primarily in monolayers. These results have shown that FSH-treated granulosa cells undergo pronounced changes in vitro that are similar to the in vivo process of differentiation. These include the development of LH receptors and the capacity for progesterone and cyclic AMP formation, as well as cell association and intercellular communication. This work has also demonstrated that GnRH agonists inhibit the specific morphological and biochemical processes that are associated with FSH-induced granulosa cell differentiation.

Significance:

These findings have shown new aspects of the regulation of receptor concentration and receptor-mediated processes in peptide hormone target tissues. The results are of general application to protein hormone receptors, and of particular relevance to the control of gonadal cell function and reproductive processes.

Proposed Course:

The mechanism by which homologous and heterologous hormones influence receptor turnover in the plasma membrane will be further investigated in the gonads. Combined biochemical and morphological studies on LH and PRL receptor regulation will be performed in the Leydig cell. The mechanism of the inhibitory actions of GnRH agonists on gonadal function will be further analyzed in cultured granulosa and interstitial cells.

Publications:

Amsterdam, A., Knecht, M., and Catt, K.J.: Hormonal regulation of cytodifferentiation and intercellular communication in cultured granulosa cells. Proc. Nat. Acad. Sci. USA. 78: 3000-3004, 1981.

Amsterdam, A., Naor, Z., Knecht, M. Dufau, M.L., and Catt, K.J.: Hormone action and receptor redistribution in endocrine target cells: Gonadotropins and gonadotropin-releasing hormone. In Middlebrook, J.L., Kohn, L.D. (Eds.): Receptor-Mediated Binding and Internalization of Toxins and Hormones. New York, Academic Press, 1981, pp. 283-310.

Catt, K.J., Harwood, J.P., Clayton, R.N., Davies, T.F., Chan, V., Katikineni, M., Nozu, K., and Dufau, M.L.: Regulation of peptide hormone receptors and gonadal steroidogenesis. Recent Progress in Hormone Research. 36: 557-622, 1980.

Chan, V., Katikineni, M., Davies, T.F., and Catt, K.J.: Hormonal regulation of testicular luteinizing hormone and prolactin receptors. Endocrinology. 108: 1607-1612, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HD 00152-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization and Radioligand Assay of Gonadotropin Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other	I. Huhtaniemi	Visiting Scientist	ERRB, NICHD
	M. Katikineni	Staff Fellow	ERRB, NICHD

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0.5	0.5	

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the development and use of analytical procedures for identification and characterization of gonadotropin receptor sites in the testis and ovary. Methods of gonadotropin labeling, characterization of the labeled tracer, and optimization of receptor assay methods, are evaluated and improved. Computerized methods for analysis of binding data, from kinetic and equilibrium experiments, plus corrections for the tracer characteristics, are applied to studies on the properties of gonadotropin receptors. Native and modified gonadotropins are evaluated for receptor binding activity, and receptors are analyzed in animal and human tissues.

PHS-6040
(Rev. 2-81)

Objectives:

To characterize the binding properties of gonadotropin receptors in gonadal cells, homogenates and membrane preparations. Also, to develop improved radioligand-receptor assays for LH/hCG and FSH, to apply such methods to assays of gonadotropin activity and structure-function studies on chemically modified gonadotropins, and to analyze the species specificity of gonadotropin receptors.

Methods Employed:

Quantitative binding studies with labeled gonadotropins. Radioligand-receptor assays of gonadotropins. Affinity chromatography upon receptor sites. Computer analysis and curve-fitting of receptor binding data. In vitro bioassay of LH in dispersed Leydig cells.

Major Findings:

1. Decreasing reversibility of hCG:receptor complexes.

The interaction of LH and other hormones with specific receptor sites is accompanied by a progressive change in the degree of reversibility of the hormone-receptor complex. This process was investigated by analysis of in vitro binding of [^{125}I]hCG to 15,000xg fractions of rat testis homogenate. During the first hour of incubation at 37°C, dilution and washing of the particles gave about 20% less specific binding than when particles were incubated with excess unlabeled hormone for a further 1 h before dilution and washing. This effect was no longer demonstrable after 4 h, suggesting that the hormone-receptor complex became less reversible to dilution with time. A decrease in reversibility was also noted when hormone-receptor complexes formed by preincubation with [^{125}I]hCG at 37 C were dissociated with $MgCl_2$ at successive intervals after binding. Thus, 90% of the complexes formed in the first 10 min could be dissociated by 1 M $MgCl_2$, whereas about 50% of the complexes was dissociable at 10-12 min, and only 20% was dissociable at 120 min. In testis fractions equilibrated with 1 nM unlabeled hCG at 24°C, available LH receptors decreased to 30% over 16 h, and the proportion of occupied sites that could be dissociated with 2 M $MgCl_2$ also showed a serial decrease. The apparent loss of LH sites during prolonged incubation was attributable to occupancy by progressively less dissociable hormone. The absence of true LH receptor loss during exposure to gonadotropin in vitro at 24°C suggests that receptor regulation is a function of the intact, metabolically active Leydig cell. The progressive formation of LH-receptor complexes with increased resistance to dissociation was temperature dependent, being more rapid at 37°C, and was coincident with the early biological actions of the gonadotropins. The progression from loose to tight binding of gonadotropins in the testis may represent an initial step in the processing and degradation of the hormone-receptor complex.

2. Receptor binding affinities of LH and hCG.

Specific binding of hCG, human LH (hLH), and ovine LH (oLH) to LH receptors was analyzed in rat testis homogenate. Equilibration studies with

each of the gonadotropins indicated that the three hormones bind to a common receptor with similar binding capacity for each hormone. Scatchard analysis of the binding data revealed a single set of binding sites for hCG and hLH, with an equilibrium association constant (K_a) of 7.6×10^{10} M^{-1} for hCG and 2.5×10^{10} M^{-1} for hLH. In contrast, two sets of binding sites were found for oLH, about 20% with a mean K_a of 7.0×10^{10} M^{-1} and the remainder with Ka of 0.08×10^{10} M^{-1}. Kinetic binding experiments gave similar association rate constants (k_1) of 3.4, 4.0, and 4.0×10^{8} M^{-1} min^{-1} for hCG, hLH, and oLH, respectively. In dissociation experiments, the half-times of bound hCG, hLH, and oLH were 25, 9.2, and 2.1 h, respectively, and two dissociation components were seen with each of the three hormones. Binding-inhibition studies with [^{125}I]iodohCG, -hLH, or -oLH tracers gave parallel and steep displacement curves for hCG and hLH, while those of oLH were shallower, consistent with binding of the ovine hormone to two sets of receptor sites. When testis LH-receptors were solubilized, the nonparallelism of hCG and oLH binding inhibition curves was significantly decreased. Although the quantitative binding of hCG, hLH, and oLH to rat testis LH/hCG receptors is similar, there are differences in the binding affinity of the three hormones. The high affinity of hCG is mainly due to its low dissociation rate constant, and contributes to the high bioactivity of the chorionic hormone. In contrast, sheep LH binds to two functional sets of LH receptors, the majority of which exhibit low affinity for the ovine hormone.

Significance:

Radioligand-receptor systems are of considerable value for hormone assay, and for structure-function studies on hormone molecules and receptor sites. The progressively more tight binding of hCG to its receptors may favor internalization of the hormone-receptor complexes in testis and ovary. The high binding affinity of hCG for gonadal receptors with its prolonged circulating half-life, renders hCG a super-agonist among the lutotropins.

Proposed Course:

Studies on the thermodynamic properties and processing of hormone-receptor complexes will be pursued. Radioligand-receptor assays and *in vitro* bioassay will be applied to the analysis of native and modified gonadotropins. The basis of the species specificity of the primate LH receptor, and the characterization of the primate FSH receptor, will be examined in rhesus and human gonads.

Publications:

Huhtaniemi, I.T., and Catt, K.J.: Differential binding affinities of rat testis luteinizing hormone (LH) receptors for human chorionic gonadotropin, human LH, and ovine LH. Endocrinology. 108: 1931-1938, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00160-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biology of the Adrenocortical Cell: Molecular Events in Steroid Biosynthesis and Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	ERRB, NICHD
Other	Tetsuo Nishikawa	Visiting Fellow	ERRB, NICHD

COOPERATING UNITS (if any)
None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-term aim of this project is to explore the biology of the steroid-synthesizing cell. There are several areas of uncertainly as to the molecular events involving 1) initiation, propagation, and termination of steroid synthesis, 2) intercompartmental movement of steroid precursors and intermediates, 3) secretion of steroid products. Other areas of uncertainty involve 1) the interaction of subcellular organelles, e.g., mitochondria, endoplasmic reticulum, Golgi apparata, microtubules, microfilaments, lipid droplets, etc. 2) cell-cell interaction or zone-zone interaction (different cells are programmed to secrete predominaely one type of steroid, i.e., glucocorticoid, mineralocorticoid, and sex steroids), 3) life-span and turnover of adrenocortical cells. Current areas of research involving: 1) identification, characterization, and physiology of noncatalytic proteins (membrane bound and/or soluble) of the adrenal cortex that interact with specific steroid ligands; 2) examination of the role of microtubules and contractile proteins in steroid synthesis; 3) identification, characterization and physiology of adrenocortical secretory proteins; 4) examination of soluble stimulatory and inhibitory factors.

PHS-6040
(Rev. 2-81)

Project Description

I. Non-catalytic steroid-binding proteins.

A. Rationale: The rate-limiting step in steroid synthesis in considered to be the conversion of cholesterol to pregnenolone (i.e., the cholesterol lyase reaction). This reaction occurs at an intramitochondrial site (possibly associated with the outer membrane). Cholesterol, which is an absolute requirement for steroid synthesis, is a ubiquitous sterol, being found in membranes and stored esterified to fatty acids in cytoplasmic lipid droplets; it can also be made available through de novo synthesis and extraction from the circulation. The exact location and pool size of cholesterol utilized in steroid synthesis are not known. It is presumed, however, that the principal source of cholesterol is extramitochondrial. If this is true, then there must be a mechanism for transporting cholesterol into the mitochondrion where the cholesterol lyase is located. The molecular events involved in this transport are not known. Similarly, pregnenolone, the product of the rate-limiting step must move to specific extramitochondrial sites for further enzymatic alterations leading to the formation of cortisol, aldosterone, or one of the sex steroids. The molecular events involved in this transport are also not known. It is conceivable that specific proteins acting as transport vehicles are involved.

B. Major Findings: Using standard techniques of subcellular fractionation and methods of protein separation, six specific proteins have been identified to date.

1. Cholesterol/cholesterol sulfate-binding protein No. 1. This is a heat stable protein which has been partially characterized. The initial characterization studies have been published in the Journal of Steroid Biochemistry, 9:721-730, 1978.

2. Cholesterol/cholesterol sulfate-binding protein No. 2 This is a heat sensitive protein whose initial characterization has been published in the Journal of Steroid Biochemistry, 13:73-82, 1980. Purification of these two proteins is now being undertaken.

3. 20 α-hydroxycholesterol-binding protein. This project remains undeveloped.

4. Pregnenolone-binding protein. This is a heat labile protein whose initial characterization has been published in the Journal of Biological Chemistry, 252: 464-470, 1977. Purification of this protein is currently being completed.

5. Pregnenolone sulfate-binding protein. This is a heat labile protein (clearly distinct from the pregnenolone-binding protein) whose initial characterization was published in Biochemistry, 17: 4557-4563, 1978. Purification of this protein is in progress.

6. 21-hydroxypregnenolone-binding protein. This is a heat labile protein whose initial characterization is being prepared for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00165-06 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Isolation and Characterization of Protein Hormones and Other Active Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	A. Chrambach	Senior Investigator	ERRB, NICHD
Other	N. Y. Nguyen	Visiting Scientist	ERRB, NICHD
	B. Ribas	Guest Worker	University of Madrid Asst. Professor 1
	S. Ben-Or	Guest Worker	The Hebrew University 2 Assoc. Professor
	R. E. Grindeland	Collaborator Senior Investigator	Ames Research Center, NASA 3

COOPERATING UNITS (if any)
1) Department of Biochemistry, Universidad Complutense de Madrid, Faculty of Pharmacy, Madrid - 3, Spain. 2) Dept. of Physiology, Hadassah Medical School, The Hebrew University, Jerusalem, Israel. 3) Moffett Field, CA.

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) Human growth hormone isohormone B is needed as a starting material for enzymatic digestion leading to bioactivation, and as a bioactivity control. A procedure for isolation of hGH-B (28%) from a mixture with isohormones C, D and E (72%) was elaborated, using isotachophoresis at acid pH. 2) Metallothionein, a Zn and Cd binding peptide from liver and brain, was characterized in a crude homogenate as a single charge isomeric species, existing in 3 size isomeric forms. 3) Glucocorticoid receptor from chick embryonic retina, purified by sucrose density sedimentation, was found to exhibit protein-protein interactions indistinguishable from the crude solubilized receptor.

PHS-6040
(Rev. 2-81)

1) Preparation of human growth hormone isohormone B by isotachophoresis at acid pH (in collaboration with N. Y. Nguyen and R. E. Grindeland):

Objectives: To work out a procedure for the isolation of hGH-B from a mixture with isohormones C, D and E, to obtain a suitable starting material for enzymatic activation as well as clinical control material.

Methods employed: Since application of the recently elaborated isolation procedure for hGH-E operative at neutral pH (Nguyen, N.Y., et al., Preparative Biochem. 11:139-157, 1980) to the isolation of hGH-B would be excessively time-consuming, its isolation was attempted at acid pH, using competitively either selective steady-state stacking or isotachophoresis.

Major findings: Selective stacking of hGH-B does not result even from extreme refinement of the Lower Stacking Limit. Thus, isotachophoresis only is applicable. Both yield and purity of hGH-B are sufficient for preparation of a starting material for enzymatic digestion, but not as homogeneous control hormone. However, stacked hGH was found to acid precipitate easily, even at very low voltages, making this a somewhat uncomfortable procedure, although yield, product purity and bioactivity are adequate.

Significance to biomedical research: The possibility of isolating hGH-B prior to enzymatic bioactivation will allow one to obtain a realistic yield value for the isolation of bioactivated hGH-E by isotachophoresis (Nguyen, N.Y., et al., Preparative Biochem. 11: 139-157, 1980). Once this yield value can be shown to be acceptably high, we are justified in proceeding to the preparation of hGH-E in amounts sufficient for clinical analysis.

Proposed course: In view of the acid precipitation of stacked hGH, we will competitively attempt the isolation of hGH-B by chromatofocusing.

2) Characterization of Cd-metallothionein from various sources (in collaboration with B. Ribas):

Objectives: Metallothionein is a well-characterized, sequenced binding protein for Cd and Zn. But the charge isomeric and aggregate forms in which the peptide occurs in tissues is unknown, and so is the distribution of these forms in different tissues. This study aimed at elucidating these points.

Methods employed: Quantitative polyacrylamide gel electrophoresis of crude extracts of the binding protein labeled with Cd in vivo and obtained from liver and brain.

Major findings: In contrast to isolated metallothionein, crude extract of liver or brain does not contain more than one charge isomeric forms. However, the crude liver extract contained three hitherto unrecognized aggregate forms of the peptide.

Significance to biomedical research: An inventory of active forms of the binding protein is needed to locate the physiological sites for Zn-detoxification, to recognize clinical deficiencies by the component pattern of the binding protein, and to be able to evluate their binding constants.

Proposed course: Extend the tissue inventory of metallothionein forms, measure their binding constants on gels (using methods previously developed for estrogen-testosterone binding protein), and isolate the most active form for metabolic studies.

3) Characterization of solubilized glucocorticoid receptor prefractionated by sucrose density sedimentation (in collaboration with S. Ben-Or):

Objectives: The analysis of the solubilized receptor system by quantitative polyacrylamide gel electrophoresis (Ben-Or and Chrambach, A., Archives Biochem. Biophys. 206:318-330, 1981) suffers from protein-protein interactions at the high concentrations of either the stack or the gel surface. An attempt was made to avoid these interactions by separating the interacting species on the basis of their known size differences, using sedimentation, prior to gel electrophoresis.

Methods employed: Sucrose density sedimentation, followed by quantitative polyacrylamide gel electrophoresis under strict temperature control at 0 degrees.

Major findings: Pre-purification of the solubilized receptor species by sedimentation does not in any way alter the protein-protein interactions revealed by the gel patterns.

Significance to biomedical research: A catalog of receptor-active species is required in the study of the protein chemical changes concomitant with acquisition of receptor function at 15 days of embryonic development in the chick.

Proposed course: It is planned to interfere with the protein-protein interactions on the gels without sacrifice of more than 1/2 of the biological activity of the solubilized receptor by use of the cholic acid analogues with amphoteric or hydroxyl functions (Hjelmeland, 1981) as dissociating agents of hydrophobic interactions and by decreasing protein concentration within the zones through application of agarose gels.

Publications:

Nwokoro, N., Chen, H-C., and Chrambach, A.: Physical, Biological and Immunological Characterization of Highly Purified Urinary Human Chorionic Gonadotropin Components Separated by Gel Electrofocusing. Endocrinology. 108:291-300, 1981.

Ben-Or, S., and Chrambach, A.: Multiple Forms of Glucocorticosteroid Receptors in the Neural Retina of the Chick Embryo, Revealed by Polyacrylamide Gel Electrophoresis. Archives Biochem. Biophys. 206: 318-330, 1981.

Kapadia, G., Vaitukaitis, J., and Chrambach, A.: One-Step Isolation of Human Chorionic Gonadotropin in Milligram Amounts, using Selective Steady-State Stacking on Polyacrylamide Gel. Prep. Biochem. 11:1-22, 1981.

Nguyen, N.Y., Baumann G., Arbegast D.E., Grindeland, R.E., and Chrambach, A.: Isolation of human growth hormone isohormones D and E in milligram amounts (I), using isotachophoresis on polyacrylamide gel. Preparative Biochem. 11:139-157, 1980.

Nguyen, N.Y., and Chrambach, A.: Protein composition of plasmin preparation "Homolysin". Preparative Biochem. 11:159-172, 1980.

Nguyen, N.Y., Grindeland, R.E., and Chrambach, A.: Isolation of human growth hormone isohormones D and E in milligram amounts (II), using isoelectric focusing on polyacrylamide gel. Preparative Biochem. 11:173-189, 1980.

Salokangas, A., Eppenberger, U., and Chrambach, A.: Isolation of active cAMP dependent protein kinases from calf ovaries: Gel electrophoresis vs. gel electrofocusing. Preparative Biochem. 12:299-320, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00170-05 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Randomized Study for the Treatment of Chromophobe Adenomas of the Pituitary Gland with Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	NICHD, ERRB
Other	Jesse Roth	Chief	NIAMDD, DB

COOPERATING UNITS (if any)
None

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Endocrinology and Reproduction Research Branch

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Office of the Chief

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NICHD, NIH, Bethesda, Maryland 20205

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0.25	0.25	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the natural history of chromophobe adenomas unassociated with visual impairment is uncertain, the approach to therapy in patients with this disorder is controversial. Complications often attributed to tumor growth may, in fact, be sequalae of the treatment administered, whether it be radiotherapy or surgery. The purpose of this project is two-fold: 1) to observe the natural history of untreated chromophobe adenomas in patients who do not present initially with visual disturbances and 2) to compare their clinical course with that of patients treated by one commonly employed modality, i.e., radiation therapy. Thus, patients will be randomized into two groups: one group will receive no therapy and one group will be treated with conventional pituitary irradiation. In a sense, each group is serving as a control for the other group. Over the study period specific clinical and laboratory abnormalities will be assessed in each group and compared in an attempt to help guide physicians in the treatment of this disorder.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00171-05 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Other PAGE Instrumentation and Procedures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	A. Chrambach	Senior Investigator	ERRB, NICHD
Other:	L. Hjelmeland,	Staff Fellow	DPB, NICHD
	B. Bunow,	Staff Fellow	DCRT, NIH
	B. An der Lan,	Staff Fellow	DBP, FDA
	A. Newby,	Lecturer	University of Cambridge 1
	J. Sullivan,	Engineer	DRS, BEIB

COOPERATING UNITS (if any)
Cooperating units: 1) Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QR, UK.

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Summary of work: 1) A monograph on gel electrophoretic methods was written (first draft 3/4 finished at this time). 2) Electrophoretic methods in detergent containing buffers were reviewed. 3) Electrophoretic methods for the characterization of native membrane proteins were reviewed. 4) A study aiming at a theory of pH gradient dynamics and at computer simulation of isoelectric focusing was initiated. 5) I edited the chapter on proteins for "Electrophoresis...Applications" (Elsevier) which summarizes for the various types of proteins the techniques, benefits and problems in gel electrophoretic analysis. 6) I reviewed Quantitative PAGE "for export" in a German journal. 7) A rapid destaining method for SDS-PAGE under conditions of protein fixation was developed.

PHS-6040
(Rev. 2-81)

Project description

1) Monograph on gel electrophoresis: The methodology of gel electrophoresis developed by our laboratory has at this time matured to comprise an objectively defined separation strategy, a sufficient armory of instrumentation and procedures, including computer programs, and the elements of a generally applicable procedure for protein isolation in milligram amounts. However, this methodology is presently available only in form of 134 papers. It remains therefore unavailable to the biochemist in need of non-arbitrary, optimized approaches to protein separation. We are therefore in the process of summarizing the needed information under a single cover.

2) Review of detergent electrophoresis (in collaboration with L. M. Hjelmeland): The possibility for characterization and isolation of native hydrophobic proteins has recently extended the usefulness of gel electrophoretic methods. It appeared necessary to succinctly define the extent and the limits of that usefulness, and to provide guidelines for a rational use of these methods.

3) Strategy for the gel electrophoretic analysis of native membrane proteins (in collaboration with A. Newby): The rationales and tools of the electrophoretic analysis of native membrane proteins needed to be summarized in a chapter of a series of monographs addressed to the membrane biochemists who remain largely unfamiliar with material hitherto mostly published in the methodological literature. In particular, multiphasic buffer systems of both polarities fitting the characteristically slowly migrating membrane proteins were included.

4) Theory of pH gradients (in collaboration with B. Bunow and L. M., Hjelmeland): Isoelectric focusing remains burdened by the uneven field strength across the pH gradient which may obscure the isoelectric position of a protein, and by the binding of carrier ampholytes of MW in excess of 700 to proteins. Buffer electrofocusing (Chrambach, A. and Nguyen, N.Y., In Electrofocusing and Isotachophoresis (B.J. Radola and D. Graesslin, eds.), Walter de Gruyter, Berlin-New York, 1977, 51-58). would allow one to overcome these impediments, but its conditions can at present be explored only experimentally and therefore rather inefficiently. Computer simulation of pH gradients is clearly needed to predict and select pH gradients for any particular separation by isoelectric focusing. Present theory is static, i.e. it neglects the continuous changes in carrier ampholyte patterns along the pH gradient (Baumann, G. and Chrambach, A., In "Progress in Isoelectric Focusing and Isotachophoresis" (P.G. Righetti, Ed.) Elsevier, Excerpta Medica, North Holland, Assoc. Sci. Publ., Amsterdam, 1975, p.13-23). We have therefore begun to devise a theory of pH gradient dynamics, a computer program for pH gradient prediction and experimental testing of the predictions.

5) Editing of the chapter on proteins for "Electrophoresis. A Survey of Techniques and Applications. Part B. Applications" (Z. Deyl, Czechoslovak Academy of Sciences, editor-in-chief). This chapter summarizes, for the first time, the impact gel electrophoresis has had on fifteen areas of protein biochemistry, the specific techniques developed for these areas and their problems.

6) Review of Quantitative PAGE in a German journal (Zeitschrift fuer Analytische Chemie). Purpose of this activity was to bridge for my field between the English language literature the communication gap between German scientists, and in particular the graduate students and younger colleagues in all but the very top institutions, with regard to know-how published in the English language.

7) A destaining device for SDS-PAGE (in collaboration with B. An der Lan and J. Sullivan):

Objectives: SDS-PAGE suffers at present from use of inefficient, partial modes of protein fixation and slow destaining. An attempt was made to utilize efficient TCA-fixation in SDS-PAGE, and at the same time to accelerate the staining procedure by removing SDS by charcoal prior to application of a staining procedure which does not require any destaining.

Methods employed: Construction of a TCA-resistant perforated cylinder for destaining in 12.5% TCA, 45% methanol.

Major findings: Even very hydrophobic SDS-proteins fix effectively in 12.5% TCA, 45% methanol, while the CMC of SDS is sufficiently augmented to allow for rapid diffusion of SDS from the gel. After adsorption of SDS on charcoal, the 1.5 h Diezel procedure for staining of proteins in 12.5% TCA can be applied. Total staining time: 5-6 h.

Significance to biochemical research: Time saving and increased pattern accuracy in SDS-PAGE.

Proposed course: Patenting of procedure for the NIH; development of a streamlined design of the destaining cell for commercial exploitation of the procedure by the NIH.

Publications:

Jackiw, B.A., and Chrambach, A.: Natural pH gradients formed by two and three aminoacids on polyacrylamide gel: Changes of pH, segmental voltages and aminoacid distributions with time. Electrophoresis. 1:150-154, 1980.

Hjelmeland, L.M., and Chrambach, A.: Electrophoresis and electrofocusing in detergent containing media: A discussion of basic concepts. Electrophoresis. 2:1-11, 1981.

Hjelmeland, L.M., Allenmark, S., An der Lan, B. Jackiw, B.A., Nguyen, N.Y., and Chrambach, A.: Electrophoresis in gels containing zwitterionic groups. Electrophoresis. 2:82-90, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00174-05 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hormonal Control of Ovarian Proteoglycan Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
	M. Yanagishita	Visiting Fellow	ERRB,
Other	V. C. Hascall	Senior Investigator	LB, NIDR

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NIDR

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hormonal factors which stimulate the biosynthesis of proteoglycans by rat ovarian granulosa cells in vitro have been examined in detail. LH, FSH, and HCG have been shown to stimulate proteoglycan synthesis. The identity of these proteoglycans has been determined, and the dose response curves have been characterized. In addition, the effects of estradiol, testosterone, various prostaglandins, and cyclic AMP have been studied in this system. The results provide a necessary background for further studies of the physiology of biosynthesis of ovarian proteoglycans in normal and abnormal states.

PHS-6040
(Rev. 2-81)

Objectives:

These studies are intended to examine the biochemical mechanisms of actions of ovarian responsiveness to follicle stimulating hormone, luteinizing hormone, human chorionic gonadotropin, and other gonadotropins. In addition, the responsiveness of the ovarian granulosa cell to a variety of sex steroid hormones and prostaglandins is examined.

Methods Employed:

1. Methods for isolation and characterization of proteoglycans from rat ovarian follicular fluid, including cesium chloride density gradient sedimentation under dissociating conditions, gel filtration, and related techniques.

2. In vitro short term culture of ovarian granulosa cells from immature rats stimulated with pregnant mares serum gonadotropin (PMSG).

Major Findings:

Ovarian granulosa cells were obtained from antral follicles of immature rats after stimulating animals with PMSG in vivo, and maintained in short term cell cultures. Proteoglycan synthesis by granulosa cells was monitored by including radioactive sulfate in the culture medium. Two distinct species of proteoglycans were observed in the cell culture medium. The larger proteoglycan (MW=3 x 10^6), which constituted approximately 60% of total proteoglycans synthesized in control cultures, had high buoyant density (>1.5 g/ml) and the smaller proteoglycan (MW=1 x 10^5), which constituted the rest, 40%, had a lower buoyant density (>1.5 g/ml). The gonadotropins (LH, FSH and hCG) stimulated exclusively the production of the smaller, low buoyant density proteoglycans up to 350% of control levels. These hormones exerted their stimulatory effects on the proteoglycan synthesis at concentrations similar to those required for other in vitro biological responses of granulosa cells. The similar stimulatory effects observed by the treatment using a cAMP analog and a phosphodiesterase inhibitor suggest that the stimulation of proteoglycans by gonadotropins is mediated, at least in part, by cAMP system. Further, testosterone and prostaglandin E_1 and E_2 stimulated proteoglycan production. No stimulatory or inhibitory effects were observed by estradiol, progesterone and prostaglandin F_1 and F_2 .

Proposed Course:

These studies will be continued together with Dr. Yanagishita, who is now an expert, and Dr. Vincent Hascall of the NIDR. The role of proteoglycans, and the mechanisms of in vitro synthesis of the proteoglycans will be examined, in order to identify the mechanism of action of the gonadotropins in terms of their effects on proteoglycan synthesis. Further, proteoglycans will be examined in a variety of models of human disease, to evaluate whether there are changes in proteoglycan structure in diseases such as polycystic ovarian disease, ovarian cystadenomas, and related disorders.

Publications:

Yanagishita, M., Hascall, V., Rodbard, D.: Biosynthesis of Proteoglycans by Rat Granulosa Cells Cultured In Vitro: Modulation by gonadotropins, steroid hormones, prostaglandins and a cyclic nucleotide. Endocrinology. 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00176-04 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Evaluation of Adrenocortical Hyperfunction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI Charles A. Strott Senior Investigator NICHD, ERRB

COOPERATING UNITS (if any)
None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A regimen of standard tests for the diagnosis and differential diagnosis of the hyperfunctioning pituitary-adrenal axis has been designed to provide data for comparison of the various determinations and procedures now commonly employed. In addition the results of these tests will be compared to investigative procedures to be carried out under protocols which will be separately submitted. Patients will be treated by standard clinical methods, or by investigative methods also to be covered by future protocols.

PHS-6040
(Rev. 2-81)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00179-03 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Inferior Petrosal Sinus Catheterization in Diagnosis and Treatment of Pituitary Tumors.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Charles A. Strott	Senior Investigator	NICHD, ERRB
Other	John R. Doppman	Chief, Diagnostic Radiology Dept.	CC

COOPERATING UNITS (if any)
None

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Adrenal

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Inferior Petrosal sinus opacification by retrograde venography has been used at several centers as an alternate technique for opacification of orbital veins. In addition, the procedure delineates the anatomy of the cavernous and intrasellar venous sinuses and provides an opportunity for obtaining blood samples directly from the cavernous sinuses. We propose that it may offer definitive methods for the diagnosis of pituitary tumors and that sampling the effluent for hormones will provide evidence for the source of hypersecreted hormones when that source is in doubt. To establish whether this be the case, patients with disorders of the pituitary-adrenal axis who are undergoing continuous venous catheterization for other reasons will have sampling of the inferior petrosal sinus for hormone measurement, and, when indicated opacification of the cavernous sinus.

PHS-6040

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00184-03 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of Pituitary Hormone Secretion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	K. J. Catt, Chief	ERRB, NICHD
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COOPERATING UNITS (if any)

Naval Medical Research Institute.
National Naval Medical Center, Bethesda, MD 2014

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project examines the mechanisms by which gonadotropin releasing hormone (GnRH) controls the secretion of LH and FSH by anterior pituitary cells. This includes characterization and analysis of the GnRH receptors in anterior pituitary cells and membranes, and the roles of calcium, cyclic nucleotides and other intracellular regulators in the mechanism of action of GnRH. Receptors for GnRH have been shown to be regulated by the releasing hormone, which causes an increase in its pituitary binding sites, and by gonadal steroid hormones. The mechanism by which GnRH stimulates gonadotropin release has been shown to be unrelated to cyclic nucleotides, but is highly dependent on calcium, and current studies suggest that phospholipid metabolites, especially arachidonic acid and its derivatives, may have an important function as mediators of GnRH action.

PHS-6040

Objectives:

To characterize the nature and regulation of GnRH receptors, and to elucidate the mechanisms by which receptor occupancy leads to release of gonadotropic hormones and trophic effects in anterior pituitary cells.

Methods Employed:

Radioligand assay of GnRH receptors in pituitary homogenates and cultured cells. Measurement of gonadotropins and cyclic nucleotides by radioimmunoassay. Isolation and culture of anterior pituitary cells.

Major Findings:

1. Characteristics of cellular GnRH receptors.

The pituitary binding sites for GnRH have been characterized in rat anterior pituitary glands, both in membrane fractions and in homogenates of the whole gland. The latter method permits assay of GnRH receptors to be performed in single pituitaries with good precision. Non-degradable GnRH agonist analogs (GnRHa) employed for readioligand assay bind to pituitary GnRH receptors with Ka of about 5×10^9 M^{-1}, and concentration of about 100 fmol/gland. This method has been applied to measurement of pituitary GnRH receptors in various physiological states, and to the assay of GnRH sites in cultured rat pituitary cells. The latter approach is being applied to correlative studies on the binding affinity and bioactivity of GnRH analogs, and to analysis of the direct regulatory actions of GnRH on its own receptor sites in vitro.

2. Pituitary GnRH receptors during the estrous cycle.

A radioiodinated superagonist analog of gonadotropin-releasing hormone, [D-Ser (t-Bu)^6]des-Gly^{10}-GnRH N-ethylamide, was used to quantitate the GnRH receptor content of single pituitary glands. This ligand binds with high affinity ($Ka=4.9 \times 10^9 M^{-1}$) to a single class of sites in pituitary homogenates, without significant tracer degradation during equilibration for 80 min at 4 C. The GnRH receptor content of adult male rat pituitaries [112 $\pm$ 6.4 (SE) fmol/gland] was similar to that of adult female rat pituitaries obtained at estrus or metestrus (104.8 $\pm$ 6.4 fmol/gland). Between 1800 h on metestrus and 1800 h on diestrus, the pituitary content of GnRH receptors increased to 200 fmol/gland or greater. The pituitary content of GnRH receptors remained elevated until the time of the serum LH surge, then fell sharply to metestrus levels. These data indicate the physiological relevance of the GnRH-binding sites measured by radioassay, and demonstrate their relationship to the events of the estrous cycle. The increased GnRH receptor content from the evening of diestrus until late in the afternoon of proestrus may contribute to the enhanced sensitivity of the pituitary to GnRH during proestrus. The close temporal relationship between rising blood estrogen levels and increasing pituitary GnRH receptors suggests that steroid-mediated receptor induction occurs before proestrus. Such an effectof estrogen could be exerted directly upon the pituitary gland or indirectly via the hypothalamus to increase the release of endogenous GnRH, which subsequently induces its own receptors in pituitary gonadotrophs.

3. Regulation of pituitary GnRH receptors by gonadal hormones.

The regulatory role of pituitary GnRH receptors in the control of gonadotropin secretion was investigated in male and female rats after castration and sex steroid hormone replacement. GnRH receptors were measured in homogenates of individual pituitaries by equilibration with ^{125}I-labeled[D-Ser(tBu)^{6}]des-Gly^{10}-GnRH N-ethylamide, and compared with serum and pituitary LH concentrations. The equilibrium association constants (K_a) were 6.1 and 5.1 x $10^{9}m^{-1}$ for intact and castrate male rat pituitaries, respectively. After orchidectomy, pituitary GnRH receptor concentration increased by 75% at 24 h, from 150 fmol to 250 fmol/gland, while serum LH levels increased 10-fold (30 to 300 ng/ml). There was a further slight increase in the GnRH receptor concentration (to 370 fmol/gland) and serum LH (to 500 ng/ml) over the ensuing 10 days, and at 15 and 20 days when GnRH receptors were 304 and 306 fmol/gland, respectively. There was a highly significant ($P<0.001$) positive correlation between basal serum LH and FSH concentrations and the pituitary GnRH receptor content measured in individual animals. Treatment with testosterone propionate (100 µg/day) completely prevented the GnRH receptor and serum LH responses to castration, while 50 µg testosterone propionate/day produced variable results. 17β-Estradiol (5 µg/day), diethylstilbestrol (5 µg/da y) and dihydrotestosterone (50 µg/day prevented the increase in GnRH receptors 5 days after orchidectomy, while serum LH levels were only partially suppressed. In adult female rats, ovariectomy caused a 2- to 4-fold increase in serum LH in the first 3 days, followed by a larger secondary increase of 10- to 15-fold after 5 days. GnRH receptor concentration increased from 130 to 240 fmol/gland on the third day after operation, just before the major rise in serum LH. 17β-estradiol (1 µg/day), progesterone (2.5 mg/day), and estradiol plus progesterone inhibited the post-ovariectomy rise in GnRH receptors for up to 11 days. Estradiol or progesterone given alone prevented the initial rise in serum LH but not the secondary rise from 5 days on, while the combination of estradiol plus progesterone was effective in this regard for the entire treatment period. These results indicate that increased pituitary binding of GnRH is a significant component of the mechanism responsible for postcastration elevations of gonadotropin secretion.

4. Calcium-dependence of GnRH action.

The effects of GnRH on cGMP production and LH release in cultured rat pituitary cells are markedly dependent upon the extracellular calcium concentration. The absence of calcium from incubation media caused almost complete loss of the GnRH effects on cGMP production and LH release, but did not change the stimulation of cAMP accumulation by GnRH in the pituitary of the adult male rat. In female rat pituitary cells, reduction of the extracellular calcium concentration increased the concentration of GnRH required to produce half-maximal LH release and decreased the maximal gonadotropin output, but had no significant effect on basal LH release. The divalent cation ionophore A23187 stimulated LH release, and this action was dependent on extracellular calcium. Both GnRH and A23187 were found to have maximal effects when the calcium concentration was 0.6 mM, and their actions were not additive. The calcium antagonists, verapamil and lanthanum, caused conentration-dependent inhibition of the actions of GnRH, with half-maximal blockade values of 10^{-5} and 3 x 10^{-6} M, respectively, and had no effect on basal LH release. The

binding of a radioiodinated GnRH analog, [D-Ser(t-Bu)^6]des-Gly^{10}-GnRH-N-ethylamide, to pituitary GnRH receptors was unchanged in the absence of extracellular calcium. These observations demonstrate that stimulation of pituitary cGMP production and LH release by GnRH is dependent on extracellular calcium. The site at which calcium is required during GnRH action is at a postreceptor locus before cGMP formation.

5. Role of arachidonic acid in GnRH action.

The action of GnRH upon luteinizing hormone (LH) secretion is calcium-dependent, but is not mediated by cyclic AMP, cyclic GMP, or prostaglandins. The role of calcium-mediated phospholipid turnover in GnRH action was investigated in 2-day cultured pituitary cells, in which the production and target cell effects of arachidonic acid were analyzed in relation to GnRH-stimulated LH release. Addition of 10^{-8} M GnRH, which stimulated LH release 5-fold, caused a 35% increase in the rate of [^{3}H]arachidonic acid release from prelabeled phospholipids. The effects of GnRH on arachidonic acid and LH release, which occurred within 10 min, were calcium-dependent and were not accompanied by a change in [^{3}H]prostaglandin formation. The phospholipase A_2 inhibitors, chloroquine and quinacrine, prevented the effect of GnRH on arachidonic acid formation and LH release. Addition of exogenous arachidonic acid or phorbol myristate acetate (which increases endogenous arachidonic acid) caused a dose-related release of LH, whereas prostaglandin E_2 had no effect. Although GnRH action was highly calcium-dependent, stimulation of LH release by arachidonic acid was unaltered in the absence of extracellular calcium. The effect of arachidonic acid on LH release did not depend on conversion to prostaglandins and was not additive with the action of a maximal stimulating concentration of GnRH. These results demonstrate that GnRH increases arachidonic acid formation from the phospholipids of pituitary gonadotrophs, and indicate that the fatty acid or its metabolites can initiate LH release.

6. Internalization of GnRH in cultured pituitary cells.

The actions of GnRH upon pituitary gonadotropin secretion depend on specific binding to receptors located in the plasma membrane of the gonadotrophs. We have demonstrated that labeled GnRH agonists bind to receptors in cultured pituitary cells, and that these sites are specific, saturable, and activated by the releasing hormone. An additional approach to receptor analysis has been provided by the use of fluorescent-conjugated proteins and video intensification microscopy to analyze cell-protein interactions, and has particular value for studies on hormone receptor binding. For this purpose, we prepared a biologically active rhodamine derivative of the [D-Lys^6]-agonist analog of GnRH and studied the uptake of the fluorescent peptide by living cultured pituitary cells. The rhodamine-derivatized peptide (Rh-GnRH) retained 40% of the receptor binding activity of [D-Lys^6]GnRH ,and 50% of the luteinizing hormone-releasing activity assayed in cultured pituitary cells. When the fluorescent analog was employed to visualize the distribution of GnRH receptors in cultured pituitary cells, binding of Rh-GnRH was confined to the large gonadotrophs which comprised 15% of the cell population. The specificity of the binding was shown by the absence of significant fluorescence fluorescence in the presence of a 100-fold excess of [D-Lys^6]GnRH, or when Rh-GnRH was incubated with choriocarcinoma, neuroblastoma, or 3T3 cell

lines devoid of GnRH receptors. The interaction of Rh-GnRH with living pituitary cells was characterized by an initial diffuse distribution, followed by the formation of polar aggregates that later appeared to be internalized. These observations emphasize the value of fluorescent derivatives of GnRH for elucidating the course of the interaction with specific receptors on pituitary gonadotrophs. The initial results indicate that GnRH-receptor complexes undergo aggregation during stimulation of luteinizing hormone release, and are later internalized for subsequent degradation and/or intracellular actions.

Significance:

Clarification of the mechanisms of action of GnRH will improve understanding of pituitary regulation and its disorders, and will permit the development of improved methods for the control of gonadotropin secretion.

Proposed Course:

The relationship between GnRH binding affinity and bioactivity assayed by LH release will be evaluated with agonist and antagonist analogs of the releasing hormone. The role of the GnRH receptors during pregnancy, and the effects of agonists and antagonists on reproductive processes, will be analyzed. The calcium-dependent mechanism by which GnRH stimulates LH release will be investigated in purified pituitary gonadotrophs by assay of calcium fluxes and related responses. The role of phospholipid metabolites, including arachidonic acid and its products, in the actions of GnRH will also be analyzed in purified gonadotrophs, together with studies on the metabolism of arachidonic acid to lipooxygenase products in anterior pituitary cells. The regulatory actions of GnRH on its own receptors will be studied both *in vivo* and in cultured pituitary cells.

Publications:

Clayton, Richard N., and Catt, Kevin J.: Gonadotropin-releasing hormone receptors: characterization, physiological regulation, and relationship to reproductive function. Endocrine Reviews 2: 186-209, 1981.

Clayton, Richard N., and Catt, Kevin J.: Regulation of Pituitary Gonadotripin-Releasing Hormone Receptors by Gonadal Hormones. Endocrinology 108: 887-895, 1981.

Clayton, Richard N., Solano, Angela R., Garcia-Vela, Alfonso, Dufau, Maria L., and Catt, Kevin J. Regulation of Pituitary Receptors for Gonadotropin-Releasing Hormone during the Rat Estrous Cycle. Endocrinology 107: 699-706, 1980.

Naor, Zvi, Atlas, Daphne, Clayton, Richard N., Forman, David S., Amsterdam, Abraham, and Catt, Kevin J. Interaction of Fluorescent Gonadotropin-Releasing Hormone with Receptors in Cultured Pituitary Cells. J. Biol. Chem. 256: 3049-3052, 1981.

Naor, Zvi and Catt, Kevin J. Mechanism of Action of Gonadotropin-Releasing Hormone. Involvement of Phospholipid Turnover in Luteinizing Hormone Release. J. Biol. Chem. 256: 2226-2229, 1981.

Naor, Zvi, Clayton, Richard N., and Catt, Kevin J.: Characterization of GnRH receptors in Cultured Rat Pituitary Cells. Endocrinology 107: 1144-1152, 1980.

Naor, Zvi, Leifer, Arthur M., and Catt, Kevin J. Calcium-Dependent Actions of Gonadotropin-Releasing Hormone on Pituitary Guanosine 3',5'-Monophosphate Production and Gonadotropin Release. Endocrinology 107: 1438-1445, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00185-02 RR

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Temperature and Other Physical Factors on Localization, Natural History and Susceptibility to Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
Other	L. Brill	Guest Worker	ERRB, NICHD
	R. J. Sherins		DEB

COOPERATING UNITS (if any)

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Endocrinology and Reproduction Research Branch

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The use of the thermographic camera has been examined in a variety of disease states. Thermography has been demonstrated to be useful in the detection and diagnosis of varicocele and testicular tumors. The thermographic camera is also useful in evaluation of temperature at the site of infectious disease (e.g. cutaneous and mucocutaneous leishmaniasis), and a variety of inflammatory diseases. Fever therapy or local heat therapy is being evaluated in a variety of disease states, including sporotrichosis, mucocutaneous leishmaniasis, and paracoccidioidomyosis.

PHS-6040
(Rev. 2-81)

Objectives:

It is intended to evaluate the role of temperature, fever, hyperthermia, and hypothermia, on the course of disease in man and experimental animals. Such information may assist in the physical diagnosis of disease, in the design of clinical and immunological studies, and the choice of experimental animals. In particular, use of thermography seems relevant to studies of patients with infertility associated with varicoceles.

Methods Employed:

The thermographic camera is utilized for clinical studies, supplemented by direct measurements of cutaneous temperature utilizing digital or analog electronic thermometers. Additional studies employ the use of heat therapy, utilizing a water jacketed system, or a heat lamp.

Major Findings:

Preliminary results indicate that the thermographic camera is effective in detecting varicoceles and testicular tumors. Further, the thermographic camera has been helpful in demonstrating the absence of varicoceles in patients who had previously been informed that varicoceles were present.

The thermographic camera has also been demonstrated to be useful in the location of veins. This can be useful in patients receiving long-term cancer chemotherapy or other agents producing phlebitis and sclerosis. The distribution of cutaneous temperature in patients with thyrotoxicosis and/or hypothyroidism is also under study, as is the role of the thermogram in other diseases.

Significance to Biomedical Research and the Program of the Institute:

The ability of hyperthermia to damage the germinal epithelium of the testes has been known since the turn of the century, when infertility was occasionally found to be one of the sequelae of febrile illnesses. The mechanisms of the damage to the testes have been studied in many laboratories. Recent studies in other laboratories have indicated that the presence of an experimentally induced unilateral varicocele can lead to bilaterally increased venous pressure, increased blood flow, and increased testicular temperature. Further, it is associated with testicular atrophy and physiological changes. Hence, there is now strong evidence that elevated temperature, either as the result or as the cause of increased testicular blood flow, is intimately associated with the pathogenesis of infertility associated with varicocele. The ability to detect varicoceles in patients by an objective method, and the ability to examine patients for recurrence of varicocele following surgical correction, should be of importance in the management of these patients and understanding the mechanisms of their disorder. The role of temperature in the treatment of various diseases is obviously of direct clinical importance.

Proposed Course:

We are considering the possibility of use of local heat therapy in treatment of leprosy, in patients available at the Leprosy Unit in Staten Island, in collaboration with Dr. William Levis, Chief of Dermatology. Studies are currently underway in collaboration with Dr. I. Dvoretzky, to examine the effect of temperature on the rate of proliferation on the human papilloma virus.

Publications:

Del Negro, G., Melo, E. H. L., Rodbard, D., Layton, J., and Wachslicht-Rodbard, H., Limited Adrenal Reserve in Paracoccidioidomycosis: Cortisol and Aldosterone Responses to 1-24 ACTH, Clinical Endocrinology 13: 553-559, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00187-02 RR

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Hormonal Regulation of Cellular Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.-P. Huang	Research Chemist	ERRB,	NICHD
OTHER:	A. Akazuka	Visiting Fellow	ERRB	NICHD
	T.J. Singh	Guest Worker	ERRB	NICHD

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J. Chou, PRB, NICHD; P.B. Chock, LB, NHLBI

LAB/BRANCH
Endocrinology and Reproduction Research Branch

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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX(c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phosphorylation and dephosphorylation of the enzymes controlling the rate-limiting step is a most important regulatory mechanism for cellular metabolism. In the diabetic animals the regulation of glycogen metabolism by this regulatory mechanism becomes defective and the defect can be corrected by the administration of insulin. The purposes of this work are: (1) to study the regulation of glycogen synthase and phosphorylase kinase activities by protein kinases and phosphoprotein phosphatases; (2) to define the defect in glycogen metabolism resulted from diabetes; and (3) to elucidate the mechanism of action of insulin.

PHS-6040
(Rev. 2-81)

Major Findings: (1) A second cAMP-independent casein kinase from rabbit skeletal muscle has been purified to homogeneity. This enzyme consists of two different subunits having molecular weights of 42,000 and 27,000, and phosphorylates glycogen synthase up to 0.8 mol of phosphate/subunit. The phosphorylation of glycogen synthase by this kinase results in the incorporation of ^{32}P into two major tryptic peptides. The sites in glycogen synthase phosphorylated by this kinase are different from those phosphorylated by cAMP-dependent protein kinase, phosphorylase kinase, and cAMP-independent casein kinase-1. Based on the structural similarities between cAMP-independent kinases which regulate glycogen synthase activity, and those kinases which regulate protein synthesis, we infer that the cAMP-independent casein kinases are multifunctional enzymes. (2) The cAMP-independent casein kinase-1, which was found to phosphorylate and inactivate glycogen synthase, has been shown to phosphorylate and activate phosphorylase kinase. This phosphorylation results in the incorporation of phosphate into a subunit of phosphorylase kinase. The finding that the activity of phosphorylase kinase can be regulated by cAMP-dependent and -independent protein kinases will provide further insight into the hormonal regulation of phosphorylase kinase. (3) Phosphorylase kinase from rabbit skeletal muscle was found to be activated by Mg^{2+}. The activation of phosphorylase kinase by this cation is synergistic with that caused by calmodulin and Ca^{2+}. This regulatory mechanism can provide a means of activation of phosphorylase kinase without covalent modification as previously observed under electrical stimulation. (4) Liver glycogen synthase from normal and diabetic rats was extensively purified. The synthase from diabetic animals, unlike that from normal animals, cannot be activated by protein phosphatase. Glycogen synthases from these two sources also differ in their molecular weights and kinetic properties. These results indicate that a less active form of glycogen synthase is present in diabetic rats. This observation provides an explanation for the presence of low levels of liver glycogen in diabetic animals.

Significance to Biomedical Research and the program of the Institute: Investigation on the regulation of the activities of glycogen synthase and phosphorylase kinase, two key enzymes controlling glycogen metabolism, provides understanding of the hormonal regulation of glycogen metabolism in normal and pathological development. Using glycogen synthase and phosphorylase kinase as model systems which are controlled by multiple site phosphorylation with multiple kinases, we learn more about the regulation of phosphorylation and dephosphorylation reactions--key processes in the regulation of gene expression, cellular metabolism, differentiation, and growth. The identification of the defect in glycogen metabolism resulting from diabetes, and studies on the mechanism of action of insulin, will enhance our understanding of this disease.

Proposed Course of Project: We will continue to explore the regulatory mechanisms of phosphorylation and dephosphorylation of glycogen synthase and phosphorylase kinase, and to apply these findings to studies of the regulation of cellular metabolism associated with fetal development and with diabetes.

Publications:

1. Ahmad, Z., and Huang, K.-P.: Dephosphorylation of rabbit skeletal muscle glycogen synthase (phosphorylated by cyclic AMP-independent synthase kinase-1) by phosphatases. J. Biol. Chem. 256: 757-760, 1981.

2. Huang, K.-P., Huang, F.L., Ahmad, Z., and Itarte, E.: Phosphorylation of rabbit skeletal muscle glycogen synthase by cyclci AMP-dependent and -independent protein kinases. Cold Spring Harbor Conferences on Cell Proliferation - Protein Phosphorylation, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00188-01 RR

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)
Development of New Analogs of Enkephalin with increased Receptor Activity and Selectivity.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	D. Rodbard	Head, Section on Biophysical Endocrinology	ERRB, NICHD
	T. Costa	Visiting Fellow	ERRB, NICHD
	S. Krumins	Visiting Fellow	ERRB, NICHD
	Y. Shimohigashi	Visiting Fellow	ERRB, NICHD
	H. Chen	Senior Investigator	ERRB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Biophysical Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of new analogs of enkephalin have been developed. These consist of dimers, crosslinked at the carboxy terminal by diaminoalkanes. A new method of synthesis has been developed. The dimeric pentapeptide enkephalins have been purified, and their structure and molecular weight have been confirmed by several physical chemical techniques. These new analogs have been assayed for activity in a variety of radioligand assays employing whole brain membrane and neuroblastoma-glioma cells. The new analogs show extremely high potency for the "delta" opiate receptor and possess the best "delta" specificity ever reported. These compounds are useful in the characterization of the opiate receptor in the membrane, and should be valuable for a wide variety of autoradiographic and pharmacological applications.

PHS-6040
(Rev. 2-81)

Objectives:

The enkephalin (opiate) receptor system provides an ideal model of other hormone and neurotransmitter receptor systems. The enormous number of analogs of opiate alkaloids and of enkephalin, endorphin, and other peptides permits rather detailed structure-activity relationships to be studied, and they provide insights into the mechanism of action at the receptor level. Further, enkephalins, endorphins and related peptides are now known to be present and of considerable physiological importance to the hypothalamus, pituitary, adrenal medulla, and probably in a wide variety of other endocrine organs. The present study was undertaken to develop new analogs of enkephalin which may show increased biological activity and/or receptor activity. Further, initial findings in the study suggest that the approach as utilized here - principally, the use of dimers or oligomers of enkephalins - may provide increased specificity.

Methods Employed:

Techniques of peptide synthesis have been utilized for the development of these new analogs in amounts sufficient for subsequent study. A wide variety of physico-chemical techniques ranging from mass spectrometry to melting point determinations, amino acid composition and sequencing, various forms of spectrometry, and techniques for peptide purification, have been employed for the characterization of these new enkephalin analogs. These are combined with techniques for assay of activity of the receptor, including a variety of radioligand assays, adenylate cyclase assays, and related techniques.

Major Findings:

A series of dimeric pentapeptide enkephalin analogs has been synthesized. These consist of the alpha, omega, diaminoalkanes attached at the carboxy terminal to the D-Ala^2,D-Leu^5-enkephalin. The methylene bridge used for cross-linking ranges in length from n=2 through n=12. The homogeneity, molecular weight, and other physical properties of these compounds have been determined. In whole brain, these compounds have been assayed for their ability to displace "mu' receptor specific ligands, such as [^{3}H]-naloxone, [^{3}H]-dihydromorphine; for the ability to displace "delta" opiate receptor ligands such as D-Ala^2, D-Leu^5-enkephalin (DADLE), and for their ability to displace compounds which are relatively nonspecific in regard to "mu-delta" specificity, D-Ala^2,Met^5 -enkephalin amide (DAMEA), and D-Ala,D-Leu-enkephalin amide (DALEA). One may regard either DADLE or DALEA as the monomer corresponding to the dimer. We find that the dimeric enkephalins do not show increased potency when assayed utilized a "mu" specific ligand, but a dramatic increase in potency ranging between three and ten-fold, when assayed using a "delta" specific ligand. When whole brain (which consists of a mixture of mu and delta receptors) is used as the source of membranes, then use of a nonspecific ligand such as DAMEA results in a potency for the dimer which is intermediate between the values obtained when "mu" or "delta" ligands are utilized. In contrast, when neuroblasto-glioma cells are utilized (which contain almost exclusively delta receptors), then the dimers also show a five to ten-fold increase in potency.

Interestingly, there is a systematic relationship between the length of the flexible crosslinking methylene chain, and the potency of the ligands in a

variety of assay systems. We find that the most potent analog is the one with the shortest crosslinking chain (n=2). As the length of the methylene chain increases to 6 or 8, there is a small decrease in potency, followed by a dramatic loss of potency for n=8,10,and 12. Specificity also varies in a systematic manner, again with the best delta/mu specificity observed for a short crosslinking chain. Based on indirect competition studies, it appears that the affinity of the new dimeric pentapeptide enkephalin analogs is between four and ten-fold higher than for the monomeric analog.

Indirect studies of the dissociation rate suggest that the dimeric enkephalin analogs bind with high affinity, and with an extremely slow dissociation. Again, the slow dissociation rate is best observed when utilizing tissues which contain the delta receptor, and/or when a delta specific radioligand is utilized. This further suggests that the dimeric enkephalin analogs can crosslink the "delta" receptor but not the "mu" receptor. This finding carries considerable implications for the organization of the enkephalin receptors in the membrane, and/or for the mobility of the receptors in the membrane. We have obtained tritiated dimeric pentapeptide enkephalin analogs, for the case where the methylene bridge consists of n=2. Direct binding studies with this labeled ligand confirm an increase in affinity by approximately a factor of ten, so that studies can be conveniently performed with 50% initial binding, instead of the 5% normally employed when utilizing labeled enkephalins. Association and dissociation studies utilizing the labeled ligand suggest approximately a two-fold increase in the association rate, and a two-fold decrease in the dissociation rate. The dimeric pentapeptide enkephalin shows a "sodium shift" suggestive that it may be an agonist or at least a partial agonist. The binding of DPE2 is modulated by magnesium ion (1 mM), a probe which is known to influence the opiate receptor and in particular to alter the apparent distribution of receptors between the mu and delta states.

Significance to Biomedical Research and a Program of the Institute:

The opiate receptor system is perhaps the most actively studied of any receptor system today, of interest to Endocrinologists, Pharmacologists, and Neurobiologists, among others. The influence of enkephalins and endorphins in the pituitary, hypothalamus, adrenal medulla and possibly adrenal cortex is now an active area of investigation, using a variety of human and animal model systems. The present studies promise to add to our understanding of the structure-activity relationships of enkephalins. It remains to be seen whether these new analogs will be active in vivo in animals or man: there is a possibility that they will serve as super-active agonists if they cross the blood brain barrier. Their effects remain to be characterized in the in vivo systems.

Proposed Course:

The present studies have numerous ramifications. Under active study at the present time there are a variety of other analogs, employing rigid, as opposed to flexible, crosslinking chains. Also, in addition to the dimers of pentapeptide enkephalins, studies are now underway utilizing dimeric tetrapeptide enkephalins. Preliminary data suggests that these are also active, and show delta specificity. One can then progress to make dimers of the tripepide, and a variety of other analogs of enkephalins. Each of these can

be characterized for activity, specificity, biochemical and biological activity. We expect to assay these new compounds, looking for desensitization of neuroblastoma-glioma cells in tissue culture, evaluation of analgesic effects using a central (third ventricle) bioassay, examination of potency in classical opiate assay systems such as the in vivo analgesic assays and the in vitro guinea pig ileum and mouse vas deferens assays. It is likely that these compounds will be useful in the examination of the peculiar properties of the vas deferens opiate receptor, which may be relevant to reproduction.

Publications:

Shimohigashi, Y., Costa, T., Matsuura, S., Chen, H-C., and Rodbard, D., Dimeric Enkephalins: Synthesis of bivalent ligands with increased affinity and selectivity for the "delta" opiate receptor. Proceedings of the 1981 7th American Peptide Symposium, in press.

DEVELOPMENTAL ENDOCRINOLOGY BRANCH

ANNUAL REPORT

October 1, 1980 - September 30, 1981

Project #	Project Title	Principle Investigator
Z01 HD 00600-01	Physiology of Puberty	G. Cutler, Jr., M.D.
Z01 HD 00601-01	Pathophysiology of Gynecomastia and Hirsutism	D.L. Loriaux, M.D.
Z01 HD 00602-01	Role of Sex Steroids in Regulation of FSH and LH Levels in Man	R.J. Sherins, M.D.
Z01 HD 00603-01	Clinical Studies of Male Reproductive Disorders	R.J. Sherins, M.D.
Z01 HD 00604-01	Biology of Hormone Binding Proteins	B.C. Nisula, M.D.
Z01 HD 00605-01	Steroid Antagonists	G. Cutler, Jr., M.D.
Z01 HD 00606-01	Structure, Function, and Physiology of Glycoprotein Hormones	B.C. Nisula, M.D.
Z01 HD 00607-01	Catechol Estrogens: Physiological Effects	D.L. Loriaux, M.D.

ANNUAL REPORT
Summary

DEVELOPMENTAL ENDOCRINOLOGY BRANCH
National Institute of Child Health and Human Development

The research aim of the Developmental Enocrinology Branch is to further understanding of the role of the endocrine system in the complex processes of growth and development. The periods of primary interest include fetal and neonatal life, puberty, and senility. The current focus of research is the pubertal period. The systems under study are two; the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis.

Studies on the hypothalamic-pituitary-gonadal axis are directed toward understanding the process underlying the initiation of LH and FSH secretion that heralds the onset of puberty, the mechanism of action of these glycoprotein hormones, the gonadal response to these hormones, and the roles of the gonadal sex steroids in gametogenesis, central nervous system maturation, breast physiology, hair growth, and skeletal maturation.

Studies directed toward understanding the mechanism of initiation of LH and FSH secretion in puberty have continued to center about our growing population of children with precocious puberty. We are currently following over 50 children with this disorder. Thirty of these children are being treated with a long acting LRF analogue supplied to us by the Salk Institute. Treatment with the analogue has resulted in decreased gonadotropin secretion, decreased sex hormone levels, regression of secondary sexual characteristics, and a slowing of growth in patients with idiopathic isosexual precocious puberty. Since no satisfactory treatment is currently available for this disorder, these results extend the promise that this might represent an important therapeutic advance. An important side light of this project has been insight gained into the pathophysiology of precocious puberty. As an example, it has been shown that complete gonadotropin suppression does not alter the course of isosexual precocity in the McCune-Albright Syndrome, thus resolving the controversy of a central versus gonadal etiology for the sexual precocity associated with this disorder. Systematic investigation of the dose, frequency, duration, and chemical nature of the analog used will be necessary to realize the full potential benefit from this new form of therapy.

Studies directed toward understanding the mechanism of action of the glycoprotein hormones have centered about the exmaination of structure-function-relationships between the various glycoprotein hormones and the development of specific assays for these hormones and their metabolic products. Particular effort has been given to three aspects of glycoprotein hormone action: the role of chorionic gonadotropin in choriocarcinoma-associated thyrotoxicosis, the effects of structural modification of the chorionic gonadotropin molecule on its agonist-antagonist activity with the thyrotropin receptor, and the metabolism of hCG, including the characterization of its metabolic products.

Clinical studies designed to assess the thyrotopic activity of the hCG molecule in choriocarcinoma have been completed. In 20 patients with gestational trophoblastic neoplasms, the degree of thyroid hyperfunction correlated with

the level of serum hCG. In the mouse thyroid bioassay, the biological characteristics of the thyroid-stimulating activity in both serum and urine correlated closely with the levels of hCG. Thus, hCG was the only apparent thyrotropic factor in these patients.

Interesting findings have emerged from the study of structure function relationships with the thyrotropin receptor. Earlier studies provided clear evidence that the gonadotropin molecules, chorionic gonadotropin (hCG) and luteinizing hormone (hLH), contained the structural domains requisite not only for interacting with the human thyrotropin (TSH) receptor, but also for activation of the adenylate cyclase system in thyroid membranes. Given the similarity of their biologic effects at the LH/CG receptor in the gonad, it was surprising to find a dramatic difference between hCG and hLH with respect to their activity at the thyrotropin receptor—hLH exhibited about 65 times greater intrinsic thyrotropic activity than hCG. Structural modifications were made in the hCG molecule to probe the domains which determined this difference in function. Carboxypeptidase digestion of the hCG molecule resulted in the cleavage of residues 142-145 from the carboxyterminus of the hCG beta subunit and resulted in a dramatic increase in the thyroid-stimulating activity of the preparation. In contrast, a reduction in the carbohydrate structure of hCG, brought about by digestion with neuroaminidase, resulted in a loss of thyroid-stimulting activity. Although asialo-hCG had no adenylate cyclase-stimulating activity, it retained the ability to interact with the TSH receptor and behaved as a competitive antagonist of TSH at the thyrotropin receptor. Interestingly, this molecule has been shown to be bound more avidly to its primary gonadal receptor and to activate gonadal adenylate cyclase.

These findings suport the general concept that the glycoprotein hormones comprise a structurally homologous group of molecules with shared biological properties. Thus, hCG, hLH, and asialo-hCG exhibit secondary biological activity at the TSH receptor; they are all agonists at the gonad, while only hCG and the hLH are agonists at the thyroid. Competitive antagonists have been very useful in studies of the beta-adrenergic receptor and its adenylate cyclase system; analogous applications of asialo-hCG are expected.

Research aimed at understanding the metabolism of these glycoprotein molecules has also been fruitful. To establish the parameters of hCG metabolism and the nature of the metabolic products derived from hCG, infusions of highly purified hCG were given to normal subjects over an 8 day period. This allowed steady state conditions to be reached and sufficient time for relevant metabolites to accumulate. The parameters for distribution, metabolic clearance, disppearance curve components, and renal clearance were established. Although the total metabolic clearance rate of hCG was 10 fold less than that of its subunits, its renal clearance rate was actually several fold greater. Thus, renal excretion accounts for the metabolic fate of 20% of the hCG, but less than 1% of its subunits. There was no apparent production of subunits or fragments of hCG detectable by radioimmunoassay of serum or urine. The vast majority of the excreted hCG was indistinguishable from the highly purified material infused. Only one of seven subjects given an 8 day infusion of hCG had a detectable amount of the hCG beta core fragment which we have previously characterized as a prominent metabolic product derived from the free hCG beta subunit.

These studies give the first comprehensive analysis of the kinetic parameters and metabolic products of hCG in man and provide new insights into the sources of free subunits and fragments in pregnancy and malignant disease.

Continued emphasis will be given to the development of more accurate, sensitive, and convenient techniques for the measurement of glycopeptide hormones, their subunits, and related degradation products in the serum and urine of patients. Studies are underway which compare the clinical applicability of urinary hCG measurement to that of the standard serum hCG radioimmunoassay. The role of genetic factors and endocrinologic factors, such as diabetes, in modulating the metabolism of glycoproteins will be studied. Structure-function studies with the TSH receptor and the LH/CG receptor will be continued to gain further insight into the domains of the glycoprotein hormones which confer the abilities to interact with the receptor and to activate adenylate cyclase.

A considerable effort has been expended in improving our tools for exmaining the gonadal response to gonadotropin stimulation. Using a solid phase method for examining transport protein-steroid interactions, the binding parameters of 21 endogenous steroids and 70 assorted drugs and compounds of significance to steroid physiology and biochemistry have been determined. The solid phase method developed in this laboratory permits determination of the parameters under equilibrium conditions at physiologic pH and temperature. Thus, with parameters determined under physiologic conditions, computer simulations of *in vivo* transport based on the law of mass action can be performed andl yield a solution to the complex simultaneous interactions between multiple circulating steroids and TeBG, CBG, and albumin. Estimates of the plasma distribution of 21 circulating steroids into TeBG-bound, CBC-bound, albumin-bound, and unbound fractions in normal men, normal women during both the follicular and luteal phases of the ovarian cycle, and women during pregnancy have been produced.

Structure-binding studies of the testosterone binding site on the TeBG molecule have produced new information concerning the effects of A ring substituents on TeBG binding. The affinity of estradiol with its aromatized A ring is about one third that of testosterone. When the hydrogen at position 2 is substituted with a hydroxyl group the affinity is about one fifth that of estradiol. However, when the hydrogen at position 2 is substituted with a methoxy group, the affinity is about four-fold greater than that of estradiol, and significantly greater than that of testosterone. These findings suggests that a hydrophobic region of the binding site interacts primarily with the A ring at position 2.

A compehensive survey of drug interactions with TeBG and CBG has been completed. A wide variety of steroids showed appreciable interaction with TeBG and CBG *in vitro*. Whether or not a given compound will alter the transport of endogenous steroids *in vivo* due to this interaction depends on the concentration which it achieves during treatment. Therapeutic levels of danazol, methyltestosterone, fluoxymesterone, and norgestrel were found to displace 83%, 48%, 42%, and 16%, respectively, of the testosterone bound to TeBG in a normal man. Further, through its occupancy of 97% of the TeBG binding sites, and 56% of the CBG binding sites, danazol can markedly increase the unbound concentrations of testosterone, estradiol, and cortisol. These data strengthen the concept that drugs can modulate the concentration of the bioavailable fraction of hormones by occupying the hormone binding sites in transport hormones, independent

of any direct effects on steroid secretion, steroid metabolism, or binding protein levels.

Future studies will emphasize the evaluation of transport physiology in normal development and in a variety of disease processes. Attention will be given to the development of a practical clinical assay for human CBG, and its clinical applications. Studies of the phylogeny of CBG in primates have been initiated. Several laboratory models of transport physiology will be explored with the aim of establishing the necessary system for evaluating the role of transport proteins in steroid action, metabolism, and pituitary regulation.

Gonadotropin stimulation of the testis also eventuates in spermatogenesis. Our studies on spermatogenesis have primarily utilized experiments of nature, men with abnormal spermatogenesis, to further our understanding of this complex process.

A 10 year prospective study of the treatment of hypogonadotropic men has been completed and indicates that intratesticular testosterone alone can initiate spermatogenesis and that FSH is required only to complete the process. The finding that supplemental FSH is not usually required to maintain human spermatogenesis after initiation suggests that the completion of spermatogenesis may be a permanent induceable maturational event.

Studies on semen from infertile men with non-motile sperm (necrospermia) show that these sperm fail to metabolize fructose, the normal primary energy source in sperm, and have increased specific activity of an endogenous lipid peroxidase capable of producing peroxides of sperm phospholipid. These peroxides promptly and irreversibly immobilize spermatozoa.

Human sperm have also been shown to contain protein carboxyl methylase, an enzyme required by mammalian monocytes and bacteria for chemotaxis. This enzyme acts on a specific protein methyl acceptor and inactivation of the enzyme leads to failure of characteristic unidirectional motile progression. A deficiency of protein carboxyl methylase activity has been identified in some men with immotile sperm.

Prospective studies of the adverse effects of chemotherapy and radiotherapy on gonadal function indicates that these agents vary considerably in their potential gonadal toxicity. High-dose methotrexate appears to produce no ovarian dysfunction, and only transient azoospermia. Adriamycin is not spermicidal and does not significantly alter ovarian function. Methylhydrazines, alkylating agents and radiation, on the other hand, produce dose-dependent gonadal failure in both sexes.

Studies on the central nervous sytstem effects of the gonadal steroids have centered about the feedback actions of androgens and estrogens on the pituitary secretion of FSH & LH. In particular, we have been interested in the role of sex steroids in the regulation of FSH secretion. Studies in castrate male rats have shown that testosterone in doses which maintain normal plasma testosterone levels and normal prostate size produce normal plasma FSH and LH concentrations. These studies indicate that testicular factors other than sex steroids are not necessary for maintaining FSH and LH levels within the normal range. When estradiol is given at doses which produce slightly supranormal

estradiol concentrations, plasma LH levels are suppressed while FSH levels remain elevated into the castrate range. These data imply that androgen, not estrogen is of primary importance in maintianing tonic suppression of FSH secretion.

When testosterone and estradiol are administered together at doses which produce sub-physiologic levels of plasma testosterone and elevated levels of estradiol, a selective increase in plasma FSH is maintained similar to that seen in animals with severe germ cell depletion. A similar change in the ratio of pituitary FSH and LH is also produced at a time when GnRH content of the hypothalamus is within normal limits.

These studies lend new insight into the control of gonadotropin secretion in the male. The results indicate that testosterone can maintain both gonadotropins within the normal range in the absence of other testicular factors and, further, that a selective increase in plasma FSH can be induced when there is subnormal replacement of testosterone but increased production of estradiol. This is exactly the situation in mild Leydig cell failure and suggests that, in the rat, it is unnecessary to postulate a testicular non-steroidal regulator of gonadotropin secretion (inhibin).

Another class of steroids that may be important in the regulation of FSH and LH secretion is the catechol estrogens.

It was initially speculated that catechol estrogens may represent the "missing" link between secreted estrogens and central nervous system estrogen action in that they interact with both the cytosol estrogen receptor and the membrane bound catecholamine receptors. On the basis of earlier studies in rodents, it has been speculated that these compounds may act as endogenous antiestrogens.

We have previously shown that both 2-OH estrone and 2 OH estradiol interact with the rat uterine cytosol estrogen receptor with about 5% the affinity of estradiol, that $2OHE_1$ and $2OHE_2$ are weak estrogen agonists in man, and that the potency of these estrogens is much less than would be predicted on the basis of their interaction with the estrogen receptor. This has been shown to be the result of an extremely rapid metabolic clearance rate for the catechol estrogens, on the order of 20,000-40,000 L/day, the highest known clearance rate for any of the naturally occurring steroid hormones. There findings are also consistent with the very low plasma concentrations that we have found for both 2OH-estrone and 2-OH-estradiol.

We have also exmained the role of catechol estrogens in regulating prolactin secretion. Recent studies by investigators prominent in the field of catechol estrogen physiology purport to show that prolactin is released by an infusion of 2OH estrogen. We have infused large doses of 2-OH estrone into eight normal volunteers and cannot corroborate these findings.

Our findings would categorize the catechol estrogens as metabolic products of estradiol, and to a lesser extent, estrone, with low estrogenic activity. They do not appear to subserve a unique physiologic role.

It still remains unclear how the catechol estrogens, weak as they are, effect their central nervous system action. Two hypotheses prevail. The first proposes that these steroids act exclusively via catecholamine receptors or by influencing the metabolism of endogenous CNS catechol amines. The second proposes that all of the actions of the catechol estrogens can be accounted for by their interaction with the estrogen cytosol receptor. We propose to test these two hypotheses by examining the action of the catechol estrogens in systems wehrein one or the others of these receptor systems has been blocked. Initial studies will use the rat as the experimental animal.

Studies on the peripheral action of steroid hormone have yielded new and interesting findings. Earlier studies revealed that one of the causes of abnormal sex steroid expression is resistance of the peripheral tissue to sex hormone. Androgen resistance in men, for example, is usually associated with feminization manifest as gynecomastia. Steroid hormone resistance has been described for androgens, mineralocorticoids, and progestins. Naturally occurring examples of resistance to glucocorticoids and estrogens have not been described. Since glucocorticoids are essential for life, individuals surviving with the defect would be expected to have compensated for the abnormality. Theoretically this could only be done if the primary defect were one of receptor affinity rather than receptor number, the compensatory mechanism being an increase in the plasma concentration of the appropriate glucocorticoid. Following this reasoning, we have used plasma cortisol concentration as a screen for this condition in animals and man.

Several new world primates were found to have elevated plasma cortisol levels. These include the squirrel monkey, the marmoset, and the owl monkey. Plasma cortisol levels ranged between 100 and 300 μg/dl in these animals. Plasma free cortisol levels were equally elevated and the hypothalmic-pituitary-addrenal axis was found to be resistant to dexamethasone suppression.

When glucocorticoid receptors were exmained in circulating white cells and cultured fibroblasts, they were found to be of normal number but reduced affinity. The receptor molecule also appeared to be unstable in that receptor number rapidly diminished in broken cell preparations.

An example of glucocorticoid resistance has also been identified in man. A 56 year old man under evaluation for hypertension was found to have elevated plasma cortisol levels. He exhibited none of the clinical stigmata of Cushing's Syndrome. Evaluation of other family members revealed a son with the same abnormality. Both subjects had elevated plasma cortisol levels, elevated free cortisol concentrations, and were resistant to the suppressive effects of dexamethasone. Examination of the glucocorticoid receptor in white cells and fibroblasts from these men showed an abnormality similar to that seen in the new world primates. There appeared to be a normal number of labile glucocorticoid receptors having a reduced affinity for cortisol.

These are the first known examples of glucocorticoid resistance. The defect, one of decreased receptor affinity for cortisol, is the first known affinity defect for steroid hormones. These models promise to provide new insight into the physiology of glucocorticoid hormone action.

A number of ongoing studies are directed toward understanding the role of adrenal androgen secretion in growth and development and toward understanding the physiological principles underlying this important feature of adrenal function.

The role of adrenal androgens in idiopathic hirsutism remains controversial. The clinical observation that a link exists between hyperprolactinemia and hirsutism is being reported with increasing frequency. In reviewing our clinical experience it became apparent that little is known about the natural history of spontaneous hyperprolactinemia and its influence on androgen production.

We examined, retrospectively, the clinical course of 16 women with hyperprolactinemia. None of these patients were treated with pituitary surgery or x-irradiation. Three patients received courses of bromocryptine lasting from 6 months to 3.5 years. None had received this drug for at least 2 months before reevaluation. The mean interval between the onset of symptoms and the final evaluation was 11.3 yrs (range 3 to 26). No patient, over this interval, worsened clinically. The number of patients with galactorrhea dropped from 12 to 7 and the number with amenorrhea dropped from 12 to 9. Five of the 16 women had a total of 7 pregnancies since the onset of their symptoms. Six were spontaneous and 1 was induced with clomiphene. No patient complained of headaches. At the time of reevaluation two complained of impaired libido. None complained of hirsutism. The mean prolactin level was 123.2 ng/ml (range 30.6 to 207.7). One patient had subtle progression of a sella abnormlaty and 1 patient with a grade III sella showed improvement. Visual fields were normal in all patients.

We conclude that hyperprolactinemia may have a benign clinical course. In our subjects, prolactin levels tended to decrease with time. Small tumors did not progress to large ones even with the stimulus of pregnancy. Conservative management of hyperprolactinemia, in contrast to the current standard of therapy, seems indicated. Intervention should be reserved for patients with disabling symptoms or documented progression.

Prolactin is known to cause bilateral adrenal hypertrophy in rats, and specific prolactin binding has been found in the adrenal glands of this species. Several reports show that lowering plasma prolactin with bromocriptine in man results in decreased plasma adrenal androgen concentrations. Whether this is due to a decrease in the production rate of these steroids or to an increase in their metabolic clearance rate was unclear.

We investigated this problem by measuring basal and ACTH stimulated serum dehydroepiandrosterone and dehydroandrosterone sulfate and the prodution rate of dehydroepiandrosterone sulfate in hyperprolactinemic patients before and during treatment with bromocriptine. Seven women, aged 22-34 years, had prolactin levels ranging between 100-507 ng/ml (mean 201). Bromocriptine was administered for an average of 7 months. Serum prolactin fell to 36 $\pm$ 15 ng/ml ($\bar{x}$ $\pm$ SD). Serum dehydroepiandrosterone, dehydroandrosterone sulfate and cortisol (F) were measured in blood samples obtained every hour for 24 hours and 8 serum samples were obtained during maximal ACTH stimulation. The production rate of dehydroepiandrosterone sulfate was detemrined using the technique of urinary isotope dilution.

Basal serum dehydroepiandrosterone and dehydroepiandrosterone sulfate were normal before and after treatment. The metabolic clearance rate of dehydroepiandrosterone sulfate did not change significantly with treatment but the production rate of DHAS decreased by about 50%.

These data show that decreasing plasma prolactin concentrations are associated with a decreasing production rate of dehydroepiandrosterone sulfate, the predominant adrenal androgen in man. This is, to our knowledge, the first documented extramammay effect of prolactin in humans.

The initiation of adrenal androgen secretion is termed adrenarche. This process begins, in man, at about 5 years of age. The enzymatic changes underlying this change in adrenal steroidogenesis have been the topic of much speculation. We have measured the activities of 5 microsomal enzymes in human adrenal glands obtained from subjects between the ages of 3 months and 60 years. The preodmnant enzymatic change is an increase in the activities of both 17-hydroxylase and 17-20-desmolase. This observation focuses attention on the factors that regulate these enzymes as a new approach to exploring the mechanism of adrenarche.

Studies exploring the mechanism of adrenarche are underway in the chimpanzee, the only known animal model for human adrenarche. We hope to determine (1) whether human pituitary extract can initiate adrenarche and (2) whether non-ACTH pituitary factors are required to maintain adrenarche in adult chimpanzees. Ten chimpanzees have been entered into the study. Basal measurements of plasma adrenal androgen levels and cortisol and DHA metabolic clearance rates have been completed. Determinations during the experimental period are in progress. The available data suggest that hypophysectomy with replacement of ACTH will maintain cortisol but not adrenal androgen secretion supporting the hypothesis that a pituitary hormone other than ACTH regulates adrenal androgen secretion.

Our final project is the development of clinically useful steroid hormone antagonists. Antagonists exist for androgens, estrogens and mineralocorticoids. They do not exist for glucocorticoids and progestins.

We have spent considerable efort in trying to identify a glucocorticoid antagonist that is active *in vivo*. 11-deoxycortisol is a known glucocorticoid antagonist *in vitro*. It has not previously been shown to be an antagonist *in vivo*. We found that the reason for the apparent failure of 11-deoxycortisol to act as a glucocorticoid antagonist *in vivo* is that it undergoes 11-hydroxylation by the adrenal gland, thus being converted to an agonist. This has been confirmed by showing that 11-deoxycortisol retains full antagonist activity in adrenalectomized rats. Work has progressed toward developing effective *in vivo* antagonists by synthesizing 11-deoxycortisol analogues that resist 11-hydroxylation such as $^{1,9(11)}$S. This compound acts as a glucocorticoid antagonist both *in vivo* and *in vitro*. $\Delta^{1,9(11)}$S, however, is notsufficiently resistant to 11-hydroxylation to have potential clinical use.

Several newly synthesized 11-deoxycortisol and cortisol analogs which are antiglucocorticoids *in vitro* have been examined for glucocorticoid antagonist activity *in vivo*. These compounds include 2'-p-fluorophenylpyrazole[3,2c]-11-deoxycortisol, cortisol-21-mesylate, and dexamethasone-oxetanone. The first

two compounds exhibited partial agonist-antagonist activity. The latter compound exhibited only agonist activity *in vivo* despite potent antagonist activity *in vitro*.

Our effort to synthesize the most promising compound, 11-oxa-11-deoxycortisol, has produced a low yield from a 20-step synthesis. The amount of material made thus far is not sufficient for biologic testing, but will permit development of an assay for the compound and pharmacokinetic studies. The experience gained during the initial synthesis has suggested methods which should increase the yield of the compound sufficiently to allow biological studies. The available evidence suggests that 11-oxa-11-deoxycortisol will have pure glucocorticoid antagonist activity *in vivo*, a property not yet found in any other compound.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00600-01 DEB (formerly 00157-05)

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Physiology of Puberty

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI G. B. Cutler, Jr. M.D., Senior Investigator — DEB, NICHD, NIH

Other
- D. Lynn Loriaux, M.D., Ph.D., Chief — DEB, NICHD, NIH
- B. Albertson, DHHS Post Doctoral Fellow — DEB, NICHD, NIH
- F. Cassorla, Clinical Associate — DEB, NICHD, NIH
- F. Comite, Clinical Associate — DEB, NICHD, NIH
- J. Levine, Clinical Associate — DEB, NICHD, NIH
- J. Winterer, Clinical Associate — DEB, NICHD, NIH
- J. Booth, Visiting Associate — DEB, NICHD, NIH

COOPERATING UNITS (if any)

Dr. Erhard Gross, ERRB, NICHD

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS XX ☐ (b) HUMAN TISSUES XX ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to gain insight into the physiologic processes underlying normal and abnormal pubertal progression. Principal areas of investigation include the mechanisms of adrenarche, the temporal changes in hypothalamic function with respect to gonadotropin regulation, the mechanism of the pubertal growth spurt, the treatment of idiopathic true precocious puberty with an analog of luteinizing hormone releasing hormone, and the development and use of luteinizing hormone releasing hormone antagonists.

PHS-6040
(Rev. 2-81)

Objectives:

This project was started in 1976 in an attempt to begin to understand some of the fundamental processes of pubertal development. The three immediate goals are the study of adrenarche, hypothalamic-pituitary maturation, and the mechanism of the pubertal growth increase. In adition, a newly available analog of luteinizing hormone releasing hormone is being used to treat children with true precocious puberty.

Methods Employed:

1) Adrenarche - Methods consist of steroid radioimmunoassay, determination of steroid metabolic clearance rate and production rate, and enzymatic assay of 17-hydroxylase, 21-hydroxylase, 17,20-desmolase, and 3β-hydroxysteroid dehydrogenase/ Δ^5, Δ^4-isomerase in adrenal microsomes.

2) Treatment of true precocious puberty-steroid and peptide hormone radioimmunoassay, solid-phase synthesis of luteinizing hormone releasing hormone (LHRH) analogs, and LHRH analog bioassay.

3) Mechanism of the pubertal growth spurt - This project will depend on the accurate measurement of growth over very short time intervals (3 weeks) using the recently invented ulnar measuring device of Dr. Ignatius M. Valk (Nijmegen, Netherlands) which is able to measure the length of the ulna with a standard deviation of $\pm$ 0.2 mm.

Progress:

1) Adrenarche - Studies of adrenal androgen secretion in response to ACTH in adults with pituitary disease have shown a preadrenarchal pattern, suggesting that pituitary hormones other than ACTH may be required to maintain the postadrenarchal response.

Measurement of 17-hydroxylase, 21-hydroxylase, 3β-hydroxysteroid dehydrogenase/ Δ^5, Δ^4-isomerase, and 17,20-desmolase activities in human adrenal microsomes has revealed that the predominant enzymatic alteration during adrenarche is a marked increase in the activities of both 17-hydroxylase and 17,20-desmolase.

To investigate the role of prolactin in adrenal androgen secretion, dehydroepiandrosterone (DHA) sulfate blood production rates have been measured before and after hypophysectomy and before and during bromocryptine treatment in hyperprolactinemic patients. The study has shown a fall in DHA sulfate production rate after prolactin levels were lowered.

Investigations are underway in the chimpanzee, the only known model for human adrenarche, to determine (1) whether human pituitary extract can initiate adrenarche and (2) whether non-ACTH pituitary factors are required to maintain adrenarche in adult chimpanzees. Ten chimpanzees have been entered into the study. Basal measurements of plasma adrenal androgen levels and cortisol and DHA metabolic clearance rates, have been completed. Follow-up determinations during the experimental period are in progress. The available data suggest that hypophysectomy with replacement of ACTH will maintain cortisol but not

adrenal androgen secretion, which supports the hypothesis that a pituitary hormone other than ACTH regulates adrenal androgen secretion.

2) Treatment of true precocious puberty - approximately 30 patients have begun treatment with a long-acting LHRH analog, to our knowledge the first use of this agent to treat precocious puberty. The results thus far have been promising, with decreased pituitary gonadotropin secretion, decreased sex hormone levels, and favorable clinical changes.

Significance:

1) Adrenarche - adrenal androgens play an important pathogenetic role in certain hormone-dependent cancers such as carcinoma of the breast and prostate, and in a variety of endocrine disorders such as congenital adrenal hyperplasia, premature adrenarche, hirsuitism, and Cushing's syndrome. Increased understanding of the mechanism of adrenarche may lead to new methods of controlling adrenal androgen secretion in these disorders.

The observation that increased 17-hydroxylase and 17,20-desmolase are the major enzymatic changes during adrenarche focuses attention on the factors that regulate these enzymes as a new approach to exploring the mechanism of adrenarche. At present very little is known about how the activity of these enzymes is regulated.

2) Treatment of true precocious puberty - Since no generally satisfactory treatment is available for this disorder, the promising early results of LHRH analog therapy may represent a major therapeutic advance. Future systematic investigation of the dose, frequency, duration, and chemical nature of the analog used will be necessary to realize the full potential benefit from this new form of therapy.

The study has also been enlarged to include different etiologies of precocious puberty, such as McCune-Albright Syndrome, CNS hamartoma, and familial male isosexual precocity. This will determine whether the promising results seen in idiopathic precocious puberty will apply to other forms of precocious puberty.

Proposed Course:

1) The investigation of the role of the hypothalamic - pituitary system in the induction and maintenance of adrenarche in the chimpanzee. This study, currently underway in collaboration with the primate research facility at Holloman Air Force Base, Alamagordo, New Mexico, will be continued.

2) The investigation of the functional properties of the human adrenal zona fasciculata and zona reticularis. Portions of human adrenal zona reticularis and zona fasciculata will be removed from surgical specimens with the aid of a dissecting microscope. Androgen secretion rates will be measured during organ culture in vitro to compare directly the ability of each zone to secrete adrenal androgens. The concentration of receptors of prolactin, a known stimulator of adrenal androgens, will also be measured in each adrenal zone.

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Significance:

1) Adrenarche - adrenal androgens play an important pathogenetic role in certain hormone-dependent cancers such as carcinoma of the breast and prostate, and in a variety of endocrine disorders such as congenital adrenal hyperplasia, premature adrenarche, hirsuitism, and Cushing's syndrome. Increased understanding of the mechanism of adrenarche may lead to new methods of controlling adrenal androgen secretion in these disorders.

The observation that increased 17-hydroxylase and 17,20-desmolase are the major enzymatic changes during adrenarche focuses attention on the factors that regulate these enzymes as a new approach to exploring the mechanism of adrenarche. At present very little is known about how the activity of these enzymes is regulated.

2) Treatment of true precocious puberty - Since no generally satisfactory treatment is available for this disorder, the promising early results of LHRH analog therapy may represent a major therapeutic advance. Future systematic investigation of the dose, frequency, duration, and chemical nature of the analog used will be necessary to realize the full potential benefit from this new form of therapy.

The study has also been enlarged to include different etiologies of precocious puberty, such as McCune-Albright Syndrome, CNS hamartoma, and familial male isosexual precocity. This will determine whether the promising results seen in idiopathic precocious puberty will apply to other forms of precocious puberty.

Proposed Course:

1) The investigation of the role of the hypothalamic - pituitary system in the induction and maintenance of adrenarche in the chimpanzee. This study, currently underway in collaboration with the primate research facility at Holloman Air Force Base, Alamagordo, New Mexico, will be continued.

2) The investigation of the functional properties of the human adrenal zona fasciculata and zona reticularis. Portions of human adrenal zona reticularis and zona fasciculata will be removed from surgical specimens with the aid of a dissecting microscope. Androgen secretion rates will be measured during organ culture in vitro to compare directly the ability of each zone to secrete adrenal androgens. The concentration of receptors of prolactin, a known stimulator of adrenal androgens, will also be measured in each adrenal zone.

3) The treatment of precocious puberty with a long-acting LHRH analog. Patients currently under treatment will continue to be followed to assess the long-term effects of therapy. New patients will be added to the study so that the effects of differing etiology of precocious puberty and differing dose, frequency, and duration of treatment can be evaluated.

4) The investigation of the mechanism for the pubertal growth spurt. Patients with delayed puberty will receive short-term treatment (one week) with estrogen, testosterone, or a non-aromatizable androgen (dihydrotestosterone). The growth response to each steroid will be quantitated using a uniquely sensitive device for the measurement of ulnar growth. Dose-response curves will be determined for each sex steroid to determine which contributes most to the pubertal growth spurt.

5) Patients with gonadal dysgenesis will be used as a model to study the pubertal maturation of gonadotropin secretion. Changes in the frequency, amplitude, and diurnal pattern of gonadotropins will be measured to provide further insight into the altered neuroendocrine activity that underlies the onset of puberty.

6) Theoretical considerations suggest that an effective LHRH antagonist would be useful for the treatment of precocious puberty, hirsutism, endometriosis, fibrocystic mastopathy, and endocrine-dependent neoplasms such as breast and prostate cancer. However, none of the available LHRH antagonists are sufficiently potent for clinical use. A new series of LHRH analogs will be synthesized in collaboration with Dr. Erhard Gross in the hope of developing structure-function relationships that will permit synthesis of a clinically useful LHRH antagonist.

Publications:

Crowley, W.F., F. Comite, W. Vale, J. Rivier, D.L. Loriaux, and G.B. Cutler, Jr.: Therapeutic Use of Pituitary Desensitization with a Long-Acting LHRH Agonist in Idiopathic Precocious Puberty. J. Clin. Endocrinol. Metab. 52, 370-372, 1981.

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Schiebinger, R.J., B.D. Albertson, K.M. Barnes, G.B. Cutler, Jr., and D.L. Loriaux: Developmental Changes in Rabbit and Dog Adrenal Function: A Possible Homologue of Adrenarche in the Dog. Am. J. Physiol. 240: E694-E699, 1981.

Chrousos, G.P., D.L. Loriaux, R.J. Sherins, and G.B. Cutler, Jr.: Unilateral Testicular Enlargement Resulting from Inapparent 21-Hydroxylase Deficiency. Journal of Urology, in press.

Castracane, V.D., G.B. Cutler, Jr., and D.L. Loriaux: Pubertal Endocrinology of the Baboon: Adrenarche. Am. J. Physiol., in press (October or November 1981).

Cutler, G.B., Jr., F. Comite, J. Rivier, W.W. Vale, D.L. Loriaux, and W.F. Crowley, Jr.: Pituitary Desensitization with a Long-Acting Luteinizing Hormone Releasing Hormone (LHRH) Analog: A Potential New Tretment for Idiopathic Precocious Puberty, In Brooks-Gunn, J. and A. Petersen (eds.): Female Puberty, Plenum Publishing Corp., New York, 1981, in press.

Comite, F., G.B. Cutler, Jr., J. Rivier, W.W. Vale, D.L. Loriaux, and W.F. Crowley, Jr.: Suppression of Pituitary Gonadotropin Secretion with a Long-acting Luteinizing Hormone Releasing Hormone (LHRH) Analog: A Potential New Therapy for Idiopathic Precocious Puberty. N. Engl. J. Med., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00601-01 DEB (formerly 00158-05)

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pathophysiology of Gynecomastia and Hirsutism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G. Cutler, Jr., M.D., Medical Officer	DEB, NICHD, NIH
	M. Koppelman, M.D., Clinical Associate	DEB, NICHD, NIH
	M. Lipsett, M.D., Director	Clinical Center, NIH
	G. Merriam, M.D., Clinical Associate	DEB, NICHD, NIH
	R. Schiebinger, M.D., Clinical Associate	

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION
Developmental Endocrinology Branch

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

XX

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide information for the understanding of disordered phenotypic secondary sexual expression, including such disorders as gynecomastia and hirsutism. The current principal area of investigation is end organ steroid-receptor interaction.

PHS-6040
(Rev. 2-81)

Progress:

1 - Gynecomastia:

Earlier studies revealed that one of the causes of abnormal sex steroid expression is resistance of the peripheral or responding tissue to sex hormone. Androgen resistance in men, for example, is usually associated with feminization manifest as gynecomastia. Steroid hormone resistance has been described for androgens, mineralocorticoids, and progestins. Naturally occurring example of resistance to glucocorticoids and estrogens have not been described. Since glucocorticoids are essential for life, individuals surviving with the defect would be expected to have compensated for the abnormality. Theoretically this could only be done if the primary defect were one of receptor affinity rather than receptor number, the compensatory mechanism then being an increase in the plasma concentration of the appropriate glucocorticoid. Following this reasoning, we have used plasma cortisol concentration as a screen for this condition in animals and man.

Several new world primates were found to have very elevated plasma cortisol levels. These include the squirrel monkey, the marmoset, and the owl monkey. Plasma cortisol levels ranged between 100 and 300 μg/dl in these animals. Plasma free cortisol levels were equally elevated and the hypothalmic-pituitary-adrenal axis was found to be resistant to dexamethasone suppression.

When glucocorticoid receptors were examined in circulating white cells and cultured fibroblasts from these animals, they were found to be of normal number but reduced affinity. The receptor molecule also appeared to be unstable in that receptor number rapidly diminished in broken cell preparations.

An example of glucocorticoid resistance has also been identified in man. A 56 year old man under evaluation for hypertension and found to have elevated plasma cortisol levels. He exhibited none of the clinical stigmata of Cushing's Syndrome. Evaluation of other family members revealed a son with the same abnormality. Both subjects had elevated plasma cortisol levels, elevated free cortisol concentrations, and were resistant to the suppressive effects of dexamethasone. Examination of the glucocorticoid receptor in white cells and fibroblasts from these men showed an abnormlaity similar to that seen in the new world primates. There appeared to be a normal number of labile glucocorticoid receptors having a reduced affinity for cortisol.

Significance: These are the first known examples of glucocorticoid resistance. The defect, one of decreased receptor affinity for cortisol, is the first known affinity defect for steroid hormones. These models promise to provide new insight into the physiology of glucocorticoid hormone action.

Proposed Course: Over the next year we will attempt to characterize the abnormality of the glucocorticoid receptor protein from new world primates.

Progress:

2 - Hirsutism: The clinical observation that a link exists between hyperprolactinemia and hirsutism is being reported with increasing frequency. In reviewing our clinical experience it became apparent that little is known

about the natural history of spontaneous hyperprolactinemia and its influence on androgen production.

A - We examined, retrospectively, the clinical course of 16 women with hyperprolactinemia. None of these patients were treated with pituitary surgery or x-irradiation. Three patients received courses of bromocryptine lasting from 6 months to 3.5 years. None had received this drug for at least 2 months before reevaluation. The mean interval between the onset of symptoms and the final evaluation was 11.3 yrs (range 3 to 26). No patient, over this interval, worsened clinically. The number of patients with galactorrhea dropped from 12 to 7 and the number with amenorrhea dropped from 12 to 9. Five of the 16 women had a total of 7 pregnancies since the onset of their symptoms of which 6 were spontaneous and 1 induced with clomiphene. At reevaluation no pt complained of headaches. Two complained of impaired libido. None complained of hirsutism. The mean prolactin level was 123.2 ng/ml (range 30.6 to 307.7). One patient had subtle progression of a sella abnormalities and 1 patient with a grade III sella showed improvement. Visual fields were normal in all patients.

Significance: We conclude that hyperprolactinemia may have a benign clinical course. In our subjects, prolactin levels tended to decrease with time. Small tumors did not progress to large ones, even with the stimulus of pregnancy. Conservative management of hyperprolactinemia; in contrast to the current standard of therapy, seems indicated. Intervention should be reserved for patients with disabling symptoms or documented progression.

2B - Adrenal androgen secretion:

Prolactin is known to cause bilateral adrenal hypertrophy in rats, and specific prolactin binding has been found in the adrenal glands of this species. Several reports show that lowering plasma prolactin with bromocriptine in man results in decreased plasma adrenal androgen concentrations. Whether this is due to a decrease in the production rate of these steroids or to an increase in their metabolic clearance rate is unknown.

We investigated this problem by measuring basal and ACTH stimulated serum dehydroepiandrosterone and dehydroandrosterone sulfate and the production rate of dehydroepiandrosterone sulfate in hyperprolactinemic patients before and during treatment with bromocriptine. Seven women aged 22-34 years had prolactin levels ranging between 100-507 ng/ml (mean 201). Bromocriptine was administered for an average of 7 months. Serum prolactin fell to 36 $\pm$ 15 ng/ml ($\bar{x} \pm$ SD). Serum dehydroepiandrosterone, dehydroandrosterone sulfate and cortisol (F) were measured in blood samples obtained every hour for 24 hours and is 8 serum samples obtained during maximal ACTH stimulation. The production rate of dehydroepiandrosterone sulfate was determined using the technique of urinary isotope dilution.

Basal serum dehydroepiandrosterone and dehydroepiandrosterone sulfate were normal before and after treatment. The metabolic clearance rate of dehydroepiandrosterone sulfate did not change significantly with treatment but the production rate of DHAS decreased by about 50%.

Significance:

These data show that decreasing plasma prolactin concentrations are correlated with a decreased production rate of dehydroepiandrosterone sulfate, the predominant adrenal androgen in man. This is, to our knowledge, the first well documented extramammary effort of proalctin in man.

Proposed Course:

1 - Natural history of hyperprolactinemia: The study will be expanded to a total of 25 patients. On the basis of the conclusions derived from this retrospective study, a prospective study will be designed to examine potential liabilities associated with long term untreated hyperprolactinemia.

2 - Modulation of adrenal androgen secretion by adrenal androgens: The locus of the effect of prolactin on dehydroepiandrosterone sulfate secretion will be determined using appropriate tracer techniques. The site of prolactin binding in human adrenal tissue will be sought. The effect of prolactin on the steroid secretory products of the three adrenal zones in short term organ culture will be examined.

Publications:

Chrousos, G.P., D. Renquist, D. Brandon, C. Eil, M. Pugeat, G.B. Cutler, D. Loriaux, and M.B. Lipsett, (1981). Glucocorticoid Hormone "Resistance" in 2 Primate Species. Clin Res 29: 504 (Abstract).

Chrousos, G.P., A. Vingerhoeds, D. Brandon, J. De Regt, M. Pugeat, C. Eil, D.L. Loriaux and M.B. Lipsett, (1981). Primary Cortisol Resistance in Man: A Glucocorticoid Receptor-Mediated Disease. Endocrine Society, Cincinnati, Ohio (Abstract).

Cassorla F., B. Albertson, G.P. Chrousos, J. Booth, M.B. Lipsett and D.L. Loriaux, (1981). The Mechanism of Hypercortisolemia in Squirrel Monkeys. European Society of Pediatric Endocrinology and Lawson Wilkins Society Jount Meeting, Geneva, Switzerland (Abstract).

Chrousos, G.P., A. Vingerhoeds, D. Brandon, M. Pugeat, C. Eil, D.L. Loriaux and M.B. Lipsett, (1981). Primary Cortisol Resistance in Man: A Glucocorticoid Receptor-mediated Disease, New Engl. J. Med. (submitted for publication).

Chrousos, G.P., D. Renquist, D. Brandon, C. Eil C, R. Vigersky. D.L. Loriaux and M.B. Lipsett (1981). Glucocorticoid Resistance and Primate Evolution: Receptor-mediated Mechanisms. Proc. U.S. Acad. Sci. (submitted for publication).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00602-01 DEB (formerly 00161-05)

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Sex Steroids in Regulation of FSH and LH Levels in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of studies in the rat have been utilized to ascertain the extent to which testosterone and estradiol can regulate the secretion of FSH and LH in the absence of the testes. The data demonstrate that 1) testosterone alone at physiologic doses can maintain both gonadotropins within the normal range, 2) that estradiol alone preferentially suppresses LH, but not FSH concentrations and 3) that a selective increase in plasma FSH is produced when there is reduced androgen production in association with increased estradiol production. Similar changes are seen in the pituitary content of FSH and LH suggesting that these altered sex steroid levels effect gonadotropin synthesis, storage and release. These data provide an alternate explanation for the inhibin hypothesis which suggests that there is a specific non sex steroid inhibitor of FSH secretion arising from the seminiferous tubules.

PHS-6040
(Rev. 2-81)

Objectives:

A considerable body of data exists suggesting that androgens regulate pituitary LH secretion in men by negative feedback inhibition. Estradiol, however, also significantly suppresses LH secretion. Since plasma estradiol in men is derived primarily from testosterone aromatized in peripheral tissues, any study of the role of testosterone per se on gonadotropin secretion measures the effect of both testosterone and estradiol. Accordingly, a series of studies have been developed to compare the relative contributions of testosterone and estradiol in the regulation of FSH and LH concentrations in normal men and male rats.

Methods Employed:

Testosterone (T) and Estradiol (E) are administered to castrate adult male rats via silastic capsules. Plasma levels of T, E, and FSH and LH are measured by radioimmunoassay from samples obtained during one month of study and correlated with changes in hypothalamic content of GnRH and pituitary content of FSH and LH.

The effects of physiological alterations on the recruitement of gonadotrophs is assessed by stereological techniques, using immunohistocytochemistry to identify LH and FSH - specific gonadotrophs.

Major Findings:

When T alone is given at a dose which maintains mid normal plasma T levels and normal prostate size in the castrate rat, plasma FSH and LH are both normal, indicating that testicular factors other than sex steroids are not necessary for maintaining FSH and LH levels within the normal range in the rat.

By contrast, when E is given alone at a dose which increases plasma E levels slightly above normal, plasma LH concentrations are suppressed, while FSH levels remain unexpectedly elevated into the castrate range. The data indicate that androgen (not estrogen) is critical in maintaining tonic suppression of FSH secretion.

When T and E are administered together at doses which produce subphysiologic levels of plasma T, a selective increase in plasma FSH is maintained similar to that seen in male animals with severe germ cell depletion. A similar change in ratio of pituitary FSH and LH is also produced at a time when GnRH content of the hypothalamus is within normal limits, suggesting that altered ratios of testosterone and estradiol primarily effect synthesis, storage and release of pituitary gonadotropins.

Preliminary findings indicate that alteration in the sex-steroidal millieu within the physiological range markedely alters pituitary size due to changes in the number and size of functioning gonadotrophs as well as lactotrophs.

Significance:

These data add considerable insight into the mechanism of control of gonadotropin secretion in the male. The results indicate that testosterone

can maintain both gonadotropins within the normal range in the absence of other testicular factors and, further, that a selective increase in plasma FSH level can be induced when there is subnormal replacement of T but increased production of E. The data provide an alternate explanation for the inhibin hypothesis.

Proposed Course:

1) Using in vitro cultures of rat anterior pituitary cells, the effects of T and E on synthesis of FSH and LH will be investigated.

2) Using castrate men, men with germ cell depletion and with Klinefelter's syndrome a systematic study of androgen production in relationship to estrogen production will be made to determine if the selective increase in FSH seen in men with germinal aplasia can be accounted for by a decrease in T production in association with increased E production.

3) In the human, testosterone is transported in the circulation bound to TEBG (a binding protein). T reduces while E increases TEBG concentrations. Thus, subtle changes in T/E ratio can markedly alter T transport. Accordingly, TEBG binding will be assessed in men with reproductive disorders to provide a more quantitate estimate of changes in testicular function.

It is our intention to show that a subtle decrease in Leydig cell function in men with germ cell depletion produces decrease T production and increased E production leading to a selective increase in plasma FSH concentration similar to data obtained from our studies in the rat. In the human, however, T binding to TEBG in plasma may obscure the subtle changes in sex steroid production.

Publications:

Sherins, R.J., Patterson, A.P., Brightwell, D., Udelsman, R. and Sartor, J. Alteration in the Plasma Testosterone/Estradiol Ratio: An Alternative to the Inhibin Hypothesis. Annals New York Acad. Sci. (In press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00603-01 DEB (formerly 00163-05)

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Studies of Male Reproductive Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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C. Gagnon - Visiting Scientist, DEB, NICHD
J.L. Ziegler - Senior Investigator, NCI, NIH
N. Javadpour - Senior Investigator, NCI, NIH
D. Poplack - Senior Investigator, NCI, NIH
T. Kinsella - Senior Investigator, NCI, NIH
S. Rosenberg - Senior Investigator, NCI, NIH

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LAB/BRANCH
Developmental Endocrinology Branch

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A variety of studies of clinical disorders of male reproduction are in progress. These include 1) studies of the hormonal regulation of spermatogenesis in hypogonadotropic men, 2) analysis of biochemical parameters of sperm metabolism in men with non-motile sperm, 3) longitudinal evaluation of the adverse effects of cytotoxic drugs on testicular function in men receiving chemotherapy for malignant disorders, and 4) the evaluation of treatment of men with idiopathic infertility.

PHS-6040
(Rev. 2-81)

Objectives:

The primary objective of the project is to provide a rational basis for the diagnosis and treatment of disorders of male infertility.

Methods Employed:

The evaluation of patients with infertility and/or hypogonadism begins with a thorough examination in our outpatient clinic. Subsequent admission to our metabolic ward is used when more detailed study is required. About 100 new patients are studied annually. A variety of techniques are employed which include: measurement of hormones (FSH, LH, testosterone, estradiol, etc.) in biological fluids, semen analysis, testicular biopsy, chromosome analysis, electron microscopy of sperm and/or testis, and selected enzyme activity measurements in sperm.

Major Findings:

1) A 10 year prospective study of the treatment of hypogonadotropic men has now been completed which indicates that intratesticular testosterone alone is required to initiate human spermatogenesis and that FSH is required to complete spermiogenesis (in concert with abundant levels intratesticular testosterone). By contrast, supplemental FSH is not usually required to maintain human spermatogenesis once it has been initiated.

2) Studies of semen from infertile men with non-motile sperm (necrospermia) show 1) that such cells fail to metabolize fructose, the normal primary energy source in sperm, and 2) have increased specific activity of an endogenous lipid peroxidase, capable of producing peroxides of sperm phospholipid. These peroxides promptly and irreversibly immobilize spermatozoa.

Human sperm have recently been shown also to contain protein carboxyl methylase, an enzyme required for chemotaxis by mammalian monocytes and bacteria. This enzyme acts upon a specific protein methyl acceptor and inactivation of the enzyme leads to failure of characteristic unidirectional motile progression. A deficiency of protein carboxyl methylase activity has been identified in some men with immotile sperm.

3) On-going prospective studies of men, women, boys and girls, of the adverse effects of chemotherapy and radiotherapy on gonadal function indicates that these agents vary considerably in their potential gonadal toxicity. High-dose methotrexate appears to produce no ovarian dysfunction, and only transient azoospermia; and adriamycin does not appear to produce spermatonial killing in men or significantly alter ovarian cyclicity. By contrast, methylhydrazines, alkylating agents and radiation produce dose-dependent gonadal failure in both sexes.

4) Ten year prospective studies of human male reproductive function indicates the need to exhaustively evaluate testicular function (hormonal and germinal functions) prior to the institution of any therapeutic intervention in order to properly assess baseline function. The concurrent need to estimate the reproductive potential of the spouse is essential. Our data demonstrate that empirical therapy of male infertility is irrational and unsuccessful, and that

treatment must be based on a fundamental understanding of the biochemical abnormality.

Significance:

These observations suggest that there may be a biochemical marker for certain of the male infertility states in which sperm motility is altered.

Proposed Course:

1) Establish a laboratory designed to provide hormone to hypogonadotropic subjects via an implantable programmable infusion pump. Such a highly sophisticated computer programmable delivery system is now available for phase I testing, in collaboration with the Applied Physics Laboratory, Johns Hopkins University, Baltimore, Maryland. This laboratory will be established to assess the hormonal regulation of spermatogenesis, but as a core laboratory will also be available to other NICHD investigators interested in utilizing the principle of continuous infusion systems to study and/or treat other endocrine disorders.

2) Pursue studies of human sperm biochemistry and attempt to identify correlations between the parameters of fructolysis, caffeine acivation of motility, the specific activity of protein carboxyl methylase and lipid peroxidase among men with necrospermia as well as infertile men with less severe disorders of sperm motility. We will include electron microscopic evaluation of the sperm in view of recent findings in other laboratories that the sperm of some necrospermic patients show ultrastructural abnormalities (i.e., absence of the dynein arms of axial filaments, known to contain ATPase).

Most would now agree that semen analysis is often an inadequate and insensitive tool in judging the adequacy of an ejaculate for impregnation. Accordingly, we are evaluating a spectrum of biochemical assays of human sperm function in an attempt to refine our diagnostic abilities in this regard.

3) Assess the effect of a variety of classes of cytotoxic drugs, used as chemotherapy for cancer patients, on human reproductive function. Such patients will also be utilized to assess changes in endocrine function of the testis and the subsequent alterations in regulation of FSH and LH secretion.

4) Recent evidence indicates that reduction of estradiol production from aromatization of testosterone actually enhances testosterone production because of an auto regulatory action of estradiol on T synthesis within the testis. A preliminary clinical study has shown that Teslac, an aromatase inhibitor, can increase sperm production in some men with idiopathic infertility by increasing T production. Accordingly, a double blind clinical trial of Teslac in infertile men will be established.

The aromatase inhibitor will also be used to assess its role in potentiating the testicular response to gonadotropin in hypogonadotropic men and men with seminiferous tubular sclerosis.

Publications:

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Cassorla, F.G., Golden, S.M., JOhnsonbaugh, R.E., Heroman, W.H., Loriaux, D.L. and Sherins, R.J. Testicular volume during early infancy. J. Pediatrics (In press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00604-01 DEB (formerly 00180-02)

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biology of Hormone Binding Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bruce C. Nisula	Sr. Investigator	DEB, NICHD
Other:	James F. Dunn	Clinical Associate	DEB, NICHD
	Michel M. Pugeat	Visiting Fellow	DEB, NICHD
	Richard Sherins	Sr. Investigator	DEB, NICHD
	D. Lynn Loriaux	Head	DEB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Summary:

The long-range goal of this project is to understand the role of serum hormone binding proteins in the regulation of normal physiologic processes and in the pathophysiology of various human disease states. A unique solid phase method for characterizing ligand interactions with testosterone-binding globulin and corticosteroid-binding globulin under equilibrium conditions at physiologic pH and temperature has been developed. Using a computer program, the plasma distribution of 21 endogenous circulating steroids has been computed for men, cycling women, and pregnant women. The physiological significance of the interaction of 70 drugs with these carrier proteins has been assessed. Future research will initiate exploration of the biological significance of hormone transport proteins.

PHS-6040
(Rev. 2-81)

Objectives:

The objective of this project is to investigate the biological relevance of hormone transport proteins to hormone action in normal physiology and pathophysiological states. Specific attention has been focused on the steroid binding proteins, testosterone-binding globulin (TeBG) andd corticosteroid-binding globulin (CBG).

Methods:

Total steroid hormone levels are measured by standard radioimmunoassay techniques employing separative procedures when necessary. Ligand interactions with the steroid-binding proteins are studied by a variety of techniques, including dialysis across semi-permeable membranes, gel filtration chromatography, and a solid phase method developed in this laboratory which utilizes the lectin concanavalin A to insolubilize the transport proteins. With the parameters of ligand interaction with the various proteins, a law of mass action based computer program is used to compute the in vivo simultaneous distribution of multiple ligands into TeBG-bound, CBG-bound, albumin-bound, and unbound fractions.

Participants in this study include normal volunteers and patients with a variety of developmental and reproductive abnormalities.

Progress:

The binding parameters of 21 endogenous steroids and 70 assorted drugs and compounds of significance to steroid physiology and biochemistry have been determined. The solid phase method developed in this laboratory permits determination of the parameters under equilibrium conditions at physiologic pH and temperature. Thus, with parameters determined under physiological conditions, computer simulations of in vivo transport based on the law of mass action can be performed, yielding a solution to the complex, simultaneous interactions between multiple circulating steroids and TeBG, CBG, and albumin. In this manner, for the first time, estimates of the plasma distribution of 21 circulating steroids into TeBG-bound, CBG-bound, albumin-bound, and unbound fractions in normal men, normal women during both the follicular and luteal phases of the ovarian cycle, and women during pregnancy have been produced.

A comprehensive survey of drug interactions with TeBG and CBG has been completed. A wide variety of steroids, some unexpected, showed appreciable interactions with TeBG and CBG in vitro. Whether or not a given compound will alter the transport of endogenous steroids in vivo due to this interaction depends on the concentration which it achieves with therapy. Indeed, therapeutic levels of danazol, methyltestosterone, fluoxymesterone, and norgestrel were found capable of displacing 83%, 48%, 42%, and 16%, respectively, of the testosterone bound to TeBG in a normal man. Further, through its occupancy of 97% of the TeBG binding sites, and 56% of the CBG binding sites, danazol can markedly increase the unbound concentrations of testosterone, estradiol, and cortisol. These data strengthen the concept that a variety of drugs can modulate the concentration of the bioavailable fraction of hormones by virtue of occupancy of binding sites on transport proteins, independent of any direct effects on steroid secretion, steroid metabolism, or binding protein levels.

Structure-binding studies of the testosterone binding site on the TeBG molecule have revealed new information concerning the substituents on the A ring. The affinity of estradiol with its aromatized A ring is about one third of that of testosterone. When the hydrogen at position 2 is substituted with a hydroxyl group, the affinity is about one fifth of that of estradiol. However, when the hydrogen at position 2 is substituted with a methoxy group, the affinity is nearly four-fold greater than that of estradiol and significantly greater than that of testosterone. These data suggest a key hydrophobic region of the binding site interacts with the A ring at position 2.

Men with azoospermia secondary to exposure to the nematocide 1,2-dibromo-3-chloropropane exhibited elevated basal gonadotropin levels in the face of increased total estradiol and testosterone levels. However, since the TeBG levels were also increased, the resulting unbound testosterone levels were not significantly different from controls.

Significance:

The development of technology to resolve the distribution of multiple steroid hormones among their various physical states in the circulation (TeBG-bound, CBG-bound, albumin-bound, and unbound) is a substantial step forward towards elucidation of the biological role of each of these physical components. Initial clinical studies have suggested that the TeBG-bound component of the sex steroids does not participate in the feedback regulation of gonadotropin regulation, but the relative roles of the other components remains to be assessed. The newly acquired facility of measuring the distribution puts resolution of this problem on the horizon. The data generated on the distribution of multiple steroids in plasma cannot be gleaned intuitively or reasoned, as multiple variables need be considered simultaneously. Of particular interest from these studies of normal human physiology was the finding that the distribution patterns of testosterone and cortisol differed substantially with respect to the impact of ligand concentration on the unbound hormone concentration. The unbound cortisol concentration increases in an exponential fashion as a function of total cortisol while the unbound testosterone concentration is better characterized as a linear function of the total testosterone level.

Proposed Course:

Clinical investigations will emphasize the evaluation of transport physiology in normal development and in a variety of disease processes. Attention will be given to the development of a practical clinical assay for human CBG, and its clinical applications. Studies of the phylogeny of CBG in primates have been initiated. Several laboratory models of transport physiology will be explored with the aim of establishing an appropriate system for evaluating the role of transport proteins in steroid action, metabolism, and pituitary regulation.

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1. Dunn, J.F., G.R. Merriam, C. Eil, S. Kono, D.L. Loriaux, and B.C. Nisula, Testosterone-Estradiol Binding Globulin Binds to 2-Methoxyestradiol with Greater Affinity than to Testosterone, Journal of Clinical Endocrinology and Metabolism 51: 404, 1980.

2. Dunn, J.F., B.C. Nisula, and D. Rodbard, Transport of Steroid Hormones: Binding of 21 Endogenous Steroids to Both Testosterone Binding Globulin and Corticosteroid Binding Globulin in Human Plasma, Journal of Clinical Endocrinology and Metabolism, 53: 58, 1981.

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4. Leroith, D., G. Potashnik, J. Dunn, I.M. Spitz, The Exaggerated Prolactin Response to Thyrotropin-Releasing Hormone and Metochlopramide in 1,2-Dibromo-3-Chloropropane-Induced Azoospermia, Journal of Clinical Endocrinology and Metabolism 52: 38, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00605-01 DEB (formerly 00181-02)

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Steroid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other D. Lynn Loriaux, M.D., Ph.D., Chief DEB, NICHD, NIH
G. Chrousos, M.D., Clinical Associate NPMB, NICHD, NIH

COOPERATING UNITS (if any)
Marvin Karten

LAB/BRANCH
Developmental Endocrinology Branch

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. No antagonists have been identified for the glucocorticoids or the progestins. The objective of this project is to develop antagonists for both of these classes of steroids.

PHS-6040
(Rev. 2-81)

Objectives:

Clinically useful antagonists have not been developed for glucocorticoids or progestins. Antagonists for both of these classes of steroids exist in vitro. The objectives of this project are to understand why the known in vitro antagonists fail to act in vivo, and using this knowledge, to produce effective in vivo antagonists.

Methods Employed:

Methods consist of glucocorticoid and progesterone radioreceptor assays, in vivo tests of glucocorticoid action (thymus involution, glycogen deposition, growth retardation) and progesterone action (endometrial maturation index).

Progress:

Several newly synthesized 11-deoxycortisol and cortisol analogs which are antiglucocorticoids in vitro have been examined for glucocorticoid antagonist activity in vivo. These compounds include 2'-p-fluorophenylpyrazole[3,2c]-11-deoxycortisol, cortisol-21-mesylate, and dexamethasoneoxetanone. The first two compounds exhibited partial agonist-antagonist activity. The latter compound exhibited only agonist activity in vivo despite potent antagonist activity in vitro.

A major effort to synthesize 11-oxa-11-deoxycortisol has produced a low yield of this compound after an approximately 20-step synthesis. The amount of compound made thus far is not sufficient for biological testing, but will permit development of an assay for the compound and pharmacokinetic studies. The experience gained during the initial synthesis has suggested methods which should increase the yield of the compound sufficiently to allow biological studies. The available evidence suggests that 11-oxa-11-deoxycortisol will have pure glucocorticoid antagonist activity in vivo, a property not yet found in any other compound.

Significance:

The early results of these studies suggest that it will be possible to develop effective antiglucocorticoids. These compounds, if active in man, would have great promise as a therapeutic tool and as an adjunct to studying adrenal physiology.

Proposed Course:

1) Identification of glucocorticoid antagonists. Efforts to synthesize 11-oxa-11-deoxycortisol will be continued. As soon as available this analog will be tested for antiglucocorticoid activity.

Publications:

Chrousos, G.P., G.B. Cutler, Jr., S.S. Simons, Jr., M. Pons, L.S. John, R.M. Moriarity, and D.L. Loriaux: Development of Antiglucocorticoids with Potential Clinical Usefulness, In Lee, H.J. (ed): Progress in Research and Therapeutic Application of Corticosteroids (Proceedings of the Sixth Annual Clinical Pharmacy Symposium, Tallahassee, Florida, February 20-22, 1981), Heyden: Philadelphia, 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00606-01 DEB (formerly 00182-02)

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Structure, Function, and Physiology of Glycoprotein Hormones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Bruce Nisula	Senior Investigator	DEB	NICHD
Other:	Sania Amr	Visiting Fellow	DEB	NICHD

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental Endocrinology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project is to understand the structure, function, and physiology of the glycoprotein hormones: thyrotropin, luteinizing hormone, follicle stimulating hormone, and chorionic gonadotropin. Recent research developments include the following: deduction from evidence obtained by the study of patients with choriocarcinoma that the thyrotropic activity of the chorionic gonadotropin molecule mediates hyperthyroidism, demonstration that desialylation of chorionic gonadotropin creates a pure competitive antagonist, while treatment with carboxypeptidase creates an improved agonist of thyrotropin action, and characterization of the kinetic parameters and metabolic products of chorionic gonadotropin metabolism. Future investigations will emphasize the development of clinically applicable methodology to measure the glycoprotein hormones and their metabolites, and characterization of the heterogeneous forms associated with various diseases.

PHS-6040
(Rev. 2-81)

Objectives:

The broad objective of this project is to understand the biology of the glycoprotein hormones and the role of glycoprotein hormone derangements in human diseases. The main research effort in the current year has been directed toward: 1) Elucidation of the putative causative role of the chorionic gonadotropin molecule in choriocarcinoma-associated thyrotoxicosis; 2) Characterization of the effects of structural modifications of the chorionic gonadotropin molecule on its agonistantagonist activity at the thyrotropin receptor; and 3) Determination of the metabolic parameters of highly purified chorionic gonadotropin in human subjects, and definition of its metabolic products.

Methods Employed:

The investigative focus is on the glycoprotein hormone group and related molecules with thyroid-stimulating, luteinizing, or follicle-stimulating activity. The properties of these hormones and related molecules are characterized in radioimmunoassays, radioreceptorassays, and bioassays. The hormones, their subunits, and related secretory or degradation products are extracted, concentrated, or purified using a variety of separative procedures including gel filtration, adsorption chromatography, poyacrylamide gel electrophoresis, or affinity chromatography. Molecular modifications for purposes of structure-function studies, are brought about by chemical or enzymatic treatments.

Progress:

Novel findings have emerged from the study of structure-function relationships at the thyrotropin (TSH) receptor. Earlier studies from this laboratory provided clear evidence that the gonadotropin molecules, chorionic gonadotropin (hCG) and luteinizing hormone (hLH), contained the structural domains requisite not only for interaction with the human TSH receptor, but also for activation of the adenylate cyclase system in thyroid membranes. Given the similarity of their biologic effects at the LH/CG receptor in the gonad, it was surprising to find a dramatic difference between hCG and hLH with respect to their activity at the TSH receptor—hLH exhibited about 65 times greater intrinsic thyrotropic activity than hCG. Structural modifications were made in the hCG molecule to probe the domains which determined this difference in function. Carboxypeptidase digestion of the hCG molecule resulted in the cleavage of residues 142-145 from the carboxyterminus of the hCG beta subunit, and a dramatic increase in the thyroid-stimulating activity of the preparation. In contrast, a reduction in the carbohydrate structure of hCG, brought about by digestion with neuraminidase, resulted in a loss of thyroid-stimulating activity. Although asialo-hCG was bereft of adenylate cyclase-stimulating activity, it retained the ability to interact with the TSH receptor and behaved as a competitive antagonist of TSH at the thyrotropin receptor. Interestingly, this molecule has been shown to bind more avidly to its primary gonadal receptor and to activate gonadal adenylate cyclase.

Clinical studies aimed at assessing whether the thyrotropic activity of the hCG molecule mediates the thyrotoxicosis which occurs in choriocarcinoma have been completed. In 20 patients with gestational trophoblastic neoplasia, the degree of thyroid hyperfunction correlated with the level of serum hCG. In the mouse thyroid bioassay, the biological characteristics of the thyroid-stimulating activity in both serum and urine correlated closely with the levels

of hCG. Thus, hCG was the only thyrotropic factor apparent in these patients.

The phase of our research project aimed at understanding the metabolism of these glycoprotein molecules has been fruitful. To establish the parameters of hCG metabolism and the nature of the metabolic products derived from hCG, infusions of highly purified hCG were given to normal subjects over an 8 day period of time. Heretofore, no data have been available on highly purified hCG given by constant infusion to permit the achievement of steady state conditions and to allow sufficient time for relevant metabolites to accumulate. The parameters for distribution, metabolic clearance rate, disappearance curve components, and renal clearance rate were established. Interestingly, although the total metabolic clearance rate of hCG was more than 10 fold less than that of its subunits, its renal clearance rate was actually several fold greater. Thus, renal excretion accounts for the metabolic fate of 20% of the hCG, but less than 1% of its subunits. Surprisingly, there was essentially no apparent production of subunits or fragments of hCG detectable by radioimmunaossay of serum or urine. The vast majority of the excreted hCG was indistinguishable from the highly purified material infused. Only one of seven subjects given an 8 day infusion of hCG showed a barely detectable amount of the hCG beta core fragment which we have previously characterized as a prominent metabolic product derived from the free hCG beta subunit. No carboxy-terminal fragments were found.

Investigations of the metabolism of glycoprotein hormones have been extended to include a glycoprotein enzyme, dopamine-beta-hydroxylase. The role of thyroid function in modulating the serum levels of this glycopeptide has been studied in patients and a model developed in rats to elucidate the mechanism of the alteration. Serum dopamine-beta-hydroxylase activity was shown to be inversely related to thyroid function in both humans and rats. The low level of serum enzyme in hyperthyroid rats was associated with an increased metabolic clearance while the high levels of enzyme in hypothyroid rats was associated with slowed clearance. Thus, altered disposal mechanisms appear to account for the effect of thyroid function on serum dopamine-beta-hydroxylase.

An unusual molecular form of TSH has been observed and characterized. In an otherwise healthy young man, there was a high-molecular weight form of immunoreactive TSH which seemed to bind to the TSH receptor, but showed decreased stimulation of the adenylate cyclase system in the membranes.

Significance:

Progress over the past year has extended our knowledge of glycoprotein hormone biology in useful directions. Analysis of structure-function relationships at the TSH receptor has revealed that asialo-hCG behaves as a competitive antagonist of TSH. This supports the general concept that the glycoprotein hormones comprise a structurally homologous group of molecules with shared biological inter-relatedness. Thus, hCG, hLH, and asialo-hCG exhibit secondary biological activity at the TSH receptor; they are all agonists at the gonad, while only hCG and the hLH are agonists at the thyroid. Competitive antagonists have been very useful in studies of the beta-adrenergic receptor and its adenylate cyclase system; analogous applications of asialo-hCG are expected. Our original observations of the greater intrinsic thyrotropic activity of hLH relative to hCG prompted the hypothesis that the extra 30 amino acids that the hCG beta

carboxy-terminus served to attenuate the thyrotropic activity of hCG. In support of this hypothesis is the observation that digestion of a portion of the carboxy-termini of hCG does indeed give rise to enhanced thyrotropic activity. The results of studies of the metabolism of hCG and its subunits have given the first comprehensive analysis of their kinetic parameters and metabolic products in humans, and provide new insights to the sources of free subunits and fragments in pregnancy and malignant diseases.

Proposed Course:

Continued emphasis will be given to the development of more accurate, sensitive, and convenient techniques for the measurement of glycopeptide hormones, their subunits, and related degradation products in the serum and urine of patients. Studies are underway which compare the clinical applicability of urinary hCG measurement to that of the standard serum hCG radioimmunoassay. The role of genetic factors and endocrinologic factors, such as diabetes, in modulating the metabolism of glycoproteins will be studied. STructure-function studies at the TSH receptor and the LH/CG receptor will be continued to gain greater insights into the domains of the glycoprotein hormones which confer the abilities to interact with the receptor and to activate adenylate cyclase.

Publications:

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8. Wehmann, R.E., and B.C. Nisula, The Metabolic and Renal Clearance Rates of Human Chorionic Gonadotropin, Journal of Clinical Investigation, 68: 184, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00607-01 DEB (formerly 00183-02)

PERIOD COVERED

October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Catechol Estrogens: physiological effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other	D.D. Brandon	DEB, NICHD
	S. Kono, MD., Visiting Scientist	DEB, NICHD
	M.B. Lipsett, M.D.	Director, Clinical Center
	G. Merriam, M.D., Clinical Associate	DEB, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

Developmental Endocrinology Branch

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NICHD, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5 1.5		

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X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The catechol estrogens, 2 or 4 hydroxy derivatives of estrone and estradiol, represent major metabolites of the naturally occurring estrogens. Little is known of the origin, disposition, and metabolic effects of these compounds. The objective of this project is to address these questions.

PHS-6040
(Rev. 2-81)

Objectives:

Catechol estrogens have been identified in urine, and have been shown to be metabolic products of both estrone and estradiol. The biological effects of these compounds remains a controversial issue. The objectives of this project are to define the metabolic origin of the catechol estrogens, and to delineate their biological effects in man, using gonadotropin suppression as a response parameter, and in the rat, using uterine weight as an end point.

Progress:

We have previously shown that both 2-OH esrone & 2 OH estradiol interact with the rat uterine cytosol estrogen receptor with about 5% the affinity of estradiol, that 2OH E_1 and 2OHE_2 are weak estrogen agonists in man, and that the potency of these estrogens is much less than would be predicted on the basis of their interation with the estrogen receptor. This has been shown to be the result of an extremely rapid metabolic clearance rate for the catechol estrogens, on the order of 20,000-40,000 L/day, the highest known clearance rate for any of the naturally occurring steroid hormones. There findings are also consistent with the very low plasma concentrations that we have found for both 2OH-estrone and 2-OH-estradiol.

Most recently we have examined the role of catechol estrogens in regulating prolactin secretion. Recent studies by investigators prominent in the field of catechol estrogen physiology purport to show that prolactin is released by infusion of 2OH estrogen. We have infused large doses of 2-OH estrone into 8 normal volunteers and cannot corroborate these findings.

Significance: It was initially speculated that catechol estrogens may represent the "missing" link between secreted steroids and central nervous system steroid action in that they interact with both the cytosol estrogen receptor and the membrane bound catecholamine receptors. On the basis of earlier studies in rodents, it has also been speculated that these compounds may act as endogenous antiestrogens.

Our findings would categorize the catechol estrogens as metabolic products of estradiol with low estrogenic activity. They do not appear to subserve a unique physiologic role.

Other reported action such as antiestrogen activity or prolactin releasing activity, have failed to be confirmed.

Projected Course:

The mechanism of action of the catechol estrogens in the central nervous system remains unclear. Two hypotheses prevail. The first proposes that these steroids act exclusively via catecholamine receptors or by influencing the metabolism of endogenous CNS catechol amines. The second proposes that all of the actions of the catechol estrogens can be accounted for by their interaction with the estrogen cytosol receptor. We propose to test these two hypotheses by examining the action of the catechol estrogens in systems wherein one or the others of these receptor systems has been blocked. Initial studies will use the rat as the experimental animal.

Publications:

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S. Kono, D. Brandon, G.R. Merriam, D.L. Loriaux, and M.B. Lipsett. Uterotrophic activity and metabolic clearance rate of 2-hydroxy-estrone in rats. Endocrinology, in press, 1980.

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G.R. Merriam, D.D. Brandon, S. Kono, S.E. Davis, D.L. Loriaux, and M.B. Lipsett. Rapid metabolic clearance of the catechol estrogen 2-hydroxyestrone. J. Clin. Endocrinol. Metab., in press, 1980.

S. Franks, C.G. Goodyer, F. Naftolin, and G.R. Merriam. Stimulation of LH release from cultured pituitary cells by 2- and 4-hydroxylated estrogens. Submitted for publication, 1980.

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