

ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF
CHILD HEALTH AND HUMAN
DEVELOPMENT

FISCAL YEAR 1973

PART I

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
ANNUAL REPORT

July 1, 1972 through June 30, 1973

	<u>Page</u>
Table of Contents	
OFFICE OF THE DIRECTOR	
Summary Report	A-1 to A-28
<u>Office of Public Information</u>	
Summary Report	AA-1
Scientific Publication Section	AA-1
Public Information Section	AA-1
Gerontology Research Center	AA-1
Communications Plan	AA-2
Training and Personnel Development	AA-2
Scientific Publications Edited	AA-2
Contributions to NIH Products	AA-3
International Reports	AA-4
Congressional Reporting	AA-4
Media Activities	AA-4
Public Inquiries	AA-5
Other Functions and Accomplishments	AA-5
<u>Office of Program Planning and Evaluation</u>	
Summary Report	AB-1
OFFICE OF ASSOCIATE DIRECTOR FOR EPIDEMIOLOGY AND BIOMETRY	
Summary Report	B-1
<u>Epidemiology Branch</u>	
Summary Report	BA-1
Non-Project Activities	BA-1
Bibliography	BA-3
Talks	BA-3
Contracts and Collaborative Research:	
Relationship of Heterozygosity in Phenylketonuria to Intelligence Quotient	BA-5
Conversion of Conversational Computer Statistical System (CCSS) Package to the PDP-10 and IBM-370 Computers at NIH	BA-6
The Epidemiology of Birth Weight with Particular Reference to the Effect of Induced Abortion on Subsequent Offspring	BA-7
A Study of Monozygous and Dizygous Twins to Ascertain Possible Neurophysiologic Factors for the Sudden Infant Death Syndrome	BA-8

	<u>Page</u>
Development of Sleep and Cardiopulmonary Regulation within Sleep: Clinical Studies of a Functional Mechanism for Risk of Sudden Infant Death	BA-9
Analysis of State Integration and Related Cardiopulmonary Functions in Infants at High and Low Risk for the Sudden Infant Death Syndrome	BA-10
 Project Reports:	
HD-EB-1(c) The Role of Maternal and Infant Coagulation Systems in Determining Morbidity Among Newborns	BA-11
HD-EB-2(c) Season of Birth and Incidence of Respiratory Distress Syndrome	BA-13
HD-EB-3(c) Low-Weight-for-Gestational-Age in the U.S.	BA-15
HD-EB-4(c) Relative Efficacy of Two Prenatal Care Regimens	BA-17
HD-EB-7(c) The Independent Effects of Birth- weight and Gestational Age on Risk of Mortality in the First Year of Life, New York City, 1964-67	BA-19
HD-EB-8 Father's Age and Infant Mortality	BA-21
HD-EB-9 Spina Bifida, Anencephaly, and Potato Blight	BA-22
HD-EB-10 Gonadotropins and Anencephaly/Spina Bifida	BA-23
HD-EB-11 Infant Mortality Rates and Hardness of Local Water Supplies	BA-24
HD-EB-12 The Susceptibility of Twins to the Sudden Infant Death Syndrome	BA-25
HD-EB-13 Geographic Differences in Mortality Rates and Rates of Aging - a Possible Relationship?	BA-26
 <u>Biometry Branch</u>	
Summary Report	BB-1
Activities of the Branch	BB-1
Consultations	BB-2
 Contracts and Collaborative Research:	
Epidemiologic Study of Breast Cancer and Benign Breast Lesions in Relation to the Use of Ovarian Hormones	BB-5
Oral Contraceptives and Tumors of the Breast	BB-6
A Retrospective Study of the Risks for Cancer of the Breast, Body of the Uterus, Ovary, and Cervix Among Users of Oral Contraceptives	BB-8
A Comparison of the Medical Effects of Induced Abortion by Two Methods, Curettage and Suction	BB-9

	<u>Page</u>
Outcome of Pregnancy of Women Who Have Previously Used Steroid Contraceptives	BB-10
A Collaborative Study of Oral Contraception and Cerebrovascular Disease	BB-11
Publications and Talks	BB-12
 OFFICE OF ASSOCIATE DIRECTOR FOR PROGRAM SERVICES	
Summary Report	C-1
 <u>Grants and Contracts Management Branch</u>	
Summary Report	CA-1
Negotiation of Research and Training Grants	CA-1
Research Grants	CA-1
Training Grants	CA-2
Fellowships	CA-2
Research Career Awards and Research Career Development Awards	CA-2
Research Contracts	CA-2
Summary of Institute Supported Projects (Dollars in Millions)	CA-3
 <u>Program Statistics and Analysis Branch</u>	
Summary Report	CB-1
Program Analysis Section	CB-1
Statistical Analysis Section	CB-4
Information Systems Section	CB-5
NICHD Grants and Contracts Active During March 1973	CB-8
 CENTER FOR POPULATION RESEARCH	
 <u>Office of the Director</u>	
Summary Report	D-1
Budget	D-1
Staffing	D-2
Liaison and Scientific Information	D-3
Annual Summary Progress Reports of Population Research Centers and Project Programs	D-4
Book: Human Sterilization, published 1972	D-5
Book: Regulation of Mammalian Reproduction, published 1973	D-5
 <u>Behavioral Sciences Branch</u>	
Summary Report	DA-1
Fertility, Abortion, Illegitimacy and Family Planning	DA-3
Socio-economic Correlates of Fertility	DA-4
Sex Roles and Socio-psychological Correlates of Fertility	DA-4
Demographic and Attitudinal Factors Affecting Family Formation	DA-6

	<u>Page</u>
Migration, Social Mobility, and Residential Patterns	DA-6
Research Oriented Toward the Possible Development of Population Policies	DA-8
Contract and Collaborative Research:	
National Fertility Survey, 1970-1975	DA-9
Analysis of the Decline of Fertility in Europe, by Province	DA-10
A Study of Differentials and Trends in Marital Disruption, Remarriage, and the Fertility of Remarriage	DA-11
New Estimation Techniques for Demographic Analysis	DA-12
Urbanization, Migration and Fertility in Thailand	DA-13
An Economic Analysis of Migration from Rural Eastern Kentucky to Selected Urban Centers	DA-15
Processes of Demographic Development in Imperial Russia and the Soviet Union	DA-16
Sex Role, Family Structure, and Fertility Control	DA-18
Socio-economic Factors in the Reduction of Natality in the Less Developed Areas	DA-19
Illegitimacy-Demographic and Sociological Studies	DA-20
Relationship Between Age at Marriage, Birth Intervals and Total Fertility	DA-21
The Distribution and Differentiation of Population Within Metropolitan Areas	DA-22
Human Fertility in Poland and the U.S.S.R.	DA-23
The Effect of Income Payments to Reduce or Increase Natalty	DA-25
The Kin Network and Population Dynamics	DA-26
Explorations in Fertility Values	DA-27
The Correlates of Low Fertility in a Developing Country	DA-28
Rates and Consequences of Population Change: The Growth and Decline of Counties in the Mountain Region	DA-30
An Analysis of the Spacing of Pregnancies, Births, and Completed Fertility in Relation to Socio- economic Status, Intergenerational Mobility and Aspirations for Children, Aberdeen, Scotland	DA-31
Attitudinal Factors Affecting Family Size	DA-32
A Study of Physicians' Attitudes Toward Abortion	DA-33
Socioeconomic Status and Family Factors in Residential Mobility	DA-34
Economic Cost and Value of Children in Four Societies	DA-36
A Study of the Growth of Mexican-American Families	DA-37
An Investigation of the Determinants of Fertility in the United States	DA-38

	<u>Page</u>
The Determinants of Fertility, Migration, and Population Change of Counties in the United States, 1950 to 1960	DA-39
The Effects of Purposive-Rationality, Traditionalism, and Economic Aspirations on the Process of Family Formation	DA-40
An Investigation of the Factors Involved in the Moral Judgement of Marital Status and Family Size	DA-42
Support of Publication of <u>Population Index</u>	DA-43
Women's Labor Force Participation and Fertility	DA-44
Social-Psychological Correlates of Urban Fertility	DA-46
Study of Family Formation and Fertility - Key Trends and Patterns	DA-48
A Study of Social Mobility and Fertility Control	DA-49
Family, Career and Sexuality	DA-50
Social and Psychological Factors Affecting Fertility, Family Planning and Clinic Utilization	DA-51
Analysis of Migratory Responses to Employment Opportunities	DA-53
Methods and Techniques for Using the 1960-1970 Census One Percent Public Use Samples	DA-55
Correlates of Family Size and Childspacing in the United States and Puerto Rico	DA-56
The Employed Woman: Family Planning and Careers	DA-57
An Analysis of the Causes and Patterns of Rural to Urban Migration of the Poor	DA-58
Role of Fertility Patterns of Urban Mothers	DA-59
College Women in the 1950's: Attitude and Behavior With Respect to Marriage, Children, Divorce, and Sexual Practice	DA-60
Psychological Studies of Social Norms Influencing Family Size	DA-61
A Study of Optimum Population Levels	DA-62
Occupational Experience as a Determinant of Fertility Among American Women	DA-63
Population Redistribution and Depopulation in Non- Metropolitan Pennsylvania: Social and Economic Correlates and Policy Implications	DA-64
Review of Research Findings on Rural-Urban Migration with Annotated Bibliography	DA-65
Determinants of Fertility Change in Tamil Nadu, India	DA-66
A Study of Fertility of Spanish-American Populations in the United States and Their Socio-Economic Correlates	DA-67
Age at Marriage and Time to First Birth: A Longitudinal Study of Parental Attitudes and Children's Personality	DA-69
The Effect of Birth Order on Mother-Child Relationship	DA-70

	<u>Page</u>
Research on the Consequences of Childbearing and Child-Spacing Patterns	DA-71
Development of Methodology for Evaluating the Demographic Impact of Organized Family Planning Programs in the United States	DA-72
A Review of Actual and Accepted Consequences of Family Size	DA-73
Fertility Dynamics of Cuban Refugees	DA-74
The Work and Childcare Patterns of Women After the Birth of Their First Child	DA-75
Patterns of Emotions and Feelings in Sex-Related Behaviors	DA-76
Childspacing and Current Fertility: 1970 Census	DA-77
On the Consequences of Reproduction: A Utility Model of Reproductive Behavior	DA-78
Demography of the Black Population	DA-79
The Demographic Study of the Mexican-American Population (Conference)	DA-80
The Study of Fertility-Related Decision-Making Among Married Couples	DA-81
A Study of Child-bearing and Labor Force Participation of Women	DA-82
Longitudinal Analyses of Variables Predictive of Attitudes Regarding Fertility and Actual Fertility Patterns	DA-83
A Final Household Survey to Complete the Family Planning Evaluation Project	DA-84
 <u>Contraceptive Development Branch</u>	
Summary Report	DB-1
A. Directed Fundamental Research	DB-1
The Corpus Luteum (CL)	DB-2
Prostaglandins	DB-3
Studies on Male Reproduction	DB-4
Mechanism of Hormone Action	DB-5
B. Product-Oriented Research	DB-7
Drug Development Program	DB-7
Testing Facility	DB-9
Drug Delivery System	DB-10
Development of Sterilization Techniques (Female)	DB-10
Development of Sterilization Techniques (Male)	DB-10
Clinical Studies	DB-11
Hormone Distribution	DB-12
References	DB-13
 Contract and Collaborative Research:	
Conference on Biorhythms and Human Reproduction	DB-17
Symposium on Mechanism of Ovulation	DB-18
Symposium on the Biology and Pathology of Aging Gametes	DB-19

	<u>Page</u>
Estrogen-Receptor Substances of Oviduct Tissue	DB-20
Factors Influencing Mammalian Oviductal Secretions	DB-21
A Bioengineering Approach to the Study of Tubal Activity and Contraception	DB-22
Oviduct Cilia: Structure, Function, Development	DB-23
Measurement of Blood Flow in the Mammalian Oviduct	DB-24
Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovalecto- mized-Hormone Treated Rabbit and Pig-Tailed Monkey, <u>Macaca-Nemestrina</u>	DB-25
An Interdisciplinary Program Project on the Biology of the Oviduct and Gamete Transport	DB-27
Physiology of the Oviduct	DB-29
Steroid Hormone Binding in Reproductive Organs	DB-31
Immunopharmacology of the Human Fallopian Tube	DB-32
Studies on Steroid Hormone Receptors and Their Role in Reproductive Biology	DB-33
Morphochemical Analysis of Oviductal Mucins	DB-34
The Effects of Intrauterine and Embryonic Temperature Elevation on Pregnancy in Experimental Animals	DB-36
Assay of Progesterone Receptor Activity in the Guinea Pig Uterus	DB-37
Detection of Ovulation in Chronically Instrumented Animals	DB-39
Development of Techniques for Study of Oviduct Transport and Detection of Ovulation in Primates	DB-41
The Study of Progesterone-Binding Proteins as a Tool for the Detection of Progesterone Antagonists and Agonists	DB-42
Prostaglandin: Its Role and Effect in Contraception	DB-44
Oviductal Fluid in the Rat and Mouse: A Study of the Chemical and Physical Properties and Mechanism of Formation	DB-45
Hypothalamo-Hypophysial Factors in Reproduction	DB-46
New Methods for Fertility Regulation	DB-47
Control of Ovulation in the Monkey	DB-49
The Study of Neural Regulation of Ovarian Function	DB-50
Anti-luteinizing Activity of Follicular Ovum	DB-51
Isolation of a Bovine Uterine Luteolytic Factor	DB-52
Endocrine Regulation of the Corpus Luteum	DB-53
Relationships of Endometrial Prostaglandins and Luteal Function	DB-54
The Actions of Estrogen and Progesterone Upon Uterine Function in Pregnancy	DB-55
A Simultaneous Theoretical and Empirical Approach to the Study of the Rat Estrous Cycle	DB-57
Development of a Laboratory Screening Procedure for Inhibitors of Progesterone Synthesis	DB-58
Development of Radioimmunoassays for Rhesus Monkey Gonadotropins	DB-60

	<u>Page</u>
Pilot Study for the Large Scale Preparation of Purified Human Chorionic Gonadotropin	DB-61
The Control of Ovulation and Corpus Luteum Function	DB-62
Regulation of the Changes in Corpus Luteum Physiology Required for the Establishment of Pregnancy in the Rat	DB-63
Development of an Automated Immunoradiometric Assay System	DB-64
Quantification of Prostaglandins	DB-65
A Model for Studying Inhibition of LH Action on the Corpus Luteum	DB-67
Effect of Prostaglandins on Ovarian Function	DB-69
The Role of Prostaglandins in Ovarian Physiology	DB-70
Biochemistry and Mechanism of Action of Human Gonadotropins	DB-71
Molecular Biology of the Human Corpus Luteum	DB-72
The Role of Prostaglandins in the Secretion of Gonadotropins	DB-73
Mammalian Sperm Motility and Ultrastructure	DB-74
Hormonal Control of Sperm Maturation	DB-75
Biochemical Studies on Spermatogenesis and Spermatozoa	DB-76
Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male	DB-78
Formation of Dihydrotestosterone in Male Sex Organs and Transport of Steroid Androgens from the Testis to the Epididymis	DB-80
Formation of the Subacrosomal Mass (Perforatorium) of the Golden Hamster Spermatozoa	DB-82
Fine Structure and Function of the Accessory Glands of the Male Reproductive Tract	DB-83
Ultrastructural and Electron Cytochemical Studies of Mammalian Spermiogenesis	DB-84
Role of the Epididymis in Sperm Maturation	DB-85
Control Mechanisms of Sperm Motility	DB-86
Ultrastructural and Immunological Investigation of Spermatogenesis, Sperm Transport, and Fertilization in the Mammal	DB-88
Enzymes of the Sperm Acrosomes, Occurrence of the Inhibitors of These Enzymes in Seminal Plasma and the Role of the Enzymes and Inhibitors in Capacitation of Sperm and in the Penetration and Fertilization of Ova	DB-90
Neurochemical Control of Sperm Motility	DB-91
Electron Spin Resonance and Radioisotope Labelling Studies of Mammalian Reproductive Cell Membranes in the Evaluation of New Methods of Contraception	DB-93
Testis and Sperm Specific Antigens of Man and Certain Other Primates	DB-95

	<u>Page</u>
Contraceptive Development Studies for Males: Oral Steroid Hormone Administration	DB-96
Immunologic Localization of Prostaglandins in the Reproductive Tract of Rabbit	DB-97
Ultrastructural Changes in the Sertoli Cells in Rats Under Various Hormonal Conditions	DB-98
Hormonal Control of the Epididymis	DB-100
Control of Sperm-Fertilizing Capacity	DB-102
Analysis of Anti-Sperm Antibodies in Monkey Sera	DB-103
Study on Human Acrosomal Proteinase	DB-104
Dynamics of Spermatogenesis: Quantitative Morphological and Physiological Characterization of Isolated Meiotic Cells	DB-105
Macromolecular Synthesis in Spermatogenic Cells Studied by Cell Separation Methods	DB-106
Androgen Receptors in the Testis	DB-107
Metabolism of Sperm Nuclear Proteins During Spermiogenesis	DB-108
Biochemical Requirements for Fertilization and Development of Rabbit and Human Ova	DB-109
Control of Ovulation and Capacitation in Non-Human Primate	DB-110
Morpho-Physiologic Studies on Mammalian Gametes Prior to and During Fertilization <u>In Vivo</u> and <u>In Vitro</u>	DB-111
Sperm Capacitations as Target for Contraception	DB-113
The Effect of Progesterone and Estradiol on RNA Synthesis in the Rabbit Oviduct and its Relationship to Early Embryonic Development	DB-115
Transfer of Maternal Macromolecules to Mammalian Eggs	DB-116
Endocrine Control of Human Oocyte Maturation	DB-117
Fertilization of Mouse Ova <u>In Vitro</u> by Epididymal Sperm	DB-119
Hormonal Requirement for Implantation in the Rhesus Monkey (Macaca Mulatta)	DB-120
Steroid Hormone Receptors in Human Cervix	DB-121
New Methods of Fertility Regulation: Specific Binding of Luteinizing Hormone to Cells of Corpus Luteum and Inhibition of the Process	DB-122
The Development of Inhibitors to Estrogen Biosynthesis	DB-123
Synthesis and Antifertility Testing of Prostanoid Acid Derivatives	DB-124
Synthesis and Testing of Antifertility Compounds	DB-125
Synthesis of Prostaglandin Analogs for Biological Studies	DB-126
Synthesizing and Testing of Antifertility Compounds	DB-127
Synthesize Nitrogen-Containing Analogs of Prostaglandins	DB-128

	<u>Page</u>
Synthesis of Novel Compounds of Potential Anti-Fertility Activity	DB-129
Synthesis of Prostaglandin Analogs	DB-130
The Synthesis of Potential New Agents for the Control of Fertility	DB-131
The Synthesis and Testing of Silicon Substituted Steroids as Potential Antifertility Compounds	DB-132
Synthesize and Furnish Steroidal Compounds with Antifertility Activity	DB-133
Synthesize and Furnish Peptide Inhibitors of Luteinizing Hormone-Releasing Factor/Follicle Stimulating Hormone Releasing Factor	DB-134
Contract Program for Obtaining, Synthesizing, and Testing Antifertility Compounds	DB-135
Synthesize Novel Derivatives of Progesterone and Testosterone	DB-136
Production of New Type of Contraceptives Based on Derivatives of Luteinizing Hormone-Releasing Hormone (LH-RH)	DB-137
Structure-Activity Studies of Small Peptides which Release LH and FSH	DB-138
Synthesis and Testing of Gonadotropin Releasing Hormone (Gn-RH) to Establish Antifertility and Other Endocrine Activity	DB-139
A Study of the Factors that Control Diffusion Rates in Controlled-Release Contraceptive Systems	DB-140
Novel Steroidal Gestagens	DB-141
The Synthesis of Novel Steroids with Potential Antifertility Activity	DB-142
Synthesis of Ring A Bi-and Tricycle Steroids with Potential Antifertility Activity	DB-143
Synthesis of Novel Steroids for Fertility and Population Control	DB-144
Synthesis of Steroidal Compounds Possessing a Bridged Bicyclic A-ring with Potential Antifertility Activity	DB-145
Synthesis of 12 α -Methyl Prostaglandins Related to PGF ₂ α	DB-146
Synthesis and Testing of Potential Inhibitors of Luteinizing Hormone Releasing Factor	DB-147
Non-Steroidal Nitroheterocycles with Potential Antifertility Activity	DB-148
Novel Steroids as Potential Antifertility Agents	DB-149
Synthesis and Testing of Irreversible Inhibitors of Uterine Estradiol Binding Factors	DB-150
Study of Compounds for Inhibition of Spermatogenesis	DB-151
Device and Technique for Blocking Fallopian Tubes	DB-152
Grafted Hydrogels in Contraceptive Application	DB-153
Development of an Intra-uterine Sterilization System for the Female	DB-154

	<u>Page</u>
Research and Development of Contraceptive Devices and Materials. Development of New Sterilization Techniques	DB-156
Design and Development of Implantable Contraceptive Device for Use in the Male	DB-157
The Use of Reversible Vasectomy Device in the Guinea Pig	DB-158
Reversible Sterilization by an Intravasal Spermicidal Device	DB-159
Development and Evaluation of a Reversible Vasectomy Prosthesis	DB-160
A Proposal for the Study of a Long Acting Injectable Progesterone Contraceptive	DB-162
Feasibility Study of Intrauterine Microcapsules as a Prolonged Drug Release Form	DB-163
Biological Testing Facility for the Evaluation of New Antifertility Drugs and Devices	DB-164
Implantable Bio-Absorbable Capsules as Slow-Release Contraceptive Drug Delivery Systems	DB-165
Development and Testing of an Implantable Contraceptive Delivery System	DB-166
A Study of Biodegradable Polymers for the Sustained Delivery of Contraceptive Drugs	DB-167
Clinical Evaluation of a Fluid-Filled Intrauterine Device	DB-168
Intrauterine Release of Estriol for Contraception	DB-169
Androgen Polydimethyl-siloxane Implants: Metabolic Fate of Testosterone and Contraceptive Efficacy	DB-170
Systems Approach to Vaginal Delivery of Contraceptive Drugs--Methodology and Mechanism for Absorption	DB-171

Population and Reproduction Grants Branch

Summary Report	DC-1
Population Research Center Award Program	DC-1
Program Project Grants	DC-2
Approved but Unfunded Applications	DC-3
Scope of Current Funding	DC-3
Behavioral-Social Science Population Research Grants Program	DC-3
Staff Changes	DC-4
Grants Associate Program	DC-4
Conferences, Symposia and Workshops	DC-5
Biology of Reproduction	DC-5
Research Excerpts	DC-7
Currently Supported Research Grants in the Biomedical Field	DC-7
Examples of Some of the Current Research. Corpus Luteum	DC-9
Culture of Cells <u>In Vitro</u>	DC-9
Receptors	DC-10

	<u>Page</u>
Oviduct	DC-10
Preimplantation Embryo	DC-11
IUD	DC-12
Vasectomy	DC-12
Oral Contraceptives	DC-13
Development	DC-14
Oocytes	DC-16
Photoperiods	DC-16
Pheromones	DC-16
Reproduction in Aged Animals	DC-17
<u>Fertility Regulating Methods Evaluation Branch</u>	
Summary Report	DD-1
Contract and Collaborative Research:	
Five-Year Prospective Study of Abnormal Cervical Cytology	DD-3
Contraceptive Drug Study	DD-4
Interaction of Contraceptive Steroids with Metabolic Functions of Vitamin B ₆	DD-5
Interaction of Contraceptive Steroids with Essential Dietary Nutrients	DD-8
Human Metabolism of Steroid Contraceptives	DD-10
Relationships of Contraceptive Steroids to Nutritive State	DD-11
Study of Differential Effects of Oral Contraceptives on Carbohydrate Metabolism	DD-14
An Epidemiologic Case-Control Study of Thrombo- embolism Related to the Use of Oral Contraceptives	DD-15
Effect of Oral Contraceptive and Conjugated Estrogens on Renin Activity and Aldosterone	DD-18
Evaluation of Effects of Oral Contraceptives on Vitamin B ₆ Nutrition	DD-21
Interaction of the Contraceptive Steroids and Vitamin K Absorption and Utilization	DD-24
Investigation of a Possible Interaction of Contraceptive Steroids with Vitamin E and Polyunsaturated Fatty Acids	DD-26
The Effects of Contraceptive Steroids on Trace Elements	DD-28
Effect of Oral Contraceptive Steroids on Calcium, Phosphorus, Magnesium and Zinc Metabolism with Emphasis on Bone Mineralization	DD-30
Biochemical Mechanism of Contraceptive Steroid- Induced Abnormalities in Absorption, Metabolism and Function of Vitamin K	DD-32
Endogenous and Exogenous Sex Steroids and Nutritional Status	DD-34
Relationship of Effect of Oral Contraceptives on Blood Lipids and Nutritional Status	DD-36

	<u>Page</u>
The Interaction of B-Vitamins with Contraceptive Steroids in Female Rats	DD-39
Oral Contraceptive Agents and Thromboembolic Complications	DD-42
Metabolism and Metabolic Effects of Steroidal Oral Contraceptives	DD-45
Studies of Dose-Response Relationships Between Low Dose Steroid Contraceptive Drugs and Plasma Gonadotropin Levels	DD-49
Postcontraceptive Reproduction in Man	DD-51
Chromosomal Breakage in Women Taking Oral Contraceptives	DD-52
A Study of the Metabolism of Steroidal Oral Contraceptives	DD-53
Biochemical and Physiological Evaluation of an Oral Chemical Contraceptive Agent	DD-54
Repository for Tissue Evaluation of Contraceptive Steroids	DD-55
Pharmapathologic Effects of a 17 α -Hydroxyprogesterone Derivative in Beagle Bitches	DD-56
Circulating Polypeptide and Steroid Hormones in Beagle Dogs	DD-57
Development of Radioimmunoassays for Norethindrone, Norgestrel, Ethynyl Estradiol and Mestranol	DD-58
The Metabolism of Derivatives of 17 α -Acetoxypregesterone in Beagles and in Rhesus Monkeys	DD-60
Biliary and Urinary Excretion, Enterohepatic Circulation, and Hepatic Effects of Contraceptive Steroids	DD-61
The Baboon: An Animal Model for the Study of Contraceptive Steroids	DD-63
Metabolism of Contraceptive Synthetic Estrogens	DD-64
Peripheral Metabolic Effects of the Ethynyl Estrogens in Subhuman Primates and Canines as Compared to Man	DD-65
Alteration of Thrombogenic Potential by Oral Contraceptive Steroids in Rhesus Monkeys	DD-66
Effects of Drugs on Contraceptive Steroid Metabolism	DD-68
Comparative Pharmacology of Steroid Contraceptive Drugs	DD-69
A Comparative Study of Progestin Metabolism in Animals	DD-71
Effects of Vasectomy on Testes, Epididymis and Vas Deferens	DD-73
Immunologic and Morphologic Effect of Vasectomy in Monkeys	DD-74
Sperm Immunology	DD-75
Ultrastructural and Immunochemical Studies of Sperm Antigens Involved in the Autoimmune Response in Vasectomized Mammals	DD-76

	<u>Page</u>
An Immunogenetic Analysis of Sperm Autoantigens in the Mouse	DD-77
Immunologic Studies on Steroid-Producing Cells	DD-78
Vasectomy and Autoimmune Disease	DD-79
Immunologic and Morphologic Consequences of Vasectomy	DD-80
Immunopathologic Consequences of Vasectomy	DD-81
Autoimmune Phenomena and Mechanisms in Human Vasectomy	DD-82
Immune Alterations Associated with Vasectomy	DD-83
Endocrine Changes in Vasectomized Men	DD-84
USC Collaborative Study of the Effects of Vasectomy	DD-85
Physiological Mechanisms Involved in Reactions to Implanted Devices in Animal Uteri	DD-86
Effects of Steroid Contraception on Cervical Dysplasia	DD-87
 The Contract and Collaborative Projects listed below are reported in detail in the Biometry Branch Reports on pages indicated:	
A Collaborative Study of Oral Contraception and Cerebrovascular Disease	BB-11
A Comparison of the Medical Effects of Induced Abortion by Two Methods, Curettage and Suction	BB-9
Oral Contraceptives and Tumors of the Breast	BB-6
A Retrospective Study of the Risks for Cancers of the Breast, Body of the Uterus, Ovary and Cervix Among Users of Oral Contraceptives	BB-8
A Study of the Outcome of Subsequent Pregnancies in Women Who Have Used Oral Contraceptives	BB-10
Epidemiologic Study of Breast Cancer and Benign Breast Lesions in Relation to the Use of Ovarian Hormones	BB-5
 OFFICE OF ASSOCIATE DIRECTOR FOR EXTRAMURAL PROGRAMS	
Summary Report	E-1
 <u>Growth and Development Branch</u>	
Summary Report	EA-1
Nutrition, Growth, and Development of North American Indian Children	EA-1
Training for Research	EA-2
Mechanisms of Biological Growth	EA-2
Developmental Immunology	EA-3
Developmental Pharmacology	EA-4
Nutrition	EA-5
Physical Growth	EA-6
Developmental Behavioral Biology	EA-7
Learning and Cognitive Development	EA-8
Human Communication	EA-9
Personality and Social Development	EA-10
Expanded Emphasis on Adolescence	EA-11

	<u>Page</u>
Contract and Collaborative Research:	
Electrophysiological Studies of Brain Function in Malnutrition	EA-13
Effects of Calorie and Protein Restriction in Pregnancy and Newborn Rhesus Monkeys	EA-14
Ecology of Malnutrition in Vulnerable Groups	EA-15
Immunologic Responses of Normal and Malnourished Infants to Standard Antigens	EA-16
Non-Human Primate Colony	EA-17
A Study of Malnutrition and Mental Development in Bogota, Colombia	EA-18
Prediction of Mature Stature in Growing Children	EA-19
Analysis of Data from an Ecological Study of Infection, Malnutrition, and Growth of Children in a Guatemalan Indian Village	EA-20
Research Materials and Assistance for Studies of Language Development in Children	EA-21
Study of Relationship Between Nutrition and Behavioral Development	EA-22
 <u>Perinatal Biology and Infant Mortality Branch</u>	
Summary Report	EB-1
Pregnancy and Maternal Health	EB-2
PBIM Program Holdings According to Mechanism of Support	EB-3
Developmental Biology	EB-5
Fetal Health and Development	EB-7
Infant Survival and Well Being	EB-9
Sudden Infant Death Syndrome	EB-10
Conferences and Publications	EB-13
Foreign Country Activities	EB-15
Personnel	EB-15
 Contract and Collaborative Research:	
Analysis of State Integration and Related Cardiopulmonary Functions in Infants at High and Low Risk for the Sudden Infant Death Syndrome	EB-16
Development of Sleep and Cardiopulmonary Regulation Within Sleep: Clinical Studies of a Functional Mechanism for Risk of Sudden Infant Death	EB-17
Current Management of Sudden Infant Death Syndrome in the United States	EB-18
Intrapartum Fetal Monitoring	EB-19
Cooperative Hospital Study on the Respiratory Distress Syndrome in Infancy	EB-21
 <u>Adult Development and Aging Branch</u>	
Summary Report	EC-1
Growth of Extramural Research on Aging	EC-1
Research and Training Programs	EC-2
Society, Aging and Health	EC-2

	<u>Page</u>
Mental Function	EC-2
Aging in Women	EC-3
Aging and Immunology	EC-3
Cellular Aging	EC-3
Animals for Aging Research	EC-4
Contract and Collaborative Research:	
Production Colony of Aging Rats in an Isolator Environment	EC-6
Development of a Production Colony of Three Genotypes and a Non-Inbred Strain of Laboratory Mouse for Aging Research	EC-8
Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)	EC-10
Prevention of Bone Loss in the Menopause	EC-12
Origin and Action of Estrogen in the Postmenopausal Woman	EC-13
A Retrospective Study of Postmenopausal Women with and without Estrogen Replacement Therapy	EC-15
5th Annual Course on the Biology of Aging	EC-16
Longitudinal Studies	EC-17
Production, Characterization, and Distribution of Human Diploid Cell Strains	EC-18
Cell Culture Procedures for Aging Research	EC-19
Review, Analysis, and Evaluation of Population Models	EC-20
Two Gerontological Society Symposia: "Current Experimental Models in Biological Aging" and "Gene Regulation"	EC-21
Accelerated Immunological Aging: A Possible Model for Research in Relation to Rejuvenation by Fetal Liver	EC-22
Analysis of Aging Associated Involution of Cellular Immunity in Mice	EC-23
Cell Culture Procedures for Aging Research	EC-24
Mathematical Models for an Aging Population	EC-25

Mental Retardation Branch

Summary Report	ED-1
Mental Retardation Research Centers	ED-3
Research Activities	
Genetics and Inborn Errors of Metabolism	ED-4
Conference on Somatic Cell Hybridization	ED-5
Teratology	ED-5
Malnutrition	ED-6
Early Diagnosis and Intervention	ED-6
Amelioration and Treatment	ED-6

	<u>Page</u>
Contract and Collaborative Research:	
Diagnostic and Intervention Studies of High Risk Infants	ED-8
Definition of a Behavioral Phenotype in the Cornelia DeLange Syndrome	ED-10
An Automated System for Chromosome Analysis	ED-11
Intellectual and Behavioral Consequences of Severe Malnutrition in Infancy: A Collaborative Study	ED-12
Prevention of Prematurity and Developmental Disorders Through Nutritional Supplementation and Laboratory Studies on Prenatal Malnutrition	ED-13
An Investigation of Certain Relationships Between Hearing Impairment and Language Disability	ED-15
The Development of Materials for Application of Behavior Modification Skills by Parents of Mentally Retarded Children	ED-18
OFFICE OF THE SCIENTIFIC DIRECTOR	
Summary Report	F-1
Developmental Immunology Branch	F-1
Social and Behavioral Sciences Branch	F-2
Reproduction Research Branch	F-2
Behavioral Biology Branch	F-3
Section on Brain and Behavior	F-3
Section on Neurobiology	F-3
Laboratory of Molecular Genetics	F-4
Laboratory of Biomedical Sciences	F-5
Section on Developmental Enzymology	F-5
Section on Intermediary Metabolism	F-5
Section on Physiological Controls	F-5
Section on Developmental Pharmacology	F-5
Pregnancy Research Branch	F-6
<u>Gerontology Research Center</u>	
Laboratory of Behavioral Sciences	F-6
Clinical Physiology Branch	F-7
Laboratory of Cellular and Comparative Physiology	F-7
Laboratory of Molecular Aging	F-8
Collaborative Guest Scientist Program	F-9
Project Reports:	
HD-DB-7	Specific Cellular Immunity in Recovery from Viral Infection F-10
HD-DB-5	Immune Mechanisms in Chronic and Congenital Viral Infections F-11
HD-DB-15	Search for a Negative DNA Strand in Cells Infected with the Minute Virus of Mice (MVM) F-13

	<u>Page</u>
HD-DB-20	F-15
HD-DB-16	F-17
 <u>Developmental Immunology Branch</u>	
Summary Report	FA-1
 Project Reports:	
HD-I-4(c)	FA-4
HD-I-8	FA-6
HD-I-9	FA-8
 Contract and Collaborative Research:	
Development and Testing of Capsular Polysaccharide of <u>Haemophilus influenzae</u> Type b	FA-10
 <u>Social and Behavioral Sciences Branch</u>	
Summary Report	FB-1
Research Activities	FB-1
Papers Presented at Professional Meetings	FB-5
Publications	FB-6
 Project Reports:	
HD-GD3(c)	FB-8
HD-GD4(c)	FB-11
HD-SB1(c)	FB-14
HD-SB2(c)	FB-17
HD-SB4(c)	FB-19
HD-SB5	FB-22

	<u>Page</u>	
HD-SB6	Factors Associated with the Development of Mother-Infant Attachment During the First Year	FB-24
HD-SB7	Early Environmental Influences on Cognitive and Personality Develop- ment During Infancy: A Replication with a Middle-Class Sample	FB-26
HD-SB8	Factors Influencing Career Choice in Women	FB-28
 <u>Reproduction Research Branch</u>		
Summary Report		FC-1
 Project Reports:		
HD-RPL1	Biology of Reproduction: Clinical and Experimental	FC-4
HD-RPL2	Physiology of Testis	FC-15
HD-RPL3	Fertilization and Embryogenesis	FC-17
HD-RPL4	The Use of the Rhesus Monkey in Studies of Pregnancy	FC-20
HD-LB3	Analytical Techniques and Instrumentation: a. and b.	FC-23
HD-LB14	Cell Organelles from Human Placenta: Formation, Isolation, Character- ization, and Function Under Normal and Abnormal Conditions	FC-25
HD-LB15	Fetal Resorption	FC-27
HD-LB5	Synthesis of Biologically Active Peptides	FC-29
HD-LB19	Chemical Modification of Amino Acids. Selective Nonenzymatic Cleavage of Peptide Bonds. Structure-Function Relationship of Biologically Active Peptides and Enzymes	FC-31
Honors and Awards		FC-34
Invited Lectures		FC-34
Publications		FC-37
Reviews and Chapters		FC-42
 <u>Behavioral Biology Branch</u>		
Summary Report		FD-1
Section on Brain and Behavior		FD-1
Section on Neurobiology		FD-2
 Project Reports:		
HD-BB2	Studies of CNS Functioning in Isolation Reared Monkeys	FD-6
HD-BB3(1)	Morphologic and Biochemical Studies of Nervous System Cells in Culture	FD-8

		Page
HD-BB4	Information Processing in the Central Auditory System of Mammals	FD-12
HD-BB5	Neurobiologic Studies of Neuronal and Other Cell Types in Cell Culture	FD-15
HD-BB6	Synapse Formation Between Nerve and Muscle Cells in Tissue Culture	FD-18
HD-BB8	Correlative Neurochemical and Electrophysiological Analyses of Individual, Identifiable Neurons in Molluscan Nervous Systems	FD-21
HD-BB9	A Correlated Study of the Fine Structure and the Function of the Endoplasmic Reticulum (ER) of Neurons	FD-26

Laboratory of Molecular Genetics

	Summary Report	FE-1
	Project Reports:	
HD-LB16	Control Mechanism in Temperate Bacteriophage λ . I-IV	FE-5
HD-LMG-2	Factors Influencing Genetic Transcription-Initiation and Termination	FE-9
HD-LMG-1	Integrative Control of Macromolecular Synthesis	FE-12
HD-LMG-4	<u>In vitro</u> Translation of Adenovirus Messenger RNA's	FE-20
HD-LMG-3	Molecular Aspects of the Differentiation of Embryonic Chick Lens Fibers <u>in vivo</u> and in Tissue Culture	FE-22
HD-LBC6	Regulated Gene Expression During Cell Growth and Differentiation (Note change in Project Title) I and II	FE-26
HD-LB20	Regulation of Antibody Biosynthesis I-IV	FE-31

Laboratory of Biomedical Sciences

	Summary Report	FF-1
	Section on Developmental Enzymology	FF-1
	Section on Intermediary Metabolism	FF-1
	Section on Physiological Controls	FF-2
	Section on Developmental Pharmacology	FF-2
	Project Reports:	
HD-LB1(c)	Developmental Enzymology	FF-4
HD-LB9	Models of Mental Retardation	FF-6
HD-LB10	Mechanism of Aromatic Hydroxylation	FF-8
HD-LB11	Biosynthesis and Function of Pteridines	FF-10

	<u>Page</u>	
HD-LB12	Biochemistry of Brain Macromolecules	FF-12
HD-LB4	Development of Mammalian Metabolic Regulation	FF-14
HD-LB18	Regulation of Neuroendocrine Metabolism	FF-18
HD-LB8(1)	Developmental Pharmacology	FF-22
 <u>Pregnancy Research Branch</u>		
	Summary Report	FG-1
 Project Reports:		
HD-PR1	Carbohydrate Metabolism During Primate Pregnancy	FG-4
HD-PR2	Steroid Hormones in Primate Pregnancy	FG-8
HD-PR3	Amniotic Fluid Fat Concentrations in Primate Pregnancy	FG-12
HD-PR4	The Effects of Induced Fetal Blood pH Changes on Fetal Blood Flow	FG-14
HD-PR5	Calcium Homeostasis in the Subhuman Pregnant Primate	FG-16
HD-PR6	Prolactin Metabolism During Primate Pregnancy	FG-18
HD-PR7	Renin-Angiotensin Relationships in Sheep Pregnancy	FG-20
 Contract and Collaborative Research:		
	Human Fetal-Maternal Metabolism Studies Under Conditions of Acute Fasting	FG-22

GERONTOLOGY RESEARCH CENTER

Office of the Chief

	Summary Report	FH-1
	Research Services	FH-1
	Technical Development Section	FH-1
	Photography and Arts Section	FH-2
	Animal Resources Facility	FH-2
	Information Services	FH-2
	Library	FH-3
	Collaborative Guest Scientist Program	FH-3
	Cardiac Performance in the Rat	FH-3
	Aging of Skeletal Muscle	FH-4
	Tumor Incidence in Senescent Rats	FH-4
	Cell Biology	FH-4
	Physiology of the Gastrointestinal Tract	FH-7
	Motility of the Gastrointestinal Tract	FH-10
	Colon Motility and Compliance	FH-10
	Osteoporosis	FH-10
	Thyroid Studies	FH-11
	Amyloidosis and Aging	FH-11

		<u>Page</u>
HD-AG1(c)7	Marital, Sexual and Social Factors in Aging	FJ-21
HD-CP13	Dermatoglyphics and Clinical Medicine	FJ-24
HD-CP14	Twin Studies: I, II, III	FJ-26
HD-CP15	Dermatoglyphics of Primitive and Developing Population	FJ-28
HD-CP16	Genetic Studies on Longitudinal Program Subjects	FJ-30
HD-CP1(c)	A Programmed Stress Test for the Electrocardiographic Diagnosis of Coronary Artery Disease	FJ-32
HD-CP6(c)	Non-invasive Evaluation of Ventricu- lar Function in Man by Measurement of Systolic Time Intervals	FJ-33
HD-CP9	Effects of Coronary Perfusion Pressure on Myocardial Oxygen Tension and Epicardial ST Segment Changes in Acute Infarction in Dogs	FJ-34
HD-CP10	Age Differences in Cardiovascular Performance and Baroreceptor Control Mechanisms in Intact Unanesthetized Rats	FJ-37
HD-CP17	Effect of Age on the Response to Inotropic Interventions and Hypoxia of Rat Trabeculae Carneae	FJ-38
HD-AG21(c)	The Renin-angiotensin System. I-IV	FJ-41
HD-AG22(c)	Relationship of Age to Thyroid Function and Thyroid Hormone Metabolism	FJ-46
HD-CP18	Hormone Receptors and Aging: Effects of Age on Hormone Mediated Alter- ations of the Adenyl Cyclase, Tissue Cyclic AMP and Factors Controlling These Metabolic Regulators	FJ-48
HD-CP7(c)	The Kinetics of Glucose and Insulin Metabolism in Man	FJ-51
HD-AG10(c)	Effect of Age on Carbohydrate Metabolism	FJ-55
HD-CP12(c)	Cytoxan-induced Water Intolerance	FJ-58
HD-CP19	Multiple Myeloma and the Kidney	FJ-60
HD-CP20	Uremia and Carbohydrate Intolerance	FJ-63
HD-CP21	The Response to Solar-stimulating Radiation as a Function of Age	FJ-66

		<u>Page</u>
Project Reports:		
HD-CCP12	Characterization of Age-related Defect in the Proliferative Capacity of Lymphocytes	FK-5
HD-CCP13	Characterization of Cellular Deficiencies in Age-related Decline of the Humoral Immune System	FK-8
HD-CCP14	Cellular Basis of Regulation of the Humoral Immune Response	FK-12
HD-CCP15	Cellular Aspects of the Immune Response of Long-term Radiation Induced Allogeneic Chimeras	FK-15
HD-CCP16	<u>In Vitro</u> Studies on the Capacity of Aged Mice to Develop Immunological Memory	FK-18
HD-CCP17	Pathology of Age-associated Kidney Disease of Wistar Rats Reared at GRC	FK-21
HD-CCP18	Effects of Age on Steroid Hormone Binding and Responsiveness in Target Cells and Tissues	FK-23
HD-CCP19	Effect of Chromosomal Aneusomy on Cellular Growth, Metabolism and Aging	FK-25
HD-CCP20	Effects of the Agouti Locus on Development and Aging in Mice	FK-27
HD-CCP2	Dietary Proteins and Longevity	FK-29
HD-CCP21	Effect of Dietary Manipulations on Life Span, Incidence of Diseases and Immunologic and Related Functions	FK-31
HD-AG13	Aging in the Rotifer (This project has been discontinued)	FK-33
HD-CCP22	Ageing and Genetically Defective Mammalian Skeletogenic Cells and Connective Tissues	FK-34
HD-CCP1	Changes in Cells and Extracellular Matrices of the Crayfish Gastro-lith Disc During Growth, Development and Aging	FK-36
HD-CCP11	Ultrastructural Studies of the Regenerating Sea Spine During Growth and Development Sequences	FK-39
HD-CCP8	Age-related Changes in Mammalian Myocardial Cells	FK-41
HD-AG37	Studies on the Comparative Physiology of Ageing and Environmental Effects on the Ageing Process	FK-44

<u>Laboratory of Molecular Aging</u>		<u>Page</u>
Summary Report		FL-1
Project Reports:		
HD-GMA1	Interaction of Metal Ions with Poly-nucleotides and Related Compounds	FL-4
HD-GMA2	Degradation of Nucleic Acids by Metal Ions	FL-8
HD-GMA3	Structure of Nucleoprotein, Ribosome, and Interaction of Components Related to Protein Synthesis	FL-10
HD-GMA5	Polymers as Biological Reagents	FL-13
HD-GMA6(1)	Mispairing of Nucleotide Bases	FL-16
HD-AG17(8)(c)	Relation of Structure and Function in Hemoglobin	FL-18
HD-AG30	The Function of Metal Ions in Enzymatic Reactions	FL-21
HD-GMA7	Cellular Regulation of Intermediary Metabolism	FL-25
HD-AG23(8)	Mechanisms of Membrane and Cellular Transport	FL-30
HD-GMA4(2)	Control of RNA Synthesis in Senescent, Developing, and Neoplastic Systems	FL-34
HD-GMA8	Investigations on the Biochemical Mechanisms of Hormonal Regulation of Metabolism	FL-35



NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Office of the Director

In fiscal year 1973 a number of significant events directly affected Institute program and administrative activities:

- recognition by Congress, the Office of the Secretary, DHEW, and the public concerning the need for more research on sudden infant death syndrome;
- legislative action by Congress to establish a separate Institute for Aging Research;
- continued restrictions on employment compounded by requirements to reduce average grades;
- discontinuation of training grants, fellowships, and Research Career Development Awards announced by the President;
- significant decreases in dollars (4.4%) and permanent full-time staff ceilings (4.9%).

Office of Administrative Management

This office, established last year to more clearly delineate administrative functions, responsibilities and reporting relationships has undergone some reorganization this year. The Administrative Management Section has been abolished and the services and responsibilities housed there have been split three ways:

- (1) Office services are now assigned to the Office Services Section.
- (2) Management analysis, reports, and special projects are now the responsibility of the Management Analysis Section.
- (3) Travel planning and services, timekeeping, and administrative policy and procedure liaison is the responsibility of the Institute Administrative Officer.

The Reference Services Section has also been abolished so that resources may be diverted to higher priority activities. This action is described more completely under Organizational Changes.

Staffing

At the outset of the fiscal year, the Institute developed its staffing plans based on a permanent full-time ceiling of 552 positions, i.e. the 1972 ceiling of 477 positions plus the restoration of the 75 Aging positions. The permanent full-time ceiling was adjusted in March 1973 to 525 positions. The following table summarizes the Institute's staffing pattern for 1973:

	7/1/72			12/31/72		
	<u>Full-time</u>	<u>Other</u>	<u>Non-ceiling*</u>	<u>Full-time</u>	<u>Other</u>	<u>Non-ceiling*</u>
OD	46	7	4	51	11	6
OSD	267	29	128	310	30	137
PS	75	6	4	70	8	3
EP	43	5	1	38	7	1
CPR	36	3	1	37	3	1
EB	15	6	3	15	4	5
TOTAL	<u>482</u>	<u>56</u>	<u>141</u>	<u>521</u>	<u>63</u>	<u>153</u>

	6/30/73 Estimate			Total Net Change	
	<u>Full-time</u>	<u>Other</u>	<u>Non-ceiling*</u>	<u>Full-time</u>	<u>Other</u>
OD	48	8	6	+2	+1
OSD	317	25	113	+50	-4
PS	71	10	2	-4	+4
EP	40	7	2	-3	+2
CPR	37	3	1	+1	
EB	12	3	6	-3	-3
TOTAL	<u>525</u>	<u>56</u>	<u>130</u>	<u>+43</u>	<u>0</u>

* Includes Visiting Fellows, SIS, MI's, Guest Workers, Guest Scientists, and Access Students.

Grade De-escalation

Fiscal year 1973 was the second year of the two-year program to reduce the average grade of the Federal government. The Institute met the average grade target for the first year of the program. The Institute anticipates no problem in meeting the target assigned for 1973. The following table presents the Institute's progress in meeting the average grade target (excludes summer program).

June 30, 1971	-	9.0773
June 30, 1972	-	8.8089
June 30, 1973 est.	-	8.6233

Upward Mobility

NICHD upward mobility efforts have been hampered by ceiling limitations as well as grade level controls. Those upward mobility efforts which do not

require ceiling positions have continued to be effective.

We have currently 17 students enrolled in the Upward Mobility College and two employees enrolled in the Stride Program (20 hr./wk. work--20 hr./wk. study toward a college degree). One employee is participating in a supply management training program. We anticipate the establishment of three new trainee positions for minorities and women in the near future as a part of the Institute's plan for affirmative action in Equal Employment Opportunity.

Some programs which may not be formally designated as Upward Mobility programs, but which offer training opportunities and job experience to individuals are the student programs. We now have 10 Junior Fellows (full-time summer employment for outstanding high school students), six Access students (six months college study--six months on the job leading to a Bachelor's Degree), and 32 Stay-in-School employees (part-time year-round employment for high school and college students).

Budget

After several years of increasing appropriations, NICHD in fiscal year 1973 experienced a decrease of 4.4 percent in its operating level, dropping from \$116.5 million in 1972 to \$111.4 million in 1973. This decrease meant reductions in all mechanisms of extramural research support, and in all the Institute's program areas except Population.

The following tables illustrate this:

NICHD - Comparison by Mechanism (dollars in thousands)

	<u>1972</u>	<u>1973</u>	<u>Increase or Decrease</u>
Research Grants	\$ 64,251	\$ 61,498	-\$2,753
Training Grants	10,142	7,708	- 2,434
Fellowships	3,652	2,900	- 752
Research Contracts	20,300	19,086	- 1,214
Labs & Clinics	8,680	9,736	+ 1,056
Biometry, Epidemiology & Field Studies	316	578	+ 262
Research Management & Program Services	4,866	5,817	+ 951
Management Fund	4,291	4,094	- 197
Total	<u>\$116,498</u>	<u>\$111,417</u>	<u>-\$5,081</u>

NICHD - Comparison by Program
(dollars in thousands)

	<u>1972</u>	<u>1973</u>	<u>Increase or Decrease</u>
Population	\$ 39,968	\$ 39,972	+\$ 4
Child Health	55,754	53,451	- 2,303
Perinatal Biology & Infant Mortality	(16,112)	(15,224)	- (888)
Growth & Development	(19,892)	(18,865)	-(1,027)
Mental Retardation	(19,750)	(19,362)	- (388)
Aging	12,505	12,448	- 57
Other			
Management Fund	4,291	4,094	- 197
General Research			
Support Grants	3,980	1,452	- 2,528
Total	<u>\$116,498</u>	<u>\$111,417</u>	<u>-\$5,081</u>

This fiscal year Institute funds available for funding all types of research and training grants, fellowships and research contracts amounted to \$91.2 million, a decrease of 7.3 percent from the 1972 level of \$98.4 million. This decrease in funds resulted in a decrease of 11.7 percent in the number of projects funded this year. The following table illustrates the distribution of these decreases.

Grants and Contracts

Type of Award	No. of Projects	+ or - Over 1972	Amount of Dollars (millions)	+ or - Over 1972
Research Grants				
Regular Program	858	-49	54.4	- .3
Special Programs:				
Mental Retardation				
Research Centers	12		5.6	
General Research				
Support Grants			1.5	-2.5
Training Grants	93	-18	7.7	-2.4
Fellowships	139	-67	2.9	- .8
Research Contracts	225	-21	19.1	-1.2

The most significant decreases appeared in the training and fellowships activities of the Institute which, along with all of NIH's training programs, are being phased out. The other major area of decrease was in General Research Support Grants which are being reduced in scope.

Funds for the research program of the NICHD, i.e. research grants and contracts decreased this year by 1.8 percent. The number of research grants funded in 1973 decreased by 5.7 percent, while the number of contracts and reimbursable agreements decreased by 8.5 percent. The funding for the 12 Mental Retardation Research Centers remained level. The Center for Population Research administered 308 research grants, and 175 research contracts, while the Child Health program accounted for 490 research grants and 38 contracts and the Aging program accounted for 60 research grants and 12 contracts.

Funding for the Institute's intramural research program increased 12.2 percent in 1973, to a level of \$9.7 million, or 8.7 percent of the total Institute budget. The Gerontology Research Center in Baltimore accounted for approximately \$3.5 million of the Intramural Research budget, or 36.2 percent.

The remainder of the Institute's funds were spent as follows for the supporting activities of the Institute: biometry and epidemiology - \$578,000 and research management and program services - \$5,817,000, including allowances for research conferences and for program evaluation. The NICHD payment to the NIH Management Fund was \$4.1 million in 1973.

Equal Employment

The Equal Employment Opportunity Advisory Committee has reached a very important plateau. During the past year there has been great activity and a tremendous amount of discussion in connection with the EEO efforts within NICHD. There has not been, however, unanimous consent as to the efficacy of the overall EEO Program as it relates to the expectation of minorities and women. It is felt that the initial Affirmative Action Plan (AAP) goals that dealt with blatant discriminatory practices were not as difficult to obtain as the more subtle kinds of practices that are historical and firmly entrenched in some of our day-to-day operations.

During that early stage of development, the EEO Committee along with the Institute staff achieved progress very quickly on those first goals. Methods of achieving those goals are not necessarily the same methods needed to assist the Institute in achieving the next level of goals that were identified by the revised AAP developed by the EEO Advisory Committee and approved by the Director. An absence of tried and proven solutions for achieving AAP items within the Institute in no way excuses abandonment of those new and innovative items that will ultimately affect the creditability of the program.

Hopefully, with the continuous and total support of management the particular items achieved during the past year will be the impetus needed for achievement of a more comprehensive AAP.

Aging Institute

Near the end of the 92nd Congress, a bill was sent to the President for the establishment of a National Institute on Aging within the Department of Health, Education, and Welfare. The President pocket vetoed the bill, H.R. 14424, citing five reasons. Mr. Nixon felt that the Technical Advisory Committee for Aging Research appointed by the Secretary, DHEW would accomplish the needed development of "a comprehensive, coordinated program of aging research--one which includes disciplines ranging from biomedical research to transportation systems analysis, from psychology and sociology to management science and economics" of which he spoke to the Congress on Older Americans in March 1972. An entirely separate institute would duplicate these activities. He felt that, if signed, the bill would increase administrative costs, fragment existing research efforts and, finally, duplicate the provision for a grant program for mental health facilities.

Pressure for a National Institute on Aging mounted early in the 93rd Congress. On March 16, hearings were held in the House on legislation introduced by Florida Representative Paul Rogers, and on March 27 Senate hearings were held on an identical bill sponsored by Missouri Senator Eagleton. The administration still opposes the creation of a new Institute. Administration witnesses, Frank C. Carlucci, Under Secretary of Health, Education, and Welfare, John S. Zapp, Deputy Assistant Secretary for Legislation (Health), DHEW, John F. Sherman, Ph.D., then Acting Director, NIH, DHEW, Gerald D. LaVeck, M.D., Director, NICHD, NIH, DHEW, Marvin Taves, Ph.D., Chief, Division on Aging, Social and Rehabilitation Service, DHEW, and James F. Garrett, Assistant Administrator for Research and Demonstrations, Social and Rehabilitation Service, DHEW, defended both the concept of research on lifespan processes as a logical organizational approach to research on the process of human aging, and the existing NIH arrangement wherein diseases of the aged are studied in the respective categorical institutes. Congressional pressure is not as great as in the previous Congress, and the NICHD has not offered an organizational alternative to the establishment of a new Institute.

During the Senate hearing, Dr. Sherman indicated that although funds are being cut, in terms of additional personnel positions, NIH has given a larger number to the aging program than to any other with the exception of the Cancer program.

Organizational Changes

The recent cutback in this Institute's employment ceiling made it incumbent upon the Institute to reevaluate internal services and associated organizational units to identify potentially dispensable activities. Eliminating these would eliminate the need to cut program efforts. As a result of this reevaluation, two functions were identified which could either be obtained outside of the Institute but within the NIH, or could be contracted for with private companies.

The Reference Services Section of the Office of Administrative Management, an NICHD "mini" library which formed the link between Institute staff and services from major library operations--NIH Library, National Library of Medicine, and local public library systems--has been phased out. The NIH Library now handles all inter-library loans and journal requests in addition to journal searches for Institute employees; indexes and reference volumes are still available in the Landow Building for staff use. Journal circulation and publication procurement services are still available through the Institute's reference assistant.

The Scientific Publications Section, Office of Public Information, which for years has provided the advice and technical assistance to staff on the production of scientific publications has been phased out. With this Institute still sponsoring more conferences than any other, we are producing an ever increasing number of publications. The section is being replaced by a publications specialist with responsibility for consulting with, advising and assisting staff in obtaining the required technical services for the production of all Institute publications. In addition, the publication specialist will obtain copyright releases and reprint permissions.

The Office of Administrative Management underwent other changes. This year the Administrative Management Section was abolished and the Institute Administrative Officer was placed on the staff of the Executive Officer. The Office Services and Management Analysis Sections were established. These changes required no new personnel. They were accomplished to allow the chiefs of the new sections more autonomy over their operations and full responsibility for their activities.

The Office of the Scientific Director has phased out a branch operation. The Children's Diagnostic and Study Branch was eliminated and the vestigial functions and personnel were reassigned to other research areas. This was a gradual phase out aimed at improving the utilization of present resources.

Although the Scientific Conference Branch in the Office of the Associate Director for Program Services was abolished during fiscal year 1973, the activities of the Branch were transferred to the Office of the Associate Director. Two special assistants were appointed to that Office: one to handle the administrative coordination and initiation of Institute-supported conferences and workshops and the second to handle the administrative functioning of the Institute's advisory committees. The abolishment of the Research Analysis Section of the Program Statistics and Analysis Branch reflected the need to transfer personnel to meet other Branch work priorities.

Training Programs Phase Out

The 1973 budget estimate is \$5,081,000 below the 1972 operating level. The reduction of \$3,186,000 in the fellowships and training grants programs represents 63 percent of the total Institute's reduction. Consistent with the Administration policy to rely on general resources for aid to students, the special program of supporting research training will be terminated after the training under previously approved grants is completed.

Sudden Infant Death Syndrome

"To expand and intensify a research program aimed at solving the problem of Sudden Infant Death Syndrome" became an objective in the DHEW Operational Planning System for FY 1973. It was among the OPS objectives designated top priority by the Secretary who has tracked progress throughout the year. NICHD, the responsible organization, identified activities (milestones) to be accomplished in attaining the goal. They have been achieved. Briefly:

- a. A series of research planning workshops were held to develop specific objectives in the areas of developmental immunology, cardiorespiratory and thermometabolic phenomena, developmental neurophysiology of sleep, epidemiology, and pathology as related to SIDS.
- b. Summary reports of the workshops have been prepared, published, and staff is in the process of distributing these.
- c. A Request for Proposals (RFP) for the development of animal models for research in the areas mentioned above was developed and advertised. The resultant contract proposals have been evaluated, and approved projects have been negotiated.
- d. A workshop on the management of SIDS cases with selected forensic pathologists and pertinent DHEW personnel has been held.

Initial indications, that is, the greatly increased number of inquiries and additional grant applications and the greater than usual number of contract proposals suggest that the objective was successfully launched.

In addition to these activities the Institute is currently supporting 66 research projects directly relevant to enhanced understanding of the Sudden Infant Death Syndrome. Also, more than 30 scientific journals have carried announcements of the NICHD's expanded research program in this disease area.

Several scientific and public information activities being conducted are also worthy of highlight:

- a. A "Blueprint for a More Extensive Approach to Research on the Cause of the Sudden Infant Death Syndrome" under the authorship of an Institute consultant and staff member will be published in the American Journal of Diseases of Children.
- b. SIDS and the Rescuer, a leaflet, provides pertinent information on SIDS cases to emergency vehicle personnel, policemen, medical personnel and emergency room staff members.

- c. A Sudden Infant Death Syndrome Slide Tape Presentation has been prepared and is being distributed to the lay community for public education purposes.
- d. Selected Annotated Bibliography on the Sudden Infant Death Syndrome: 1960-1971

Title X

PL 91-572, the Family Planning Services and Population Research Act of 1970, was signed by the President on December 24, 1970. This Act included an amendment to the Public Health Service Act. The amendment was designated "Title X--Population Research and Voluntary Family Planning Programs." Section 1004 of Title X authorized a program of research grants and contracts to promote research in the biomedical, contraceptive development, behavioral, and program implementation fields related to family planning and population. Section 1008 prohibits the use of funds in programs where abortion is a method of family planning.

The prohibition in Section 1008 has given rise to concern in Congress and the insistence that the Institute state explicitly the authority under which it is spending dollars in the population/family planning area, since some of the projects funded in that area support development, study, and evaluation of abortifacients.

There existed at the time PL 91-572 was signed and there still exists other authority under which research grants and contracts in these areas can and are being funded. Section 301 Part A Title III of the Public Health Service Act provides authorization for the appropriation of funds to support the research programs of NIH, including those for the Center for Population Research in NICHD. Authority for NICHD's support of population research has not been differentiated between Title X and Title III in fiscal years 1971-1973. Both authorities have been cited by the Institute in their budget justifications, and appropriation language has not stipulated the amount under each authority.

"Title X" expires on June 30, 1973, but the level of support for population research activities has not been enhanced by the existence of Title X. In the eight-year period from 1964 through 1971, prior to the passage of Title X, the funding of the population research program increased by more than 900 percent. For the two-year period from 1972 through 1973 covered by Title X, the funding for population research increased by approximately 40 percent. While other NIH programs are sustaining cuts in FY 1974, the program level for population research is being continued at current levels, even though Title X authority does not extend to that year.

It is the Institute's position, therefore, that Section 1004 need not be renewed. Research grants and contracts in population will continue to be supported whether or not Title X is an authority.

Advisory Council Activity

The National Advisory Child Health and Human Development Council has directed considerable energy in the direction of policy formulation and program development. The June 1972 meeting saw the consideration of the proposal prepared by the Growth and Development Branch, Extramural Programs, on an "Expanded Program in Adolescent Development." Wholeheartedly and unanimously endorsing the report, Council recommended immediate implementation. Its resolution transmitted to the Director, NIH, asked that the following steps be undertaken by the Institute to initiate a program in adolescence:

1. Actively seek additional specific funds of sufficient magnitude for an orderly implementation of an expanded research and training effort.
2. Develop an informational program to give visibility to this area and to make potentially interested persons aware of the Institute's interest in expanded research bearing on adolescence.
3. Establish a subcommittee of the Council who have interest and knowledge in the field to advise and assist in this development.
4. Employ additional staff who have expertise in this field.
5. Support several adolescent research units at institutions where there is potential for multidisciplinary research efforts.
6. Obtain additional funding for training (training grants, RCDA's and fellowships) in the area of adolescence research. /Of course this last recommendation is severely affected by the phase out of training programs/

Office of Program Planning and Evaluation

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the information needed for the planning and the formulation of the Institute's major goals and objectives and the means for appraising progress toward these objectives.

In Fiscal Year 1973 the OPPE prepared several reports on aspects of the research and training programs of the Institute in response to requests from Congressional Committees and Departmental offices. Similar material was developed for interagency coordination purposes. The Institute Research Plans (popularly called the Blueprints), requested in 1972 by the Senate Committee on Appropriations, were prepared by the OPPE. There continues to be much interest in how Institute research and training programs pertaining to early child development interface with other Federal efforts in this area. Several pieces of legislation concerned with Federal support of research and services specifically aimed at improving the health status and learning capabilities of children have been introduced in the Congress. All of these bills have had some impact on NICHD research and program activities in 1972. The Office keeps all personnel informed about this legislation. Staff of OPPE, in cooperation with the Budget Officer and Executive Officer and Program Personnel of the Institute, developed the Forward Plan for FY 1975-79 and the Operational Plan for FY 1974.

OPPE staff have initiated cooperative efforts to expand research concerned with the causes and prevention of Sudden Infant Death Syndrome (SIDS). These efforts include staff of the Epidemiology and Biometry Branches of the Office of Epidemiology and Biometry, and of the Perinatal Biology and Infant Mortality Branch (PBIM) of Extramural Programs. This team approach to an important pediatric problem includes development of collaborative research projects and the definition of research goals and needs as well as the coordination of Institute research efforts in this area. In addition, OPPE staff have been in collaboration with staff of Extramural Programs in an attempt to develop a developmental pharmacology research program that is concerned with effects of medications administered during pregnancy and childbirth.

The facilities and resources activity of the OPPE has included participation in the development of plans for an intramural clinical research unit in reproductive research and perinatal biology to be activated in the Clinical Center, NIH. This program will be housed in 15,492 net square feet, made available through construction of an 8th, 9th and 10th floor in wing G. Target date for occupancy is July 1, 1974.

Interest of OPPE staff continues in Research in Child Development and the Man-Made Environment. OPPE staff continue to exploit opportunities to explore new concepts in this area with a view to defining impact on the direction and progress of Institute programs.

Staff of OPPE participates in coordination efforts, and maintains liaison with agencies whose missions are complementary to or supportive of the Institute's objectives.

Office of Public Information

During fiscal year 1972, the Office of Public Information underwent major reorganization with the recruitment of four new information specialists and the retirement of one. The recruitment made possible a new system of assigning each program area an information specialist to carry out mutual information objectives.

This year, the OPI focused on establishing working relationships with the program staff; formally planning public information activities and services; training new staff; and, working out a new management and organization which could respond to public information requirements.

The Scientific Publications Section continued to provide editing, administrative, and advisory service to all Institute staff involved in publishing scientific publications pertaining to Institute conferences and interests. This section was abolished on June 30. The services will continue to be provided through contracts.

By the close of the year OPI had produced or had in press 10 public information pamphlets and 14 scientific publications. In addition, the OPI had prepared five special reports and the annual highlights for the Congress, material for numerous centrally produced NIH information projects and several major briefing documents for the Department. The OPI also received 250 requests from the press (radio, TV, magazines, and newspapers), and answered 5,550 public inquiries, and distributed some 73,000 publications in response to these requests.

Major public information campaigns on prevention of mongolism and bacterial meningitis were planned and underway in FY 73. The OPI also served as the public's contact with the Institute for information concerning advisory committee meetings.

The public information program of the GRC was carried out by a Public Information Officer assigned to that facility in Baltimore.

Office of the Associate Director for Epidemiology and Biometry

The Epidemiology and Biometry Branches continued their research and consulting activities during the past fiscal year, the first full year of operation since this Office was organized. Statistical consulting efforts and other service activities increased during the year, with scientists in the intramural program and with extramural program directors. In the first half of the fiscal year, the Epidemiology Branch initiated computer analyses of an increased number of research projects, the data for which had been gathered in previous years. However, the corresponding computer costs increased to such an extent that high priority projects only could be continued for the remainder of the year.

The Epidemiology Branch continues to concentrate on problems of prematurity and infant mortality. Particular emphasis is being put on investigating the effect of improved nutritional intake during pregnancy in indigent groups on birth weight, perinatal and neonatal deaths. Preliminary evidence indicates that some of the lowest rates of low birth weight babies among blacks ever attained has been achieved by one prenatal clinic in the San Francisco Bay area. In this facility, practice is to educate and persuade pregnant women to eat well, without concern for weight control, diuretics nor low calorie-low salt diets. The complete data from this clinic and from two other control clinics in the County are currently being analyzed. The Branch is also studying the relations between prematurity (low gestational age and low birth weight) and infant mortality in terms of the joint variation of both in the United States.

Other subjects receiving major attention by epidemiologists in the Branch are Sudden Infant Death Syndrome, Respiratory Distress Syndrome and the epidemiology of a selected number of congenital malformations such as anencephaly and spina bifida. Smaller studies, reflecting interests of individual investigators, have been carried out or are in process in Mental Retardation, in Aging and regarding the effects of induced abortion on subsequent offspring.

Consultative services of the Biometry Branch have been extensive. Scientists representing almost all the intramural laboratories have consulted with statistical staff of the Biometry Branch including the Branch Chief and the Associate Director, on the design and analysis of experiments. In addition, members of the Branch give advice to extramural scientists on statistical issues arising in the design of studies and the analysis of data in contract research.

In other areas, the Biometry Branch has continued to serve as the statistical center of the collaborative study to evaluate the untoward effects of the amniocentesis procedure. This project is now in its second year. The Branch has continued its high level research program in statistical methodology.

This branch also engages in substantive research, i.e., investigations of a statistical nature designed to gain fundamental knowledge in a given field. Thus far, this research has been in the population area with special attention to the side effects of the oral contraceptives.

Office of the Associate Director for Program Services

The Office of Program Services was effected by three major events during fiscal year 1973: (1) a significant reduction in the number and dollar value of institute supported projects; (2) the abolishment of the Scientific Conference Branch and the Research Analysis Section of the Program Statistics and Analysis Branch; and (3) the consolidation of all activities of the Office within adjoining physical space in the Landow Building.

The Institute's supported projects were reduced by 155 with a dollar value of approximately \$4.6 million below the fiscal year 1972 experience. The bulk of the reduction, \$3.2 million, reflected the phaseout of the various training programs which was implemented on January 29, 1973, in accordance with the President's budget presentation for fiscal year 1974. The training program phaseout policy provided for the funding of all programs with continuing commitments as of January 29, 1973, but did not provide for any additional new training programs. The research career award program, a senior research professorship program, will be continued until long-term commitments are completed. The contract program was reduced by approximately \$1.0 million and the research grant program by approximately \$0.2 million below the fiscal year 1972 levels.

Despite the abolishment of the Scientific Conference Branch, its functions have been continued. The Conference Assistant participated in the planning and arranging for twenty-one conferences and workshops directly sponsored by the Institute. The Committee Management Assistant provided member processing services for ten Institute advisory committees. As part of the "Federal Advisory Committee Act" it became necessary for the Institute to conduct a comprehensive review of the activities and responsibilities of each advisory committee. The Committee Management Assistant coordinated the preparation of material for this review.

The contracts management program has now operated a complete fiscal year with the full range of contracting functions: from early planning of Requests for Proposals to signing authority as Contracting Officers. A new Institute research contract policy was issued in January, 1973, to reflect the complete contracting cycle.

The Program Statistics and Analysis Branch produced two publications for the scientific community in association with program staff: "Inventory of Federal Population Research" and "The Extramural Program of Research on Aging." In addition, the Branch developed a variety of narrative and tabular reports indicating Institute support in important research areas, such as Sudden Infant Death Syndrome, perinatal physiology, pediatric neurology, and maternal nutrition and its effects on fetal and postnatal development. A Branch member has served on the Interagency Committee on Early Childhood Research and Development and the Interagency Committee on Adolescence.

The Office of Program Services continued to stress the area of staff development by participating in various training programs at all general schedule levels. We plan to hold one half-day session on EEO issues in which all Office of Program Services' members will be expected to be involved.

Office of the Associate Director for Extramural Programs

The Growth and Development Branch

Exciting progress has been made by a grantee in an ambitious attempt to achieve the chemical synthesis of a gene. Another grant awarded during FY'73 deals with the development of sensitivity of mammalian cells to growth hormone. These investigators have discovered that human growth hormone reverses the disintegration of muscle cells in certain types of muscular dystrophy, a finding that holds great promise for clinical application.

Other studies in the area of developmental immunology, have resulted in the recent publication of regimens for treatment of immune deficiency diseases, including such conditions as deficiencies in humoral immunity, phagocytic function, and cellular immunity.

A recent study funded by NICHD gives evidence of the needs and research possibilities within pediatric pharmacology. This investigator has developed a new, rapid, sensitive, clinically useful radioimmunoassay procedure for determination of serum and urine digoxin concentrations. Digoxin is an invaluable drug for a variety of cardiovascular diseases in children, but it has a low margin of safety. Complications from use of this drug have resulted in the past in both children and adult populations. The availability of this new assay procedure can lead to a rational use of this invaluable agent in children with cardiovascular disease.

Somewhat parallel nutrition studies are underway in animal and in human populations. A village level supplementation program in Guatemala has shown that maternal calorie intake during pregnancy is more directly correlated with birth weight than is protein intake. The latter appears more correlated with postnatal mortality, morbidity, and growth. Neurological and behavioral development are also being assessed. In another study, maternal supplementation during pregnancy and lactation not only produced a larger, more rapidly growing infant but also one who was physically much more active and demanding of maternal attention. These findings suggest that the active undernourished infant demands less and gets less attention, leading to worsened nutritional status and retarded development. Also, during the past year, preliminary data from the maternal nutrition studies indicate that protein requirements during pregnancy may be considerably lower for the rhesus monkey than heretofore considered, provided that the diet is adequate in other nutrients.

One grantee has recently reported that in the rat, maternal caloric deprivation during early lactation produced transient reduction in fat cell numbers and size in the offspring, whereas maternal restriction of both protein and calories resulted in permanent reductions in depot fat cell number and size in the offspring. These results may have important implications in human obesity in light of a prior finding by the same grantee that obesity in childhood is a function of fat cell number, size, and age at which rapid increase in fat cell number/size is initiated.

Institute-supported studies in the area of physical growth encompass developmental anatomy, cranio-facial growth, skeletal growth and studies in population genetics relating to anatomical development.

In the area of developmental behavioral biology, further progress in the clinical application of the cortical evoked response is in process where severely malnourished infants and children are being evaluated, utilizing cortical evoked response methodology. Preliminary results indicate gross abnormalities in the brain's functional ability to process sensory information in malnourished children.

In learning and cognitive development, investigators are discovering that children are capable of much finer perceptual differentiation under certain conditions than the existing literature on perceptual processes would indicate. Aspects of children's perception are also dealt with in other studies focusing on the relationships between the level of cognitive organization and the child's perception of the intention of others.

As a result of contract-supported human communication studies, a major breakthrough has occurred. There is evidence to indicate that infants are able to discriminate the acoustic cues underlying the adult phonemic distinctions between certain consonant sounds. Moreover, a Growth and Development supported grantee has now shown that the language hemisphere (left in most instances) of the cerebral cortex may be specialized to deal with grammatical coding, a conversion of information that distinguishes language from other perceptual and cognitive processes. Furthermore, the very latest finding seems to indicate that anatomical measurements of the language-mediating areas of the superior surface of the temporal lobe (planum temporale) are significantly larger in the left cortical area of the neonate just as in the adult. Therefore, it is suggested that this anatomical asymmetry is present prior to any environmental effects such as "language learning" and unimanual hand preference, and may be an important factor in determining the typical pattern of left hemisphere speech lateralization found in the majority of adults. Furthermore, this anatomical asymmetry which is present in the neonate suggests that the human infant is born with a pre-programmed biological capacity to process speech sounds.

In studies on personality and social development, one investigator found that vestibular stimulation, particularly in the context of soothing the infant, stimulates visual alertness and visual scanning. This finding has significant implications for early perceptual learning which are being actively pursued. Another investigator has discovered that there are six stages of moral thought which follow an invariant sequence in various individuals and cultures. Another investigator, supported in part by NICHD funds, has discovered that infants raised in relatively deprived environments in an isolated Guatemalan Indian village recover from retardation and attain age-norms of children in advanced countries if they are treated as normal infants by their cultural standards. This important finding indicates that we must have a more refined understanding of the effects of early deprivation in order to design effective intervention or remedial programs.

In June, 1972, the Branch made a preliminary report to the NICHD Council on needed research in the area of adolescence. This report, based on extensive consultation with experts and intensive staff discussion, recommended research initiatives in both biomedical and behavioral areas. These are found earlier in the Director's summary.

Adult Development and Aging Branch

It has been found that impairment in cognitive ability probably begins in many persons at least by the middle years of life. In late life a significant number of persons are severely demented.

Whether these dementias are in part the end result of the cognitive decline that begins relatively early in life or whether they for the most part are due to other processes is unknown. It has been thought for many years that a large fraction of the senile dementias are due to atherosclerotic vascular disease. However, many neuropathologists are now beginning to believe that much of what was attributed to vascular disease is due to primary neuronal disease.

Furthermore, concerning aging in women, the year before last NICHD supported a conference to lay the foundations for a program to study the causes, manifestations, and treatment of the results of the menopause. At the conference the hormonal changes associated with the menopause, their possible relations to atherosclerosis, osteoporosis, and other diseases, and the possible advantages and disadvantages of replacement therapy were discussed. This year a retrospective study of the effects of replacement therapy, and a study of the hormonal alternations in the post-menopausal period were continued.

Regarding aging and immunology, it has been found that the level of activity of both the cellular and humoral immune systems peaks during adolescence and thereafter declines. These decreases in immunological competence are very important in the diminished resistance of the elderly to infections and very possibly contribute to the high incidence of cancer in the elderly.

On the cellular level, at one time fibroblasts were thought to be potentially immortal in tissue culture, but this has proved to be not true of normal human fibroblasts. It is now known that they undergo only about fifty doublings in vitro before dying. It is also known from transplantation studies that at least one cell type, mouse mammary epithelium, will not continue to divide indefinitely when transferred serially from young mouse to young mouse. In the summer of 1972 NICHD supported a course on the use of tissue culture in studies of aging. A similar course will be supported in the summer of 1973.

A major limiting factor in the expansion of experimental work on aging has been the difficulties involved in getting old animals. Techniques are now available to raise rodents free of parasites and either free of bacteria or with a defined bacterial flora. However, the methodology requires a large investment of time and facilities. It is, therefore, not practical for most

investigators to breed their own animals and raise them to old age. Therefore, NICHD has exerted much effort to make it possible for investigators to obtain well-characterized, healthy, old rats and mice from commercial breeders. NICHD now has contracts with a commercial breeder to make this possible.

Mental Retardation Branch

A survey of an unselected newborn population has demonstrated that 1 out of 200 babies has a major chromosomal abnormality. In addition, about 5 percent have minor chromosomal defects, the significance of which remains to be determined. Most of these genetically determined conditions are associated with mental retardation or defects which impair a child's ability to achieve his optimal development.

Investigators have developed an animal model for study of phenylketonuria. The model involves the combined feeding or injection of increased amounts of phenylalanine and an inhibitor of phenylalanine hydroxylase. Using this model, learning deficits have been shown to occur in offspring of female rats fed the PKU diet between days 10 and 20 of pregnancy, although the deficit exceeded and could not be distinguished from that resulting from maternal malnutrition produced by pair-feeding of control animals. Neither inhibitor nor excess phenylalanine alone fed to pregnant animals produced learning deficits in offspring.

Biochemical data derived from studies employing this animal model serve to confirm previous reports that the behavioral and biochemical aspects of experimentally induced phenylketonuria are similar to those of the human disease.

In addition, investigators in California have now carried out field tests to evaluate two new low-cost, simple methods for detecting the galactosemia metabolic error in newborns. These tests can be combined easily with current screening programs for phenylketonuria to provide widespread early diagnosis of both diseases.

There is also a need to map human genes. The Institute is furthering a partial solution to the problem of linkage studies through the establishment of regional registries for a number of chromosomal and metabolic defects. A second method of gene mapping involves cytogenetic localizations. The first case reports correlate deficiency of immunoglobulin IgA with abnormalities of chromosome No. 18. Delineation of the clinical syndrome associating mental retardation and certain congenital malformations with deletion of the long arm of chromosome No. 18 was achieved. The third method of gene mapping is through somatic cell hybridization. The Institute supported a Conference on Somatic Cell Hybridization in March, 1973 to further work in this area.

Preliminary evidence suggests that doses of acetylsalicylic acid too low to produce gross CNS malformations produce behavioral impairments. The learning impairment was attributed to functional brain damage resulting from in-utero

exposure to salicylates. The damage was not accompanied by anatomic abnormalities of the CNS although minor malformations of the skeleton and viscera were observed.

Institute-supported scientists are studying the effect of maternal protein malnutrition predating mating on the behavior, neurophysiology, neuro-endocrinology, neurochemistry and morphology of rat offspring. Pups of mothers deprived for six weeks prior to mating and during both gestation and lactation exhibit disturbances in recorded EEG patterns, proportion of REM sleep and changes in the associated neuro-endocrinological patterns, along with reductions in birth weight and in brain weight at birth and at 10 days. Eight weeks deprivation results in fewer litters with fewer pups with more profound effects on brain weight at birth and 10 days.

Regarding early diagnosis and intervention working with Down's syndrome children in age groups from birth to 18 months, 19-36 months and from 3 to 5 years of age, investigators have been able to demonstrate significant developmental gains in cognitive, speech and language, motor and self-help performance at all age levels. These gains hold up under sustained programming to school age using a methodology for preschool classroom training and parental instruction in intervention procedures. The most marked gains were achieved in those children for whom intervention began early in their development.

Further, epidemiological studies have examined public institution and community residents. Data which have been collected on more than 20,000 residents with respect to diagnoses, adaptive behavior, physiological traits, social relationship and psychological characteristics probably represent the most comprehensive information available on institution populations. This data has been related to mortality, life expectancy tables, and training outcomes and has been applied effectively to institutional management and programming. A community study highlighted the discrepancy between measured intelligence and adaptive behavior for ethnic minority groups. Many children of Mexican-American descent and bilingual in speech tested poorly on standard intelligence tests because of deficiencies in the English language rather than intellectual impairment. These findings stimulated policy changes in the educational system and the decertification as mentally retarded of thousands of children.

Perinatal Biology and Infant Mortality Branch

Two major priority areas have been identified by the PBIM staff as a means of highlighting emphasis areas and guiding future program development. The first of these is to eradicate those maternal, fetal, and infant health problems which result in the highest incidence of infant morbidity and mortality. Chief among these problems are low birth weight, the sudden infant death syndrome, process of birth and extrauterine adaptation, abnormal fetal development, and complications of pregnancy.

The second major priority area is the identification of psychosocial factors in pregnancy and delivery which affect the fetus and outcome of pregnancy and contribute to infant morbidity and mortality. The emphasis in this priority area is on maternal stress, fears and conflicts and their influence

on physiological functioning, postpartum maternal behavior, and maternal-infant relationships.

One study in the area of pregnancy and maternal health centered on the relationship of genital mycoplasmas to the occurrence of postpartum fever. Their clinical data failed to demonstrate any correlation between vaginal colonization with these organisms and postpartum fever. Invasion of the bloodstream by these mycoplasmas was associated with less than 10 percent of the cases of unexplained postpartum fever. Dr. McCormack and his associates have also reported on a study of puerperal bacteremia and neonatal sepsis due to the same organism, Hemophilus parainfluenzae. Their observations suggest that this organism is capable of causing serious infections and that its pathogenicity should not be underestimated.

In other studies, approximately 12 percent of the women who were bacteriuric during a pregnancy showed evidence of pyelonephritis in the follow-up. This gave a first approximation of the rate of renal disease associated with bacteriuria in pregnancy.

Concerning fetal health and development, investigators at the University of California used pregnant rats to study the distribution of nicotine and other metabolites in maternal plasma, brain, lungs, heart, liver, adrenals, kidneys, gonads and the stomach-intestine-spleen-pancreas en bloc. They demonstrated that nicotine readily crossed the placenta and that the fetal tissues were perfused with higher concentrations of nicotine than maternal tissues within 30 minutes after the entry of nicotine into the maternal circulation. He noted that the decidua may pump nicotine from the maternal circulation into the fetal circulation, and further, that amniotic fluid may constitute a reservoir of nicotine available for recycling through the fetus. The survival of the fetus depends on adequate placental transfer of oxygen and carbon dioxide. Other investigators have described a mathematical model of carbon dioxide transfer in the placenta and its interaction with oxygen. The mathematical model, as the investigators point out, is useful as a tool in elucidating the dynamic aspects of the exchange process.

Studies of the effect of magnesium deficiency in pregnancy and its impact on the offspring revealed no living fetuses at term from rats under severe dietary deficiency during pregnancy (day of finding sperm in vaginal smear to day 21 of gestation), and congenital malformations in rat fetuses from pregnant females receiving the magnesium deficient diet included cleft lip, hydrocephalus, micrognathia, clubbed feet, adactyly, polydactyly, short or curly tail, diaphragmatic hernia, heart, lung and urogenital abnormalities. Further study of magnesium is needed to ascertain the correlations between magnesium metabolism and such pathological conditions as low serum magnesium levels, toxemia in human pregnancy, persistent convulsions in newborn infants, and habitual miscarriage.

The survival of the developing fetus depends on either absent or depressed reactivity of both fetal and maternal cellular immune systems. One investigator used phytohemagglutinin (PHA) to test the response of lymphocytes in human cord blood and adult peripheral blood. He reported that greater

responsiveness of lymphocytes is seen from younger individuals and he suggested that the cellular immune function is well developed at birth.

Infection during pregnancy is well recognized as a significant cause of fetal loss and morbidity. Studies on infant survival and well being indicate that although the mother may show little evidence of illness, infectious agents cross the placenta and invade the fetal tissue. Investigators at the University of Alabama are directing a prospective longitudinal study in pregnant women and their offspring on the pathogenesis of chronic intra-uterine infections. While serologic changes occurred in a surprising number of pregnancies, congenital involvement in infants born to these women appeared to be rare. These data suggest an inherent protective mechanism on the part of the developing conceptus.

Pulmonary surfactant facilitates a physiologic function essential to extra-uterine survival and alveolar stability. A central and possibly etiologic role for the deficiency of surfactant in the pathogenesis of respiratory distress syndrome underscores the potential clinical importance of recently devised tests of surfactant components in amniotic fluid. Specific stresses in utero have been found to alter lecithin/sphingomyelin ratios.

Neonatal hypoglycemia is a problem of major concern in the care of low birth weight infants. The discovery of a Coenzyme A transferase deficiency as a cause for ketoacidosis presents a unique opportunity for establishing a clearer definition of the metabolic pathways functioning in maturation. Such information, in achieving a greater understanding of glucose metabolism, should provide a firmer basis for disease therapy in the newborn.

During the past year, increased emphasis has been placed on solving the mystery of the sudden infant death syndrome. This effort has been described earlier in the Director's summary.

Office of the Director, Center for Population Research

The Center for Population Research (CPR) was established in 1969. The CPR administers Grant and Contract Programs supporting Research and Training in the broad spectrum of population research areas encompassed by the biomedical and social sciences.

The Center has continued to provide high-priority support to three large and complex programs of population research: the development of improved methods of fertility regulation (including the improvement of contraceptive technology and the control of infertility); studies of biologic and genetic implications of contraceptive use; and investigations of the social science aspects of population problems. However, unlike previous years, there have been no major new research programs initiated. The completion of research in some of the earlier supported projects has dictated development of additional methods for communicating research findings and reporting in depth the methodology and techniques of population sciences. Specifically, a number of CPR Monographs are planned to be published by the GPO and several manuscripts are currently in preparation. The first publication is expected in early FY 1974.

New activities of the Fertility Regulating Methods Evaluation Branch to evaluate the medical effects of fertility regulating agents presently in use declined sharply during FY 1973 in order to permit consolidation of the large number of new projects initiated in FY 1972 and due to a level budget for FY 1973. Areas currently under study include the medical effects of vasectomy, comparative pharmacology, dietary nutrients and the relationship between cancer of the breast and cervix and oral contraceptives.

The Population and Reproduction Grants Branch within the Center for Population Research supports basic research and training grants in the biological, clinical, biomedical and social (behavioral) areas relating to reproduction and population phenomena. It is concerned with all aspects of population fertility and infertility, particularly studies likely to yield new information and methods for the development of safer, more acceptable contraceptives. The number of research grants in biomedical and social research decreased from 326 at the end of fiscal year 1972 to approximately 308 in 1973.

Research activities, basic to contraceptive development and showing particular progress and promise during the past year, include gamete development and maturation, gene activation and other aspects of the mechanism of action of steroid hormones, ovulation, gamete transport, fertilization in vitro, implantation, corpus luteum initiation and maintenance, and many others.

The Behavioral Sciences Branch has maintained its efforts to examine the interrelationships among population size, growth and distribution and economic development and other social and psychological factors and to analyze ways in which individuals are motivated to reduce the size of their families. Development has continued in both major divisions of this program: the determinants of fertility and consequences of population growth, structure and distribution. Particular emphasis has been given to the development of more specifically defined research gaps that require information in the area of fertility determinants.

The mission of the Contraceptive Development Branch is the development of a number of safe, inexpensive, effective and reversible methods of contraception. A broad spectrum of studies--125 in 1973--with both long and short term goals are currently being supported through the contract mechanism. These studies range from research at a fundamental level, where gaps in our knowledge of reproductive physiology exist, the support of clinical testing of known steroidal drugs for their efficacy as contraceptives.

Office of the Scientific Director

Growth and change of the NICHD intramural research program have continued in this fiscal year. Planning has been completed and a contract awarded for construction of a new three story obstetrics and nursery unit to be added to the G wing of the Clinical Center. This will house the research and clinical activities of the Pregnancy Research Branch and a perinatology research program. In fiscal year 1973 total budgeted positions for intramural research increased from 277 to 321. In addition a total of 106 scientists worked in NICHD laboratories as Guest Workers, Guest Scientists, and Visiting Fellows.

Developmental Immunology Branch

1. Development of a means of immunization of infants against Hemophilus influenzae type b meningitis, the largest source of acquired mental retardation in the United States, remains the major research effort of scientists in this Branch. In a pilot study this year the immunogenicity of H. influenzae type b polysaccharide has been evaluated by studying the clinical condition and serum antibodies of 23 infants injected with 5-10 mg of polysaccharide when 2-3 months of age. On follow-up all injected infants had serum anti-type b antibodies well above the average level for their age, and none had levels in the nondetectable range, in contrast to 30% of uninjected infants who had no detectable anti-type b antibody at the same age. Thus, infants so treated at 2-3 months of age respond with sustained antibody levels. A controlled clinical trial of this means of immunization will begin in Mecklenburg County, N.C. in the coming year.

2. Another mechanism for inducing immunity to H. influenzae that may be simpler, more effective, and has far-reaching implications for the whole field of immunology and infectious disease, is also under study in this Branch. Strains of non-pathogenic E. coli have been isolated which have an antigen that cross-reacts with the polysaccharide antigen of H. influenzae type b. These strains of bacteria, when fed to newborn animals, produce no illness, but do induce the formation of antibodies which protect the animal against infections due to H. influenzae. These strains are ubiquitous in nature, and probably constitute the source of the "natural" immunity to H. influenzae which most humans develop by age 6.

3. Scientists in this laboratory have also identified non-pathogenic organisms with cross-reacting antigens to D. pneumoniae and N. meningitidis, raising the possibility of applying this method of immunization to diseases caused by these and other organisms as well. More extensive animal testing of this immunization technique will be carried out in the coming year, in preparation for later clinical trials.

Social and Behavioral Sciences Branch

1. Study of early environmental influences on child development has demonstrated effects of father absence during infancy. The amount of father interaction with male infants was positively related to general developmental status as measured by the Bayley Mental Development Index. It was also related to the child's social responsiveness, efforts to elicit feedback from the environment, and exploratory behavior. There were no measurable significant effects of father interaction on female infants.

2. Comparison of infant caretaking patterns of mothers and surrogates showed that on the whole mothers provided higher levels of stimulation. They were more demonstrative in expressing positive affect, spent more time in non-caretaking activities with their infants, played with their infants more, and provided them with a greater variety of play objects. When assessed for general developmental status or on specific aspects of development, however, infants in surrogate care did not differ from infants cared for by their own mothers. With the growing number of working mothers, this type of study gains added importance.

Reproduction Research Branch

1. Sub-units of HCG have been prepared and characterized chemically, immunologically, and biologically. Specific antisera have been prepared for each sub-unit. The sub-units were shown to be free of biologic activity. Free HCG α was found in plasma throughout pregnancy, a finding which accounts for previously noted discrepancies between biologic and immunologic measurements of HCG. The individual sub-units have been detected and levels measured in tissue, serum, and urine of patients with HCG-secreting tumors, providing evidence of cloning of cancer cells in human metastatic lesions.

2. Clinical studies of testicular regulatory mechanisms are continuing in infertile males. Serum FSH levels are high when germ cells are absent, but it has been difficult to decide whether the germ cell or Sertoli cell is responsible for regulation of FSH levels. Study of three men with a normal sperm count but only dead sperm exhibited high FSH levels, providing strong evidence that the Sertoli cell regulates FSH secretion. Additional evidence that this is the case was provided by other studies.

3. Prolactin has been isolated from amniotic fluid, and a practical method has been devised for preparation of the hormone.

4. For the first time techniques have been perfected permitting isolation and numerical quantitation of lysosomes from human placentas obtained at various times during pregnancy.

Behavioral Biology Branch

Section on Brain and Behavior

1. Several studies of primate vocalization have been completed. Sound spectrographic analysis of the vocalization of one pair of squirrel monkeys

indicates that each animal has a distinctive fine structure in its vocal productions. The complex synaptic connections responsible for the relatively specific responses to such stimuli as species-specific vocalizations thus represent securely organized sensory channels, rather than a diffuse system related to attention and arousal.

Section on Neurobiology

1. Biochemical analytical methods developed in the Branch have been used to study the chemical secretory patterns of individual neurons of the molluscan nervous system. Present studies are focusing on analysis of the increased membrane potential as the mechanism responsible for control of neurosecretory protein synthesis.
2. Scientists have succeeded in dissociating and establishing in tissue culture, mouse cerebellar neurons, and demonstrated that these neurons develop complex patterns of spontaneous and synaptically mediated electrical activity.

Laboratory of Molecular Genetics

1. Studies directed toward understanding the regulated expression of the globin gene have indicated that the reverse transcript of purified globin message can be used as a highly specific and sensitive probe for quantitating specific globin genes and mRNA. Application of this technique to studying the process of differentiation in tissue culture has provided evidence that transcription of the globin genes represents a rate limiting step in differentiation, and that globin message rises from an undetectable amount to 3,000-6,000 molecules per cell in the fully differentiated state.
2. Continued studies of the integration and excision of phage λ into bacteria have shown that host chromosomal material can be incorporated into the phage genome in a non-random fashion. This observation provides the initial evidence that phage λ can be used as a generalized transducing probe. These results provide a clearer picture of the process of viral insertion and excision and the elements needed for packaging the viral chromosome into immature virus particles.

Laboratory of Biomedical Sciences

Section on Developmental Enzymology

1. Optimal assay conditions were ascertained and specific activities determined for a large number of placental enzymes, and zymogram techniques devised for detecting isoenzyme variation among them. Qualitative and quantitative comparisons were made of these enzyme activities in normal term placenta and malignant trophoblast in tissue culture. The tissue in culture was found to be virtually devoid of heat stable (placental) alkaline phosphatase, and had an unusual lactate dehydrogenase enzyme, differing strikingly from that of the term placenta.

Section on Intermediary Metabolism

1. Investigations in this laboratory on the biochemistry of brain transfer RNA are designed to identify aspects that are unique to brain and appear to be related to brain function. Brain mRNA has been purified and described in terms of size, poly A sequences, and ability to be translated by a protein synthesizing system. Differences have been found between the messenger fraction from the brains of immature and of adult animals with respect to the length of the poly A sequences.

Section on Physiological Controls

1. All of the enzymes known to participate in glycogen cycle phosphorylations have been partially or completely purified.
2. A new method for measuring tryptophan hydroxylase was developed and used to show that this enzyme is rate-limiting in serotonin synthesis in the pineal gland and is controlled by tryptophan levels in the circulation.

Pregnancy Research Branch

1. Scientists in this Branch have continued to evaluate the pregnant baboon as a primate model for studying the mechanisms regulating steroid synthesis and metabolism during pregnancy. Numerous studies indicate that the approximation of the human condition is quite close, and that the model will be a useful one.
2. Comparison has been made of the relative usefulness of maternal serum and urine estrogens for assessment of feto-placental status in high risk human pregnancy. The study showed that serial 24-hour urinary estrogen determinations provide the most reliable data to monitor estrogen metabolism as a criterion of fetal well-being.
3. A new accurate and sensitive radioimmunoassay for monkey glucagon has been applied to the primate model diabetic pregnancy. Glucagon levels in the mother and fetus are not affected by fasting or induced hyperglycemia in either the normal or the streptozotocin-induced glucose intolerant animal. Glucagon administered intravenously in physiologic amounts does not cross the monkey placenta in either direction.

Gerontology Research Center

Laboratory of Behavioral Sciences

1. Studies of logical reasoning in elderly men in the Baltimore Longitudinal Study of Aging have shown that men over age 60 make more errors than younger men. Analysis of the errors indicates that older men are more likely to make premature attempts to synthesize information than are younger men.
2. Work on operant conditioning of autonomic functions has continued in this laboratory. A patient with mild essential hypertension has been able to maintain her systolic blood pressure 30 mg below initial baseline levels after training.

Clinical Physiology Branch

1. The Baltimore Longitudinal Study of Aging continues to be a major research resource. Particular efforts this year have been directed to development of a statistical method for predicting the requisite duration of a longitudinal study, the required frequency of data acquisition, and the number of subjects needed, in order to define the rates of aging changes in selected functions with specified accuracy.
2. The incidence of cutaneous malignancies increases with age. Studies of chronic actinic damage as a precursor of these malignancies have assumed that the damaging radiation spectrum is confined to the 290-320 nm wavelengths, and that longer wavelength UV radiation (320-400 nm) protects against sun damage to skin by inducing tanning. Study in this Branch of the effect of age on sensitivity to UV light of different wavelengths has shown that, on the contrary, the longer UV wavelengths have an augmentative effect rather than a protective one. These findings indicate that present preparations to prevent sun damage should be modified so that they protect against a broader spectrum of UV light.
3. A new labeled polymeric substrate has been developed to assay renin and to aid in study of this kidney enzyme involved in the regulation of blood pressure.

Laboratory of Cellular and Comparative Physiology

1. Observing that associated with aging and immunosenescence there is an increase in incidence of immunodeficiency diseases including autoimmunity, cancer, and infection, researchers in this Branch have been intensively studying changes in the immune system with aging.
2. Study of mice with an exceptionally long life span showed an age-related decline in cell-mediated immune activity and in resistance to allogeneic tumor cells.
3. A new research program in biomembrane physiology, emphasizing age effects on hormone binding, has yielded evidence that membranes from adipose tissue, skeletal muscle, brain, and prostate gland of aging rats have a progressive decline in steroid hormone binding.

Laboratory of Molecular Aging

1. Active transport of glucose in the kidney has been studied in isolated renal brush border membranes and found to consist of at least two processes, a high-affinity binding system and a carrier-mediated transport system. Kinetic studies of the binding have characterized time course, reversibility, saturability, alterability by phlorizin, and the influence of Na^+ , Ca^{2+} , and Mg^{2+} ions.
2. Relationship of the mechanism of infection to age of the cell has been studied extensively in tissue culture. Viral replication was unaffected by age of the cell, until just before cell death, when there was a decrease.

Induction of interferon, the viral defense mechanism of the cell, was possible throughout the life span, but older cells required a larger stimulus to produce a given amount of interferon, indicating that the sensitivity and/or efficiency of the process declines with age.

Collaborative Guest Scientist Program

1. The hypothesis that protein synthesis is impaired as age advances was examined by studying incorporation of ^{14}C -leucine or ^{14}C -labeled amino acids by liver microsomes from adult (12 month) and aged (20-31 month) female rats. Amino acid incorporation into protein was decreased by 12-43% in the cell-free system from senescent rats.

2. Effects of age on isolated cardiac muscle function were studied in rats. Contractility of the muscle was increased by norepinephrine and paired pacing, and decreased by hypoxia. No age differences were found in response to hypoxia or paired pacing, indicating that there are no major alterations in anaerobic metabolism with aging. By contrast, at higher dose levels of norepinephrine the inotropic response was less in the old than in the young, a finding that is probably reflected in decreased "cardiac reserve", the ability to respond to increased stress, in the older animals.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Office of Public Information (OPI)

Organization and Personnel

The Office of Public Information has two parts: a public information section and the Scientific Publications Section. The latter, under Acting Chief George Gaines, assisted in the preparation of 29 publications for the scientific community. The Section provided editing, administrative, and advisory services to all Institute staff involved in publishing scientific publications pertaining to Institute conferences and interests.

Public Information Section. A completely new staff of information specialists recruited early in the year provided a mixture of public information and administrative functions to the Institute. To serve the needs of the scientific program areas, the information specialists were assigned to one or more programs. Overall Institute projects were shared. A large proportion of activity centered on office organization and management, to better meet the demands of the many publics and programs which the office serves.

Major tasks this year were: establishing working relationships with the program staff; formally planning public information activities and services; training several new staff; and working out a new organization which could respond to public information requirements.

Gerontology Research Center (GRC). The public information program of the GRC is carried out by a Public Information Officer assigned to that facility in Baltimore. GRC internal communication was further improved this year with the advent of a Center newsletter in January 1973. This newsletter, developed by the Public Information Officer of GRC after consultation with employees and administrative staff, is proving an effective vehicle for informing staff about official GRC operations and general activities of interest to everyone working in the facility.

Media inquiries rose this year to 128, from 118 reported last year. Stories dealing with GRC or its programs appeared in the New York Times, Saturday Review/The Sciences, the National Observer, Newsweek, the Washington Star-News, the Baltimore Sun, and Natural History Magazine. Additional contacts were made with Newsday, the New York Daily News, and Wall Street Journal, Time, Fortune, and two German magazines, Der Spiegel and Stern. Medical World News and Chemical and Engineering News also carried articles on the Center.

Electronic media contacting GRC included CBS-TV, BBC-IV, WBAL-TV and WCAO-Radio (Baltimore), the Voice of America, Radio Free Europe, World Health Organization Radio, Italian RAI-TV, and Ontario Educational TV.

A total of 157 people participated in 42 tours of the facility, including visitors from Australia, Denmark, Germany, and Israel.

The GRC Public Information Officer set up and operated a working pressroom

for the annual meeting of the Gerontological Society at that group's request.

Communications Plan

In fiscal year 1973, DHEW information offices for the first time operated within a Communications Plan, prepared by each office at the beginning of the fiscal year. The NICHD Communications Plan, hailed as the best in the Department by DHEW staff, was based on a philosophy which anticipates and meets the needs of the public and scientific community for health-related information.

Although a large portion of information office activity continues to be in response to daily events and exigencies, the Communications Plan provided a framework for achieving a well-rounded program of public information on topics of NICHD expertise. The philosophy of a more active communications program which attempts to reach larger audiences with health information was adopted by the NIH Director as a goal for all NIH information activities.

Training and Personnel Development

Development and upgrading of professional skills was one goal of the OPI during the year. The opportunity to enroll in formal classes was taken by 85% of OPI staff, who completed courses in science, technical skills, public relations, and management.

In conjunction with serving on the NIH Information Training Committee, OPI staff trained two college students as summer interns, both of whom hope to continue work in medical communications. Staff also participated in the operation of a graduate degree program in public relations through American University for NIH and HSMHA employees.

An HEW management intern who requested training in public information has been assigned to the OPI for six months and will assist in the mongolism campaign.

Scientific Publications Edited

The following were produced or in process as of June 30, 1973:

1. Nutrition, Growth, and Development of North American Indian Children (book-length report of a conference and announcement flyer)
2. Nutrition, Development and Social Behavior (book-length report of a conference and announcement flyer)
3. Menopause and Aging (book-length report of a conference)
4. Behavioral Sciences and Medical Education (book-length report of a conference)
5. Respiratory Gas Exchange and Blood Flow in the Placenta (book-length report of a conference)

6. Sudden Infant Death Syndrome: Selected Annotated Bibliography 1960-1971 (booklet)
7. Research Directions Towards the Reduction of Injury (book-length report of a conference)
8. Seven workshops on the sudden infant death syndrome (booklets)

Public Information Products

The following were produced or nearing completion as of June 30, 1973:

1. "Facts About SIDS" (leaflet, completely revised)
2. "NICHD: 10 Years" (publication marking NICHD's 10th Anniversary)
3. "The First Three Days of Life" (leaflet)
4. "Facts About SIDS for Rescuers" (leaflet)
5. Slide-tape presentation on SIDS for lay public
6. "Prematurity and Low Birth Weight" (leaflet)
7. Special Report on SIDS, FY 73
8. "Relationships Between Speech and Reading" (pamphlet)
9. Campaign to prevent mongolism, including pamphlets, radio/TV, press releases, posters
10. Feature portfolio on Population Research Centers for NIH Feature Service
11. Booklet of highlights of research FY 73

Contributions to NIH Products

1. NIH Annual Report
2. NIH public brochure
3. NIH Scientific Directory-Bibliography (coordinated NICHD report)
4. NIH Almanac
5. NIH Record stories
6. NIH Guide to Grant and Award Programs
7. NIH News and Feature Service material

8. NIH Radio spots
9. NIH Publications List
10. DHEW Catalog of Publications

Internal Reports

The call for reports to be submitted to higher echelons of DHEW or the Executive Branch comes frequently. Often it is the Office of Public Information which prepares the initial response, many times within deadlines requiring the setting aside of other OPI work. A small sampling of the reports called for this year:

1. NICHD contribution to the government's bicentennial plans
2. Persons and organizations worthy of citation for Child Health Day
3. An index of all available information and assessment of public inquiries' handling by NICHD
4. A "Modular Orientation Manual" summarizing in some detail for the new HEW Secretary the programs and accomplishments of the NICHD
5. Monthly reports to the Associate Director for Communications, NIH
6. Communications Plan

Congressional Reporting

The OPI is called upon to prepare the appropriations testimony for the Director, NICHD, as well as testimony on other topics, such as an Institute on Aging. The annual updating of the Five-Year Plan for Population Research is also prepared with the assistance of the OPI. The months of October through January each year are focused on preparation of appropriations material in the form of Special Reports (five this year) and Highlights of Research Progress (22 prepared). Congressional reporting tests the knowledge and coordination skills of the OPI perhaps more than any other activity, in that tight deadlines, rigid reporting guidelines, and program staff cooperation all must be addressed or enlisted. This activity is an opportunity for the Institute each year to take stock of what it has accomplished and to explain its programs to the Congress, and ultimately, its constituents. OPI answered 96 Congressional inquiries in FY 73.

Media Activities

Contacts with the media originate either from NICHD OPI or from media representatives. Examples of both types were accomplished this year with scientific and lay media. For example, the OPI wrote and distributed several press releases and organized a science writers' seminar on reproductive biology topics, writing a fact sheet for each topic and arranging the meeting. Some press releases required coordination with other government agencies. Calls

initiated by media people showed a continued high level of interest in Institute programs as well as a reliance by the press on Institute expertise in areas related to NICHD programs. Questions on population, aging, child health, and on the sudden infant death syndrome were of special interest to large numbers of media representatives. A total of 250 media requests were responded to.

Public Inquiries

Letters and phone calls from the public are referred to the OPI from a variety of sources, including the President and Vice President, the Secretary of DHEW, and others. In addition to the continued high interest in population and the sudden infant death syndrome, this year, the public asked for a great deal of information on the menopause and on learning disabilities (a foggy area). Questions on normal development (sexual, fetal, etc.) are common, and requests for speakers on various subjects were acted upon.

Many letters are sent to OPI in error--on the part of the writer, who thinks we handle everything from child advocacy to child labor, and from other agencies. OPI receives many requests for health services and treatment. To OPI this indicates a need for making NICHD better known to the public and other health organizations.

Whatever the subject, the OPI considers the matter of responding to the public a serious responsibility. Generally each request is answered with an individual reply at the reading level in which it was written. The OPI feels that every citizen should be able to write to a government agency and get a response which is straightforward and understandable.

An aid to responding to the public is the NIH Information Index for use in all B/I/Ds of NIH as an assist in the routing of inquiries to the most appropriate and responsive sources. The OPI has compiled a list of topics which are, properly, to be referred to the NICHD; a list of topics frequently misdirected to the NICHD; and a list of topics in which we share an interest with other Institutes or agencies. The Index is to be periodically revised and updated. It has prompted the OPI to begin analyzing its information holdings and those of program staff and to build a source file for answering public inquiries.

In Fy 73 the OPI answered 5,550 public inquiries and distributed 73,000 publications on request from the public.

Other Functions and Accomplishments

In addition to activities which can be classified within the foregoing sections, the OPI was involved in many activities of note. Among these:

- coordinating public information involving other agencies, such as the the President's Committee on Mental Retardation, the Census Bureau, and the Center for Disease Control
- managing the clearance of all Institute manuscripts

- using one person virtually full-time to distribute Institute publications requested by the public and scientific community
- attending scientific conferences sponsored by or heavily attended by NICHD scientific staff, in order to help with press inquiries and aid in technical aspects of setting up the meetings. These conferences also provide background needed by information specialists in their work of developing information for various uses
- providing publicity for an NIH Lecture by Dr. Jane Goodall
- serving the photographic and graphics needs of program staff
- the Public Information Officer also chaired and organized the Women's Task Force as part of the Equal Employment Opportunity program of the NICHD and served on the Institute's EEO Committee and its Executive Committee
- the OPI gave a seminar on public information for staff of the Center for Population Research, NICHD.

Several activities are worthy of further mention:

Fetal research. As a result of NACHHD Council discussions on human experimentation in March, press interest in the possibility of research on aborted human fetuses was high. The OPI played a significant role in preparing information for the press, the public, and the NIH about the events and policies germane to the issue.

Preventive immunization campaign. Planning for a public information campaign to accompany a preventive immunization program against spinal meningitis was carried out near the end of the fiscal year. The campaign will materialize in fiscal year 1974 when the immunizations begin. The information campaign is essential to the acceptance and understanding of the vaccine trials by the residents and physicians of the trial area.

Mongolism campaign. Because mongolism (Down's Syndrome) is one of the most common causes of mental retardation, the OPI undertook a national publicity campaign to highlight the availability and importance of family planning in reducing the incidence of Down's Syndrome. The campaign will continue into FY 74.

The Institute program is designed to inform women over 35 about the relationship between childbearing age and genetic disorders such as mongolism. The OPI is preparing several pamphlets, "Having a Healthy Baby After Age 35," "Amniocentesis and Other Antenatal Diagnostic Tools," and "Mongolism," each aimed at a different audience (the general public, physicians, and low reading level). In addition, the OPI will plan and distribute posters which complement the pamphlet.

The problem of distribution has proved to be extremely complex. However, cooperation is being enlisted from other HEW programs, the PCMR, American

Nurses Association, AMA, Journal of American Nursing, and other groups.

In addition, the campaign is planning a newsworthy kickoff in October at a Down's Syndrome Conference. To coincide with the kickoff, the OPI will prepare television, radio and newspaper features on the prevention of mongolism.



NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Office of Program Planning and Evaluation

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the information needed for the planning and the formulation of the Institute's major goals and objectives and the means for appraising progress toward these objectives.

In performing these functions, the Office: (1) sponsors and conducts studies on the nature of the emerging problems and needs within the broad field of child health and human development; (2) stimulates and provides guidance for the analysis of the status, character, and extent of scientific activity in problem areas and the resources needed for further Institute efforts; (3) coordinates activities leading to the selection of Institute objectives and the development of program plans; (4) prepares and maintains a comprehensive multi-year program plan utilizing operational planning systems and other analytical techniques; (5) sponsors and conducts reviews of program activities to assess progress toward objectives and to evaluate accomplishments, and (6) maintains an awareness of ongoing Congressional activities, keeps staff informed of legislative developments, and coordinates matters of mutual concern with other interested Governmental and non-Governmental organizations.

Dr. S. W. Greenhouse is Acting Associate Director of the Office of Program Planning and Evaluation (OPPE) of the NICHD. His staff includes an Assistant Director for Clinical Programs, Facilities and Resources; an Assistant Director for the Behavioral Sciences; an Assistant Director for the Biological Sciences; a Program Analysis Officer, a Program Analyst; a Legislative Reference Assistant; and supporting secretarial staff.

In Fiscal Year 1973 the OPPE prepared several reports on aspects of the research and training programs of the Institute in response to requests from Congressional Committees and Departmental offices. Similar material was developed for interagency coordination purposes. The Institute Research Plans (popularly called the Blueprints), requested in 1972 by the Senate Committee on Appropriations, were prepared by the OPPE. There continues to be much interest in how Institute research and training programs pertaining to early child development interface with other Federal efforts in this area. Several pieces of legislation concerned with Federal support of research and services specifically aimed at improving the health status and learning capabilities of children have been introduced in the Congress. All of these bills have had some impact on NICHD research and program activities in 1972. The Office keeps all personnel informed about this legislation. Staff of OPPE, in cooperation with the Budget Officer and Executive Officer and Program Personnel of the Institute, developed the Forward Plan for FY 1975-79 and the Operational Plan for FY 1974.

The death of infants suddenly from unknown cause accounts for about one tenth of the total infant mortality in the United States. OPPE staff have initiated cooperative efforts to expand research concerned with the causes

and prevention of Sudden Infant Death Syndrome (SIDS). These efforts include staff of the Epidemiology and Biometry Branches of the Office of Epidemiology and Biometry, and of the Perinatal Biology and Infant Mortality Branch (PBIM) of Extramural Programs. This team approach to an important pediatric problem includes development of collaborative research projects and the definition of research goals and needs as well as the coordination of Institute research efforts in this area. Staff of the Biological Sciences activity of OPPE served as Project Officer for a contract to survey "The Management of Sudden Infant Death Syndrome in the United States." OPPE staff continue to assist in the development of plans for an expanded SIDS research effort.

In the past century the number of medications administered to pregnant women and to children has multiplied enormously. It is possible that at present the fetus is potentially at greater risk from the well-intentioned use of pharmaceuticals than from the stress of labor and delivery. Staff of OPPE, in collaboration with staff of Extramural Programs, is cooperating to develop a developmental pharmacology research program that is concerned with effects of medications administered during pregnancy and childbirth, and during childbirth.

The facilities and resources activity of the OPPE has included participation in the development of plans for an intramural clinical research unit in reproductive research and perinatal biology to be activated in the Clinical Center, NIH. This program will be housed in addition of 15,492 net square feet, made available through construction of an 8th, 9th, and 10th floor in wing G. Target date for occupancy is July 1, 1974. This activity has also been included in planning for remodeling of the existing Clinical Center Out Patient Department and for a future larger ambulatory care facility, as well as the design of a small temporary laboratory and animal facility near building 14G and minor rearrangement of 13N in the Clinical Center to adapt it to new program needs.

Interest of OPPE staff continues in Research in Child Development and the Man-Made Environment. Staff of OPPE continue to exploit opportunities to explore new concepts in this area with a view to defining impact on the direction and progress of Institute programs.

Staff of OPPE participates in coordination efforts, and maintains liaison with agencies whose missions are complementary to or supportive of the Institute's objectives. During the current year these included the Inter-agency Panel on Early Childhood Research and Development; the Standing Committee on Community Coordinated Child Care (4-C Standing Committee); the Interagency Task Force on Comprehensive Services for School Age Parents (membership on the Steering Committee, the Committee on Research Strategies, and the Committee on Public Awareness); The Interagency Panel on Adolescent Research and Development; the DHEW Interagency Committee on Planning for Children; the DHEW Interdepartmental Committee for Planning and Implementation of PL 91-695, Lead-Based Paint Poisoning Prevention Act, and its Subcommittee on Research and Statistics Issues (Chairman), and the Interagency Panel on Environmental Mutagenesis. An OPPE staff member also served as NICHD representative at the Organizational Committee Meetings for the Fogarty Center

Conferences of Preventive Medicine, as Chairman of the NICHD Safety Committee, as Chairman of the NICHD Scientific Publications Steering Committee, and as NICHD liaison representative to the Council on Child Health of the American Academy of Pediatrics and to its Committee on Accident Prevention.

New activities this year are service of an OPPE staff person as a member of the Technical Advisory Committee to the FDA on Poison Prevention Packaging and as a member of the DHEW Interagency Workgroup on Childhood Accidents. The Acting Associate Director continues to represent the Institute at the NIH Advisory Committee for Program Planning and Evaluation.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Office of the Associate Director
for Epidemiology and Biometry

The Epidemiology and Biometry Branches continued their research and consulting activities during the past fiscal year, the first full year of operation since this Office was organized. Statistical consulting efforts and other service activities increased during the year, with scientists in the intramural program and with extramural program directors. In the first half of the fiscal year, the Epidemiology Branch initiated computer analyses of an increased number of research projects, the data for which had been gathered in previous years. However, the corresponding computer costs increased to such an extent that high priority projects only could be continued for the remainder of the year.

Despite the increase in research and consultation activities, the professional staff of the two branches has not changed. There were 11 professionals, consisting of 3 epidemiologists, 5 statisticians and 3 computer specialists, and 5 supporting staff. In addition, the Epidemiology Branch supports a group of 5 trainees in the ACCESS, STRIDE and Stay-in-School programs. It is hoped that both branches will in the near future be able to receive an increase in positions so that there is an adequate staff to carry out the necessary research projects and to meet the demands for consultation and biometric services.

The Epidemiology Branch continues to concentrate on problems of prematurity and infant mortality. Particular emphasis is being put on investigating the effect of improved nutritional intake during pregnancy in indigent groups on birth weight, perinatal and neonatal deaths. Preliminary evidence indicates that some of the lowest rates of low birth weight babies among blacks ever attained has been achieved by one prenatal clinic in the San Francisco Bay area. In this facility, practice is to educate and persuade pregnant women to eat well, without concern for weight control, diuretics nor low-calorie-low salt diets. The complete data from this clinic and from two other control clinics in the County are currently being analysed. The Branch is also studying the relations between prematurity (low gestational age and low birth weight) and infant mortality in terms of the joint variation of both in the United States.

Other subjects receiving major attention by epidemiologists in the Branch are Sudden Infant Death Syndrome, Respiratory Distress Syndrome and the epidemiology of a selected number of congenital malformations such as anencephaly and spina bifida. Smaller studies, reflecting interests of individual investigators, have been carried out or are in process in Mental Retardation, in Aging and regarding the effects of induced abortion on subsequent offspring.

During the past fiscal year, the experimental statistics function of the Biometry Branch has probably reached an effective level of consultation given the limited number of statisticians. Scientists representing almost all the intramural laboratories have consulted with statistical staff, including the Branch Chief and the Associate Director, on the design and analysis of experiments. A mathematical statistician spends almost all his time on problems arising in the Gerontology Research Center in Baltimore. In addition, members of the Branch give advice to extramural scientists on statistical issues arising in the design of studies and the analysis of data in contract research.

The Biometry Branch has continued to serve as the statistical center of the collaborative study to evaluate the untoward effects of the amniocentesis procedure. This project is now in its second year.

The Biometry Branch has continued its high level research program in statistical methodology. During the past year, there were three areas receiving special emphasis: (1) design and analytic techniques in retrospective studies; (2) data analytic techniques applicable in longitudinal studies, and (3) the application of highly sophisticated time series techniques to analyse biological characteristics varying over time and the development of models that characterize their fundamental rhythmicities. The Branch has published research papers in all three areas during the past year.

The Biometry Branch also engages in substantive research, i.e., investigations of a statistical nature designed to gain fundamental knowledge in a given field. Thus far, this research has been in the population area with special attention to the side effects of the oral contraceptives. Biostatisticians in the Branch have continued their studies in the effect of the pill on breast cancer, cervical cancer, stroke and the side effects of different methods of abortion. The stroke study resulted in a major publication with the Chief of the Branch being the coauthor.

Members of the Branch have been senior authors or coauthors in ten papers published during the year.

The Associate Director continued to serve as the Acting Associate Director of the Office of Program Planning and Evaluation of the Institute. He continued as a consultant to the Mental Health Unit of the WHO. In addition, the Associate Director serves on the following advisory and review committees:

FDA, Chairman, Biometry and Epidemiology Advisory Committee
NCI, Biometry and Epidemiology Contract Review Committee
NINDS, Epidemiology and Statistics Advisory Committee

The Associate Director delivered a one hour invited address on Multivariate Analysis in Health Research at the Annual Meeting of the American Public Health Association. He was a coauthor with the Chief of the Biometry Branch on two methodology papers published during the year.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Epidemiology Branch

The Branch performs epidemiologic studies in areas related to the mission of the Institute and provides epidemiologic consultation to scientists in both the intramural and extramural programs of the Institute. At the present time Branch research activities focus on two segments of Institute interest: 1) the etiology and prevention of prematurity, and 2) the etiology and prevention of infant mortality. Some Branch projects aim at elucidating the factors which determine birthweight and length of gestation. Other projects are attempts to describe infant mortality trends and identify factors affecting the trends. Still other projects are concerned with the etiology of specific causes of infant mortality: 1) Respiratory Distress Syndrome, the most important single cause of death under 30 days of age, and 2) Sudden Infant Death Syndrome, the most important cause of postneonatal mortality. With the arrival of a new staff epidemiologist in July, 1972, the Branch began a modest program of epidemiologic research relevant to aging in humans.

Branch research activities received a significant boost by the addition of three persons to the staff in FY 73. On August 7, 1972, Dr. Philip S. Spiers came to us from the Department of Epidemiology at the School of Public Health, University of North Carolina. Also, between September 18, 1972 and May 1, 1973, Dr. Heinz Berendes served on the staff. Mr. Ernest Harley, a computer specialist, joined the Branch staff on December 24, 1972.

Non-Project Activities

During FY 73 a large portion of staff time was spent in "non-project" activities.

Branch Chief:

1. Served on the NICHD Scientific Publications Committee.
2. Continued his teaching activities in the Department of Pediatrics at Georgetown University Hospital.
3. Provided extensive consultation to the Perinatal Biology and Infant Mortality Branch (PBIMB) staff in the development of contract proposals to study 1) the epidemiology of Sudden Infant Death Syndrome (SIDS), 2) developmental sleep physiology in infants of high and low risk for SIDS, and 3) Autonomic Regulation During Sleep: Developmental Studies.
4. Provided consultation to staff of the Growth and Development Branch in the administration and execution of a contract to study the interactions of nutrition, infection and growth rate in the children of a Guatemalan village.

5. Provided consultation to staff of the Growth and Development Branch in the development of contracts to study the effects of gestational nutrition on the birthweight of babies and infant mortality.
6. Traveled again to Poland to develop 1) two PL 480 contract proposals to study the effect of induced abortion on the birthweight and gestational age of subsequent offspring, and 2) a PL 480 contract proposal to study the effect of "Post Partum Neurohypophyseal Syndrome" on the birthweight and gestational age of subsequent offspring.

Dr. Lundin:

1. Continued his Staff appointment of Lecturer in the Department of Epidemiology at the Johns Hopkins School of Hygiene and Public Health.
2. Acted as consultant to the University of Louisville study of uterine cancer and cervical cytologic screening.
3. Prepared manuscripts for three papers on cancer among uranium workers.
4. Served as a member of the Committee on the Environment of the Epidemiology Section, American Public Health Association.

Dr. Berendes:

1. Prepared two manuscripts on the effects of maternal disorders in pregnancy on the developing fetus.
2. Attended a symposium on Nutrition and Fetal Development in November, 1972, sponsored by the National Foundation.
3. Continued to serve on the advisory board of the Collaborative Study of Children treated for Phenylketonuria and attended meeting of study group in February, 1973.
4. In collaboration with Dr. Spiers, a design has been prepared to study retrospectively the association between potato consumption and anencephaly and spina bifida. Negotiations are under way to obtain data from the New York State Health Department in pursuit of this study.
5. Planned various analyses based on data collected by the Collaborative Perinatal Project of the NINDS which are of importance to the research program of the NICHD.

Mr. White:

1. Coordinated the data processing in the Branch. Supervised all data processing people in the Branch.
2. Provided consultation to members of Biometry Branch and other areas in the Institute.

3. Served as Co-Chairman of the NICHD EEO Advisory Committee.
4. Tested the Conversational Computer Statistical System.
5. Was responsible for all administrative activities related to data processing in the Branch. Evaluated Army Report on Data Processing at NIH.
6. Continued teaching statistics in the Upward Mobility College at NIH.
7. Organized Automatic Data Processing Plan for the Branch.
8. Continued research in tests for spread.

Bibliography:

- Archer, V.E., Wagoner, J.K., and Lundin, F.E., Jr.: Cancer mortality among uranium mill workers. J. Occupational Med. 15: 11-14, Jan. 1973.
- Archer, V.E., Wagoner, J.K., and Lundin, F.E., Jr.: Uranium mining and cigarette smoking effects on man. J. Occupational Med. 15: 204-211, Mar. 1973.
- Spiers, P.S., and Watthana-Kasetr, S.: Geographic differences in mortality rates and aging rates - a possible relationship? Accepted for publication in Social Gerontology.
- Spiers, P.S.: Father's age and infant mortality. Social Biology 19: 275-284, 1972.
- Spiers, P.S.: Letter to the Editor. Spina bifida, anencephaly, and potato blight. Lancet i: 426-427, 1973.

Talks:

- Lundin, F.E., Jr., Archer, V.E., and Wagoner, J.K.: Lung Cancer Mortality of Uranium Miners in Relation to Cigarette Smoking, Age, and Radiation Exposure, October, 1960 through September, 1968. Special Session on "Epidemiological Contributions to Environmental Health Policy," Annual Meeting of the American Public Health Association, November 14, 1972, Atlantic City, New Jersey.
- Lundin, F.E., Jr.: Lung Cancer Mortality of Uranium Miners - Cigarette Smoking, Age, and Radiation Exposure. Department of Epidemiology Seminar, The Johns Hopkins University School of Hygiene and Public Health, December 1, 1972, Baltimore, Maryland.
- White, N.B., Jr.: Test for Differences in Spread. Statistics Seminar, University of Maryland, February, 1973.

White, N.B., Jr.: Two papers on the K-Sample problem,
George Washington University, April, 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Epidemiology Branch
Contract and Collaborative Research

Contract Title: Relationship of Heterozygosity in Phenylketonuria to Intelligence Quotient

Contractor: University of Southern California in Los Angeles, California
Money Allocated: \$30,830.00

Objectives: To determine whether heterozygosity to Phenylketonuria is associated with a decrease in intelligence.

Summary: Carrier status to Phenylketonuria among siblings of Phenylketonurics will be determined by phenylalanine loading test. The intelligence scores obtained by standard intelligence tests will be compared for PKU heterozygotes and their genotypically normal siblings. This evaluation is planned to include about 100 siblings. It is hypothesized that the heterozygous fetus in utero because of his partial deficiency in tyrosine synthesis may be susceptible to damage from maternal dietary protein deficiency. In this situation neither the mother nor the fetus is able to compensate for deficiencies in maternal tyrosine intake by hydroxylation of phenylalanine to tyrosine.

Significance to Biomedical Research and the Program of the Institute: The investigation relates to the Institute's vigorous efforts to pursue testable hypotheses in the area of "non-specific" mental retardation and compliments other studies on nutritional and metabolic factors in mental retardation currently being explored by the Epidemiology Branch.

Proposed Course: Contract proposal is in the final review process. If approved, data collection will commence before the end of FY 73. It is anticipated that the study will take about one year.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Epidemiology Branch
Contract and Collaborative Research

Contract Title: Conversion of Conversational Computer Statistical System
(CCSS) Package to the PDP-10 and IBM-370 Computers at NIH.

Contractor: University of Washington, Seattle, Washington
Money Allocated: \$15,029

Objectives: The contractor will implement the CCSS package for use on the
IBM-370 and PDP-10 computers here at NIH.

Progress: The contractors are actively pursuing the objectives of the
contract and the anticipated completion date is June 15, 1973.

Significance to Biomedical Research and Program of the Institute:
Implementation of CCSS at NIH will enable investigators in the Branch and
Institute to do extensive analysis without the need for specialized
programming for each new data set. The ease of this data display will
simplify the investigation of various hypotheses of interest to the Branch.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Epidemiology Branch
Contract and Collaborative Research

Contract Title: The Epidemiology of Birth Weight with Particular Reference to the Effect of Induced Abortion on Subsequent Offspring

Contractor: Mother and Child Institute, Warsaw, Poland
Money Allocated: 1,450,785 Zlotys (first year)

Objectives: To determine whether or not therapeutic abortion affects the birthweights of subsequent offspring.

Summary: Controlling for a number of factors already known to be related to low birthweight, this study will assess the relationship between previous abortion and the occurrence of low birthweight in subsequent offspring. Information will be gathered on all pregnant women attending prenatal clinics, both during the actual pregnancy and during the first 3 months of the infant's life. Some 18,000 women will form the basis for the study.

Significance to Biomedical Research and Program of the Institute: Since birthweight is such an overriding determining risk factor for infant mortality, and since therapeutic abortion is the predominant method of birth control in many countries the results may affect future policies.

Proposed Course: During the first 9 months a pilot phase of the study will be performed. The design should be compatible with that already in use by the WHO which is examining the same question in Yugoslavia and Singapore. Continuation of this project will be subject to the satisfactory completion of the pilot phase.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Epidemiology Branch
Contract and Collaborative Research

Contract Title: A Study of Monozygous and Dizygous Twins to Ascertain Possible Neurophysiologic Factors for the Sudden Infant Death Syndrome.

Contractor: Boston University
Money Allocated: \$33,764 (first year)

Objectives: Since there is good epidemiologic evidence that genetic factors are not important in SIDS this study will examine a number of neurophysiologic factors in newborn twin pairs at 3 days, 1 month, 2 months and 3 months, and examine these factors for similarity across zygosity. Factors which significantly differ between dizygous twins but not monozygous pairs will be ruled out as possible risk factors for SIDS. A further objective of the study will be examination of the relationship between polygraphic recordings and those obtained from a behavior bed monitor.

Significance to Biomedical Research and Program of the Institute: Study results should provide indication of neurophysiologic factors which are more likely than other factors to repay further intensive study in order to elucidate etiology of SIDS. If behavior bed recordings reliably register polygraphic activity, than this device may provide a relatively cheap screening procedure.

Proposed Course: This contract has only been funded for the first year; subsequent funding will depend upon review.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Epidemiology Branch
Contract and Collaborative Research

Contract Title: Development of Sleep and Cardiopulmonary Regulation within Sleep: Clinical Studies of a Functional Mechanism for Risk of Sudden Infant Death.

Contractor: University of Southern California
Money Allocated: \$99,050.00

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of Sudden Infant Death Syndrome (SIDS).

Summary: In four groups of infants at high and low risk for SIDS, Drs. Hodgman and Sterman propose to collect large amounts of physiological data which will be used to evaluate the early maturation of sleep "states" and cardiopulmonary function during the various "states". The four study groups will be selected as follows:

1. Subsequent siblings of SIDS cases.
2. "Near miss for SIDS" cases.
3. In utero autonomic instability cases.
4. Healthy, "normal" newborns.

All study subjects except the "near miss" cases will be monitored during labor and approximately once each month during the first 6 months of life. The subsequent-sibs and low-risk subjects will also be monitored for one 12-hour period during the last trimester of pregnancy. Each monitoring session will last 12 hours and about 12 physiologic variables will be continuously recorded throughout the session.

This contract will be oriented exclusively toward data collection.

Significance to Biomedical Research and the Program of the Institute: At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make real progress in elucidating the etiology of SIDS, the Institute must open up promising new avenues of research. It is hoped that this project will do so.

Proposed Course: Renewal for planned 3-year effort is anticipated.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Epidemiology Branch
Contract and Collaborative Research

Contract Title: Analysis of State Integration and Related Cardiopulmonary Functions in Infants at High and Low Risk for the Sudden Infant Death Syndrome.

Contractor: University of California at Los Angeles
Money Allocated: \$101,285.00

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of Sudden Infant Death Syndrome (SIDS).

Summary: In four groups of infants at high and low risk for SIDS, Drs. Hodgman and Sterman propose to collect large amounts of physiological data which will be used to evaluate the early maturation of sleep "states" and cardiopulmonary function during the various "states". The four study groups will be selected as follows:

1. Subsequent siblings of SIDS cases.
2. "Near miss for SIDS" cases.
3. In utero autonomic instability cases.
4. Healthy, "normal" newborns.

All study subjects except the "near miss" cases will be monitored during labor and approximately once each month during the first 6 months of life. The subsequent-sibs and low-risk subjects will also be monitored for one 12-hour period during the last trimester of pregnancy. Each monitoring session will last 12 hours and about 12 physiologic variables will be continuously recorded throughout the session.

This contract will be oriented exclusively toward data analysis.

Significance to Biomedical Research and the Program of the Institute: At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make real progress in elucidating the etiology of SIDS, the Institute must open up promising new avenues of research. It is hoped that this project will do so.

Proposed Course: Renewal for planned 3-year effort is anticipated.

Serial No. HD-EB-1
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of Maternal and Infant Coagulation Systems in Determining Morbidity among Newborns.

Previous Serial Number: EB-1(c)

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: David Abramson, M.D., N. Vildan Erkan, M.D.

Cooperating Units: 1. Department of Pediatrics
Georgetown University School of Medicine
Washington, D.C.
2. Columbia Hospital for Women
Washington, D.C.

Man Years:

Total: 3 weeks
Professional: 3 weeks
Other: None

Project Description:

Objectives:

1. Primary: To determine whether Disseminated Intravascular Coagulation (DIC) occurs in infants with Respiratory Distress Syndrome (RDS).

To determine the extent of DIC in premature infants with and without RDS.

To determine the extent of DIC in premature infants who succumb to and survive RDS.
2. Secondary: To establish norms for coagulation factors and fibrinolytic activity in premature infants by age in hours.

To determine the effects of pitocin assisted delivery on the coagulation system of the newborn.

To determine the effects of cesarean section on the coagulation system of the newborn.

Significance to Biomedical Research and Program of the Institute:

This study will provide a partial test of the theory which holds that at least one cause of the Respiratory Distress Syndrome is intrapartum disseminated intravascular coagulation. If the study supports the theory or even if it shows nothing more than an association between RDS and DIC, many new approaches to the therapy of RDS will be possible.

Progress and Proposed Course:

A complete data set is on magnetic tape and ready for analysis.

Honors and Awards: None

Publications: None

PHS-NIH

Serial No. HD-EB-2

1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Season of Birth and Incidence of Respiratory Distress Syndrome.

Previous Serial Number: EB-2(c)

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: Nathaniel B. White, Jr.

Cooperating Units: The Commission on Professional and Hospital Activities (CPHA) in Ann Arbor, Michigan.

Man Years:

Total: 7 weeks
Professional: 7 weeks
Other: none

Project Description

Objectives:

To perform a descriptive study of 1) RDS incidence by month of birth, and 2) risk of death from RDS and other causes by month of birth.

Summary:

The 2,056,467 birth records that entered the CPHA record system between January, 1966, and June, 1968, were arrayed in a giant multidimensional table which was forwarded to the Branch on a magnetic tape. Table dimensions were: 1) live or dead at discharge, 2) birthweight $\leq 2,500$ gms or $> 2,500$ gms, 3) RDS or not RDS, 4) male or female, 5) racial origin, 6) method of bill payment, 7) U.S. census region, and 8) month of birth. This table will provide a basis for determining any association between risk of RDS and risk of death before discharge from the nursery on the one hand and all of the classification variables on the other.

Significance to Biomedical Research and Program of the Institute:

A widely held clinical impression holds that RDS occurs more frequently in summer months. If this clinical impression is in fact incorrect, the present study stands a good chance of providing strong evidence against the clinical

impression. If RDS occurs seasonally, the study will provide a firm foundation for future studies designed to elucidate the causes of the seasonality. U.S. vital statistics have long shown distinct seasonal patterns of infant, neonatal and 1-day mortality rates. The present study will answer the question, "Are these seasonal fluctuations in infant mortality risk independent of seasonal changes in mean birthweight?" Fluctuations in infant morbidity and mortality rates are of vital interest to the Institute.

Progress and Proposed Course

The data is ready for analysis and some initial tables have been created. Serious data analysis will be delayed until more professional time becomes available.

Honors and Awards: None

Publications: None

Serial No. HD-EB-3

1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Low-Weight-for-Gestational-Age in the U.S.

Previous Serial Number: EB-3(c)

Principal Investigator: Frank E. Lundin, Jr., M.D.

Other Investigators: Howard J. Hoffman, M.A., Charles R. Stark, M.D.
J. Douglas Ashbrook, M.A.

Cooperating Units: National Center for Health Statistics

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.3

Project Description:

Objectives:

The data for this study make possible the simultaneous classification of births by birthweight and gestational age calculated from the date of last menstrual period as collected by 36 states and the District of Columbia in 1968. These characteristics of the data permit us to establish norms for birthweight by gestational-age for a general population and to describe the risk of low-weight-for-gestational-age babies by race, sex, maternal age, parity, month of birth and geographic area. Additionally the risk of fetal wastage in relation to low-weight-for-gestational-age can be studied by the above mentioned characteristics.

Progress:

Extensive tabulations of live births and fetal deaths have been prepared by the Division of Computer Research and Technology, (DCRT). Sophisticated analytic techniques have been programmed in collaboration with DCRT and have been used for the analysis of substantial portions of this data. We are in the stage of manuscript preparation; additional publications are planned from existing tabulations.

Significance to Biomedical Research and Program of the Institute:

Clinically low-weight-for-gestational-age babies have increased morbidity and statistically they have much increased mortality. This descriptive study provides epidemiologic leads to factors responsible for infant mortality. Related studies planned by the Branch will allow direct estimates of mortality in low-birth-weight-for-gestational-age babies.

Proposed Course:

Additional analyses and manuscript preparation will continue into next FY.

Honors and Awards: None

Publications: None

Serial No. HD-EB-4
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Relative Efficacy of Two Prenatal Care Regimens

Previous Serial Number: EB-4(c)

Principal Investigator: Frank E. Lundin, Jr., M.D.

Other Investigators: Charles R. Stark, M.D., Charles U. Lowe, M.D.

Cooperating Units: Contra Costa County Medical Services
State of California - Human Relations Agency
Alameda - Contra Costa Medical Association

Man Years:

Total: 2.6 years
Professional: 1.7 years
Other: 0.9

Project Description:

Objective:

To determine whether pregnant medically indigent women indoctrinated by nutritional lectures and by the physician at each prenatal visit bear healthier babies than comparable women treated with a tradition regimen of weight restriction, diuretics and low calorie-low salt diets. The women treated with nutritional education were not subjected to weight control, diuretics nor low calorie-low salt diets.

Methods Employed:

Initially (Phase I) the treatment group is being compared with medically indigent deliveries in the same hospital. A data procurement contract was used to obtain coded abstracts for 5,500 case records of prenatal care and deliveries at Contra Costa County Hospital over a period of 5-1/2 years. Records of infant deaths among these births were obtained from the State of California, Department of Public Health. Later to evaluate and correct for bias due to possible differential useage of Medi-Cal payment to private hospitals computer records of births to residents of Alameda and Contra Costa Counties were obtained from the State of California. These records will form an additional control group (Phase IIA). They contain birth-weight and infant mortality and include all deliveries financed with Medi-Cal payments during the years 1967, 1968, and 1970. Finally data for a

random sample of approximately 600 Medi-Cal deliveries in Contra Costa County are being collected from private hospitals and private physicians under the data procurement contract (Phase IIB). Information being coded is comparable to that for Contra Costa County Hospital deliveries in Phase I. End points will include prenatal morbidity, infant and maternal hospital morbidity as well as birthweight, gestational-age, fetal and infant mortality. Code sheets for Phases I and IIB are forwarded to NICHD for processing and analysis to achieve the study objective.

Progress:

Data for Phases I and IIA have been collected, edited, corrected and are nearly ready for analysis. Data for Phase IIB are being collected with a completion date of July 31, 1973.

Significance to Biomedical Research and Program of the Institute:

It is anticipated that this study will show that avoidance of gestational weight control, avoidance of drugs, and special attention to nutrition will result in a low-birth-weight rate of about 3% and absence of the "toxemia of pregnancy" syndrome. This result would add significantly to the evidence which would enable the Institute to make recommendations which should lead to a major reduction in infant morbidity and mortality in the U.S.

Proposed Course:

Analysis should be completed by March 31, 1974. Preliminary reports are planned for May and October, 1973.

Honors and Awards: None

Publications: None

Serial No. HD-EB-7
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Independent Effects of Birthweight and Gestational Age on Risk of Mortality in the First Year of Life, New York City, 1964-67.

Previous Serial Number: EB-7(c)

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: Samuel W. Greenhouse, Ph.D., Frank E. Lundin, Jr., M.D.

Cooperating Units: New York City Department of Health supplied the data set at no cost.

Man Years:

Total: 4 weeks
Professional: 4 weeks
Other: none

Project Description:

Objective:

As stated in the title.

Summary:

This data set is large enough to permit us to use statistical means to separate the independent birthweight and gestational-age effects on risk of infant mortality.

Significance to Biomedical Research and Program of the Institute:

At various recent times both birthweight and gestational-age have been declared "the most important single determinant of infant mortality risk." To date, however, no comprehensive epidemiologic study designed to dissect out the independent birthweight and gestational-age effects has been undertaken. To pursue a rational course in perinatal research, the Institute needs this information.

Progress and Proposed Course:

The data set has been acquired and general plans for analysis have been discussed. Real progress cannot occur until other projects are finished.

Honors and Awards: None

Publications: None

Serial No. HD-EB-8
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Father's Age and Infant Mortality

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total: 4 weeks
Professional: 4 weeks
Other: None

Project Description:

Objectives:

After controlling for effect of mother's age to examine the role of father's age on infant mortality.

Summary:

The data sources were the infant deaths and matched birth certificates occurring among 1960 U.S. cohort of births, and births registered in North Carolina in 1968. It was found that infant mortality is positively associated with the age differences between mother and father. This probably reflects social circumstances since the age difference between parents is in turn negatively associated with both the mother's and father's level of education.

Significance to Biomedical Research and Program of the Institute:

Results provide no evidence to support the hypothesis that condition of foetus is affected through mutation in male gametes.

Honors and Awards: None

Publications: Father's age and infant mortality. Social Biology, 19:
275-284, 1972.

Serial No. HD-EB-9
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Spina Bifida, Anencephaly, and Potato Blight

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 5 weeks
Professional: 5 weeks
Other: None

Project Description:

Objectives:

To examine the epidemiology of Spina Bifida in the United States in the light of the Renwick hypothesis which links this condition to the consumption of blighted potatoes.

Summary:

By degrees longitude there is as Renwick claims a negative correlation between Spina Bifida infant mortality rates and percentage potato losses due to blight. By degrees latitude, however, the correlation breaks down. In fact, the correlation is strongly negative for states along the Eastern Seaboard.

Significance to Biomedical Research and Program of the Institute:

The epidemiologic basis for Renwick's hypothesis in the United States can be questioned.

Honors and Awards: None

Publications: Letter to the Editor. Spina bifida, anencephaly, and potato blight. Lancet, i: 426-427, 1973.

Serial Number HD-EB-10
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Gonadotropins and Anencephaly/Spina Bifida

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total: 10 Weeks
Professional: 10 Weeks
Other: None

Project Description:

Objectives:

To examine the epidemiology of Anencephaly/Spina Bifida (ASB) and Dizygous Twinning Rates.

Summary:

Contrary to the report by Stevenson et al. to the effect that dizygous twinning rates and ASB are positively associated, the present investigation finds them to be negatively associated in all instances examined. The discrepancy cannot be explained. Nevertheless, the present results seem the more reliable and it is suggested that one risk factor for the occurrence of ASB is a subnormal level of gonadotropin.

Significance to Biomedical Research and Program of the Institute:

The role of hormones in the production of congenital malformations should be explored.

Honors and Awards: None

Publications: None

Serial No. HD-EB-11
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Infant Mortality Rates and Hardness of Local Water Supplies

Previous Serial Number: None

Principal Investigator: Philip S. Spiers, Ph.D.

Other Investigators: Stephen G. Wright, B.A.

Man Years:

Total: 9 weeks
Professional: 9 weeks
Other: None

Cooperating Units: None

Project Description:

Objective:

To seek confirmation of British study which reported a negative correlation between hardness of water and infant mortality rates.

Summary:

Using complete data from as many of the 100 largest U.S. cities no significant correlate could be found between infant mortality rates and hardness of water. The lack of significant findings persisted when other factors such as income level, rainfall, etc., were controlled for.

Significance to Biomedical Research and Program of the Institute:

The British results are probably due to a secondary association.

Honors and Awards: None

Publications: None

Serial No. HD-EB-12
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Susceptibility of Twins to the Sudden Infant Death Syndrome

Principal Investigators: Philip S. Spiers, Ph.D.

Other Investigators: Karen Fetterly

Cooperating Units: None

Man Years:

Total: 8 weeks
Professional: 8 weeks
Other: None

Project Description:

Objective:

To examine the hypothesis suggested from review of the literature that the concordancy rates are the same for like and unlike twins.

Summary:

Using infant death certificates and matched birth certificates together with certificates for all births occurring in North Carolina 1959-67, it was found that the concordancy rates do not differ.

Significance to Biomedical Research and Program of the Institute:

Results strongly suggest that the foetal genotype plays no significant role in the etiology of this condition.

Honors and Awards: None

Publications: None

Serial No. HD-EB-13
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Geographic Differences in Mortality Rates and Rates of Aging - a Possible Relationship?

Previous Serial Number: None

Principal Investigator: S. Watthana-Kaestr

Other Investigator: Philip S. Spiers, Ph.D.

Cooperating Units: Department of Epidemiology, School of Public Health
University of North Carolina

Man Years:

Total: 8 weeks
Professional: 8 weeks
Other: None

Project Description:

Objectives:

To examine the hypothesis that individuals living in high mortality rate areas age at a faster rate than individuals living in low mortality rate areas.

Summary:

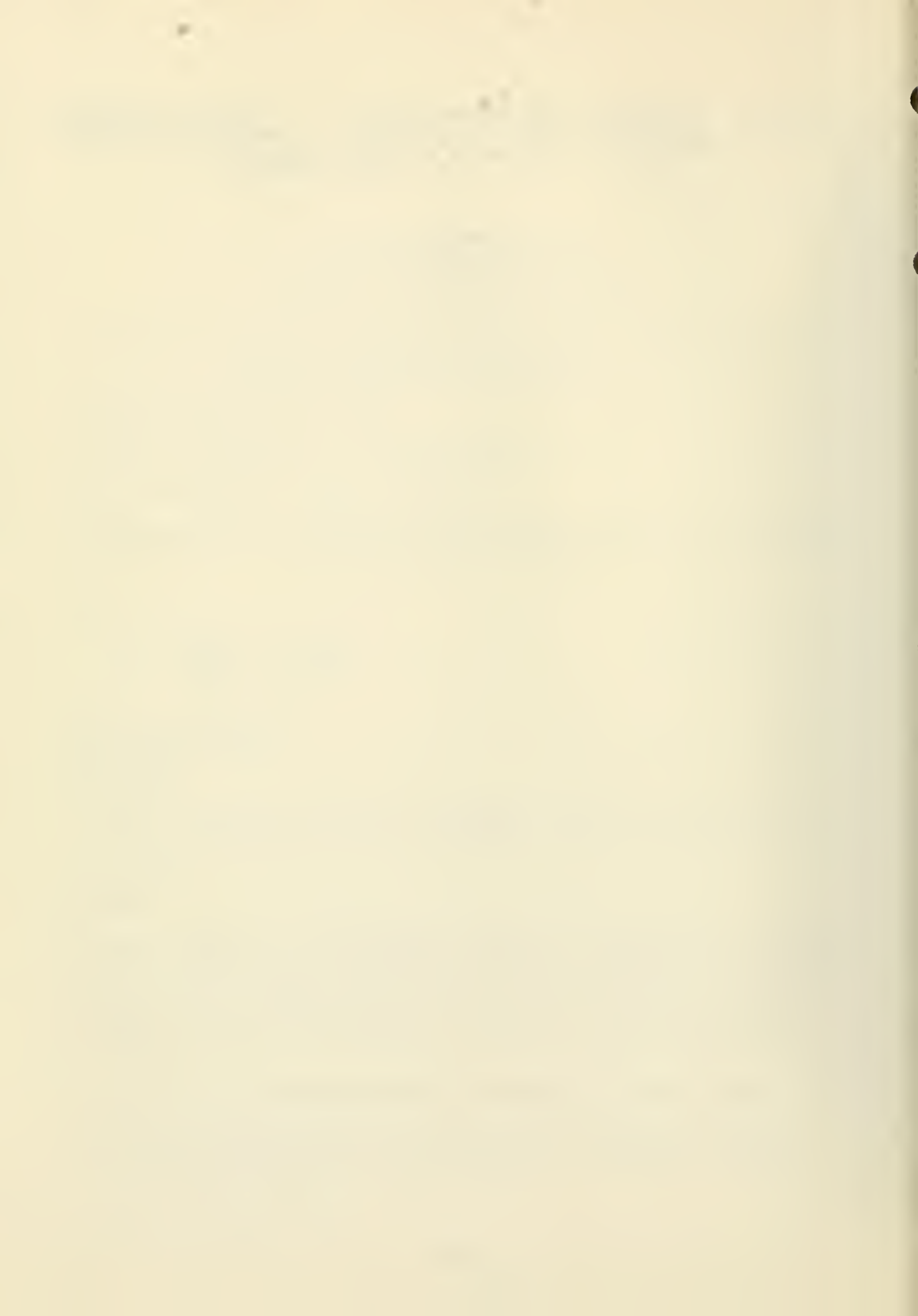
Volunteer employees of three mental health institutions in the State of North Carolina were the subjects of the study. Anthropometric, physical and sensory measurements were made upon these individuals. The results tended to confirm the hypothesis, but because of limitations in the design of the study they should be viewed as preliminary rather than definitive.

Significance to Biomedical Research and Program of the Institute:

If further studies confirm the hypothesis then investigators should concentrate upon an 'aging factor' rather than a specific geographic etiologic agent for cancer or hypertension.

Honors and Awards: None

Publications: Spiers, P.S., and Watthana-Kasetr, S.: Geographic differences in mortality rates and aging rates - a possible relationship? Accepted for publication in Social Gerontology.



NICHD Annual Report
July 1, 1972-June 30, 1973
Biometry Branch

This is the second year in which Biometry has operated as a Branch. In addition to being active in their own research, members of the Branch continue to provide statistical support to staff within the Institute. The extent of such support ranges from brief statistical consultations to long-term commitments to maintain, analyze, and describe large data files. These services are provided to staff involved in intramural, extramural, as well as administrative activities.

Contact with scientists within the program areas frequently stimulates interest in statistical theory and practice. Members of the Branch published articles on the analysis of case-control studies, time series, and longitudinal studies on aging.

Though our staff is small we are responsive to needs of the Institute to take on the analysis of large data files. The outstanding example of this is our data center on the safety of amniocentesis, in support of a contract developed by the intramural staff. Our responsibility to this study will continue for at least two more years.

Activities of the Branch

A. Major Research Activities - Health Studies

1. The Branch continues to serve as a data center for a collaborative study on the risks associated with amniocentesis, a surgical procedure used in early pregnancy.

2. A manuscript has been prepared on the risk of sudden infant death syndrome in twins.

B. Major Research Activities - Statistics

1. Research on methods for analysis of case-control studies continued to be a dominant interest in collaboration with Dr. Greenhouse, our Associate Director. This led to two publications and several invitations to describe these methods.

2. Out of the consultative services provided to the Gerontology Research Center, a comprehensive statement of the statistical issues in longitudinal studies of aging was formulated. It comprises a manuscript which has been accepted for publication.

3. Another primary research interest involves the analysis of biological variability in data collected through time. Research is being carried out to develop appropriate models to characterize these basic rhythmicities.

C. Other Significant Activities

1. Consultations, described below.
2. Staff have collaborated with scientists both in and outside of the Institute in clinical, laboratory, and epidemiologic research.
3. A course in statistical methods in clinical trials of contraceptive agents was given for the WHO, in Thailand.
4. Staff has been invited to participate in scientific reviews of contract programs in the Cancer, and Neurology Institutes.
5. We are assembling a set of basic tables appropriate to each of the program interests of the Institute. To date these have been prepared for two subjects--birthweight and mental retardation.

Consultations

Members of the Branch spent a considerable amount of their time providing consultation for other staff of the Institute. One is active almost full-time with the Gerontology Research Center in Baltimore. One is almost full-time with the Center for Population Research. Another is nearly full-time on a study for the Intramural program. In addition a very large number of requests are received by Extramural staff, by the Epidemiology Branch, and by Intramural laboratory investigators. The complete list of such consultations would be too long for this Annual Report. A selection of some of the more significant ones follows:

1. Longitudinal Study Staff at Gerontology Research Center: Completed work on a basic statistical computing system for analysis of GRC longitudinal data; variance component analysis on basal systolic and diastolic blood pressure and pulse; prepared tables and formulae on frequency of measurement and study duration.
2. Brock, M. and Barrows, C., Gerontology Research Center, Sample size estimates for planning aging studies in hamsters and mice.
3. Rosenberg, K., Gerontology Research Branch, Analysis of survival data from animal colony and derivation of formulae for adjusting for losses due to culling.
4. Robbins, J., Developmental Immunology Branch, Analysis of HL-A antigens, erythrocyte antigens, and serum anticapsular antibodies in patients with Haemophilus influenzae type b diseases. Preparation of a randomization scheme and sample sizes for a preventive immunization trial.
5. Russell, G., Office of the Director, Analyses of Equal Employment Opportunity Data.
6. Crozier, R., Center for Population Research, Review of studies of vasectomy, chromosome breakage.

7. Preterm, Washington, D. C., Planning and analyses of abortion studies.
8. McKigney, J., Growth and Development, covariance analyses of nutritional supplement data in rodents.
9. Shakhashiri, Z., Neurology Institute, Analyses of risk factors for mental retardation.
10. Lowe, C., NICHD, Programming of data on cholesterol and triglyceride data in infants, by feeding histories, sex, and race.
11. Greenhouse, Samuel W., Office of the Director, Review of journal articles on multivariate techniques in analyzing health data.
12. Crystle, Deans, and Townsley, John, NICHD, Analysis of serial hormone determinations in pregnant women for testing the presence of circadian rhythms, half-life determinations in serum, etc.
13. Gibson, Don, NICHD, Life table analysis for normative longevity information for the Fischer's 344 rat barrier colony at Charles River Animal Laboratories. Computerized data collection of fertility, morbidity, mortality and census information for the Primate Research Center, Davis, California.
14. Stark, C., NICHD, Evaluation of the time series analysis techniques which are being employed for a SIDS contract at Sepulveda V.A./L.A. County Hospital at U.C.L.A. Design of a study to sort out the genetic influence, by twin zygosity, on the ontogeny of sleep and cardio-pulmonary recordings on infants for a SIDS contract being conducted at Boston City Hospital, Boston University.
15. Alling, D., Guerry, D., and Dale, D., NIAID, Time series study of hematopoiesis for diseased grey collie dogs, and known human patients with similar disease manifestations, both conditions are presumed to result from a single recessive gene. White blood cell kinetics were investigated for one six-month's span of data collection, with simultaneous determination of counts for neutrophils, lymphocytes, monocytes, eosinophils, basophils, reticulocytes, red blood cells and platelets. Coauthored a paper on this work.
16. Lundin, F., Stark, C., and Ashbrook, D., NICHD and DCRT, Low birth-weight-for-age study of bivariate distributions of birth weight and gestational age information for 1968 U. S. single live births, contour analysis of the bivariate distributions was performed, as well as the more traditional summary measures such as means, variances, and selected percentiles. A manuscript has been coauthored on this work.
17. Ciaranello, R., NIMH, Genetic linkage data involving quantitative characters for two strains of mice: a mutant NIH strain and the original Jackson laboratory strain. Analysis of three types of enzyme determinations from adrenal medulla using the parent generation as the basis for a discriminant function analysis for the subsequent F_2 generation.

NICHD Annual Report
July 1, 1972-June 30, 1973
Biometry Branch

Contracts

Members of the Branch serve as primary project officer on six contracts. The progress of these contracts is described below.

NICHD Annual Report
July 1, 197 -June 30, 1973
Biometry Branch

Contract and Collaborative Research

Contract Title : Epidemiologic Study of Breast Cancer and Benign Breast
Lesions in Relation to the Use of Ovarian Hormones
Contractor : Institute of Oncology, Ljubljana, Yugoslavia
Money Allocated : 524,460 Yugoslavian Dinars

Objectives: To determine whether women who use oral contraceptives have an increased risk of breast cancer.

Summary: This study is similar in design to contracts funded by our Institute in the United States. Women with benign and malignant breast tumours are interviewed concerning their previous use of steroid contraceptives. A matched group of patients without breast disease will also be interviewed as well as a group with benign breast disease that are not biopsied. Analyses will be done to determine whether any increased risk of breast disease exists in women who use steroid hormones.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: Present plans are to terminate the study in 1975, but an application for much needed supplementary funds has been made, which is necessary to carry the study to its conclusion. If such funds are not provided, early termination may be necessary.

NICHD Annual Report
July 1, 1972-June 30, 1973

Contract and Collaborative Research

Contract Title : Oral Contraceptives and Tumors of the Breast
Contractor : University of California School of Public Health
Money Allocated : \$ 91,248

Objectives: To determine whether the incidence of tumors of the breast is associated with oral contraceptive use.

Summary: The frequency of oral contraceptive use in women with breast cancer and women with benign breast lesions will be compared with that of control groups of women free of cancer and/or breast lesions. Cases diagnosed in a two-year period will be selected.

Three groups of women are being used:

- 1) Women with breast cancer 49 years of age or younger in whom cancer of the breast has been diagnosed for the first time during the study period. These are patients admitted consecutively to certain designated hospitals in the San Francisco Bay Area.
- 2) Women with benign breast lesions. These are women biopsied or operated for benign lesions of the breast in the same hospitals within a specified period of the cancer cases. One woman with a benign breast lesion is matched with each cancer by age, by race, by religion, and by socio-economic status.
- 3) Women without evidence of breast lesions or cancer of any site. These women serve as controls. Two controls are chosen for each cancer case, one from surgical and one from the medical service.

The aim is to accumulate at least 400 cases in each of the study groups, women with breast cancer, with benign breast lesions, medical controls and surgical controls.

Basic data necessary for the study are derived from two main sources--review of the hospital record of each subject and a personal interview. The interview seeks information on socio-demographic, menstrual and reproductive and contraceptive variables.

Over 300 cases of malignant breast cancer have been studied, as well as appropriate controls. Preliminary tabulations have been made available to the Institute, so that current surveillance of this important problem can be maintained.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: Present plans are to complete data collection in the current fiscal year, and to then summarize and publish the findings.

NICHD Annual Report
July 1, 1972-June 30, 1973

Contract and Collaborative Research

Contract Title : A Retrospective Study of the Risks for Cancer of the Breast, Body of the Uterus, Ovary, and Cervix among Users of Oral Contraceptives
Contractor : Yale University, New Haven, Connecticut
Money Allocated : \$ 69,666

Objectives: To determine whether the incidence of carcinoma of selected sites is associated with oral contraceptive use.

Summary: Cases are being identified of women with breast, uterine, and ovarian cancer admitted to hospitals in the areas in and around Bridgeport, Hartford, and New Haven, Connecticut, and Springfield, Massachusetts, over a two-year period.

Controls are selected as the next patient with the appropriate matching variables admitted to the same hospital as the case with an acute medical or surgical condition or for an elective operation.

Interviews are administered in hospital, whenever possible, inquiring of demographic variables, obstetric history, menstrual history, contraceptive history, and a checklist of diseases.

Analysis will involve an examination of the difference in the proportions of cases and controls exposed to oral contraceptives for different lengths of time, for each site and for specific cell-types at each of the four sites. Relative risks for various lengths of exposure to the oral contraceptive will be calculated. Nearly 200 cases have been interviewed. Preliminary tabulations have been made available to the Institute, so that current surveillance of this important research problem can be maintained.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: Present plans are to complete data collection in the present fiscal year, and to then summarize and publish the findings.

NICHD Annual Report
July 1, 1972-June 30, 1972
Biometry Branch

Contract and Collaborative Research

Contract Title : A Comparison of the Medical Effects of Induced Abortion
by Two Methods, Curettage and Suction
Contractor : Dr. Franc Novak, Ljubljana, Yugoslavia
Money Allocated : 3 years @ 3,072,675 Dinars(1970-1973)

Objectives: To compare adverse effects to two methods of abortion, suction and curettage. Side effects investigated are bleeding, infection, perforation, subsequent fertility, subsequent ectopic pregnancy, isoimmunization, and cervical incompetence. Study patients to be assigned to these two surgical procedures at random.

Summary: All of the adverse effects mentioned above have been suggested as possible sequelae to abortion. Some have been indicated as being particularly a risk for suction, some for curettage. A considerable shift is occurring in preference between these two procedures with suction becoming more popular but with advantages of suction never having been resolved. This study provides an especially advantageous situation to do this research for at least three reasons: 1) the large number of abortions performed in Dr. Novak's clinic, 2) the large number of women who have had previous abortions, and 3) the willingness and competence of the staff to conduct a randomized trial.

The design has been successfully implemented; data on over a thousand abortions have already been collected.

Analyses thus far have been of a preliminary nature but useful results are already emerging. For example, it appears as though the amount of blood loss in the two procedures is similar, where it had been suggested that more bleeding would occur with curettage. Further, through a specially designed case control study the risk of ectopic pregnancy does not appear to be increased in women with a history of abortion. These and other findings will be published in a monograph as well as journal publications on selected topics.

Significance: The safety of abortions as an operative procedure is of apparent public health significance.

Proposed Course: The study will terminate at the end of the calendar year of 1973.

NICHD Annual Report
July 1, 1972-June 30, 1973
Biometry Branch

Contract and Collaborative Research

Contract Title : Outcome of Pregnancy of Women Who Have Previously Used
Steroid Contraceptives
Contractor : National Naval Medical Center, Bethesda, Maryland
Money Allocated : Discontinued

Objectives: The Institute is interested in determining whether a history of steroid contraceptive use is in any way related to the outcome of subsequent pregnancies. Variables of particular interest are fetal loss, sex ratio, and congenital malformations.

Summary: This study has not been renewed, by mutual agreement between the Institute and the contractor. Always regarded as a trial experience, it soon became clear that securing information on pregnancies, neonates, and drug histories was not as easy within military hospitals as hoped. Particularly difficult were recruiting and developing systems to obtain medical data.

NICHD Annual Report
July 1, 1972-June 30, 1973
Biometry Branch

Contract and Collaborative Research

Contract Title : A Collaborative Study of Oral Contraception and
Cerebrovascular Disease
Contractor : Duke University Medical Center
Money Allocated : \$ 25,587

Objectives: To determine whether the incidence of stroke in young women is increased by use of oral contraceptives, and if so to identify characteristics that are especially related.

Summary: The Neurologic Services of ten university medical centers have identified all young women admitted to their hospitals with definite or suspected cerebrovascular disease of any type. Four of the participating hospitals also identify patients admitted to each of the community hospitals in their metropolitan areas (Miami, St. Louis, Baltimore, and Atlanta). An estimated 200 patients with cerebrovascular disease are submitted for study each year. Clinical data on each of these patients are abstracted from the hospital and physician's records. The frequency of usage of oral contraception in these patients will be compared with that found in two selected control samples: 1) women hospitalized for other diseases and 2) women representing a "healthy" population residing in the same neighborhood as the patients selected for the study. Information regarding pill usage will be obtained from the patient and control subjects by personal interviews carried out in their homes.

All patients and controls have been selected and interviewed in this year. All clinical data have been assembled at Johns Hopkins, where a coding system has been developed.

A manuscript was published in the New England Journal of Medicine in which the basic finding, a nine-fold increase in risk of stroke for oral contraceptive users, was published. Additional analyses on further risk factors in such women, are planned.

Significance to Biomedical Research and Program of the Institute: The study is an extension of previous research on thromboembolism and oral contraceptive use in which very few stroke cases were included and is a part of our program to monitor the significant effects on health of currently used contraceptives.

Proposed Course: No further funding planned.

NICHD Annual Report
July 1, 1972-June 30, 1973

Publications and Talks

Publications

1. Orr, W. C. and Hoffman, H. J.: A 90-minute cardiac biorhythm: Methodology and data analysis using modified periodograms and complex demodulation. I.E.E.E. Trans. Biomed. Eng. In press.
2. Orr, W. C., Hoffman, H. J., and Hegge, F. W.: Sleep loss, sustained performance, and the basic rest-activity cycle (BRAC). J. Psychophysiology. In press.
3. Orr, W. C., Hoffman, H. J., and Hegge, F. W.: Methods of biorhythmic analysis in the assessment of human performance. Ann. N. Y. Acad. Sci. In press.
4. Chez, R. A., Schlesselman, J. J., Salazar, H., and Fox, R.: Single placentas in the rhesus monkey. J. Med. Prim. 1:230-240, 1972.
5. Schlesselman, J. J.: Data transformation in two-way analysis of variance. J. Amer. Stat. Assoc. In press.
6. Schlesselman, J. J.: Planning a longitudinal study: I. Sample size determinations. J. Chron. Dis. In press.
7. Schlesselman, J. J.: Planning a longitudinal study: II. Frequency of measurement and study duration. J. Chron. Dis. In press.
8. Seigel, D. and Greenhouse, Samuel W.: Validity in estimating relative risk in case-control studies. J. Chron. Dis. In press.
9. Seigel, D. and Greenhouse, Samuel W.: Multiple relative risk functions in case-control studies. Amer. J. Epid. In press.
10. Heyman, A., Seigel, D., et al.: Oral contraception and increased risk of cerebral ischemia or thrombosis. New Eng. J. Med. 288:871-878, 1973.

Talks

1. Orr, W. C., Hoffman, H. J., and Hegge, F. W.: Methods of biorhythmic analysis in the assessment of human performance. Presented to the New York Academy of Sciences for a symposium on "Instrumentation for human tolerance measurement," in New York, N. Y. on January 18, 1973.
2. Orr, W. C., Hoffman, H. J., and Hegge, F. W.: Sleep loss, sustained performance, and the basic rest-activity cycle (BRAC). Presented at the annual meeting of The Association for the Psychophysiological Study of Sleep at San Diego, California on May 3, 1973.
3. Seigel, D.: Multivariate analysis in epidemiologic studies. Presented at the annual meeting of the Society for Epidemiologic Research, at Winnipeg, Canada on June 22, 1973.
4. Seigel, D. and Greenhouse, Samuel W.: Multiple risk functions in retrospective studies. Presented at Johns Hopkins University School of Public Health on December 6, 1972.
5. Seigel, D.: Recent research on breast cancer in women using oral contraceptives. Presented at the National Academy of Science Workshop at San Francisco, California in July 1972.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Office of Associate Director for Program Services
Extramural Activities

SUMMARY

The Office of Program Services was effected by three major events during fiscal year 1973: (1) a significant reduction in the number and dollar value of institute supported projects; (2) the abolishment of the Scientific Conference Branch and the Research Analysis Section of the Program Statistics and Analysis Branch; and (3) the consolidation of all activities of the Office within adjoining physical space in the Landow Building.

The Institute's supported projects were reduced by 155 with a dollar value of approximately \$4.4 million below the fiscal year 1972 experience. The bulk of the reduction, \$3.2 million, reflected the phaseout of the various training programs which was implemented on January 29, 1973, in accordance with the President's Budget presentation for fiscal year 1974. The training program phaseout policy provided for the funding of all programs with continuing commitments as of January 29, 1973, but did not provide for any additional new training programs. The training programs effected by the phaseout were the graduate research training grant program, the research career development award program, and the postdoctoral and special fellowship program. The research career award program, a senior research professorship program, will be continued until long-term commitments are completed. The contract program was reduced by approximately \$1.0 million and the research grant program by approximately \$0.2 million below the fiscal year 1972 levels.

Although the Scientific Conference Branch was abolished during fiscal year 1973, the activities of the Branch were transferred to the Office of the Associate Director. Two special assistants were appointed to that Office: one to handle the administrative coordination and initiation of Institute-supported conferences and workshops and the second to handle the administrative functioning of the Institute's advisory committees. The abolishment of the Research Analysis Section of the Program Statistics and Analysis Branch reflected the need to transfer personnel to meet other Branch work priorities.

In the Fall, 1972 the various activities of the Office of Program Services were moved to the sixth floor of the Landow Building in Bethesda. Previously, the Program Statistics and Analysis Branch, the Conference Assistant and the Committee Management Assistant had been located in the Westwood Building; the Contracts Management Section of the Grants and Contracts Management Branch had been located in the B Wing of Building 31; and the rest of that Branch and the Office of the Associate Director had been located in the A Wing of Building 31. The move will now ease communication difficulties which existed within the office.

The Grants and Contracts Management Branch, although processing a smaller number and dollar value of extramural projects, became more involved with grant negotiations than in fiscal year 1972. The phaseout of the graduate

research training grants required individual negotiations with approximately 100 training grant program directors and/or institutions. In order to remain within fiscal year 1973 training program funds, all graduate research training grants were negotiated at a minimum rate of 18%. The reduction of the fiscal year 1973 operating budget from the fiscal year President's budget level in January, 1973, required an evaluation of the need to negotiate noncompeting research grants. These negotiations would be essential in order to maintain a viable competing research grant program following the March meetings of the National Advisory Child Health and Human Development Council. Approval was requested and given to negotiate noncompeting research grants with start dates of March 1, through June 1, 1973, at an average rate of 10% for regular research projects and of 15% to 20% for program projects and centers. Competing research grants were similarly negotiated for the same time frame as the non-competing research grants. In addition, competing research grants were negotiated at an average rate of 6% for the period July 1, 1972, through February 1, 1973.

The contracts management program has now operated a complete fiscal year with the full range of contracting functions: from early planning of Requests For Proposals to signing authority as Contracting Officers. The unique relationship between program and management staff which was characteristic of our Institute prior to contracting decentralization made it simpler and natural for contract management staff and project officers to work together closely to conduct their appropriate roles. A new Institute research contract policy was issued in January, 1973, to reflect the complete contracting cycle.

The Program Statistics and Analysis Branch continued to emphasize methods for the improvement of their data processing system and to provide statistical and analytical services to Institute staff and other interested individuals and organizations within and outside the Federal establishment. The Branch produced two publications for the scientific community in association with program staff: "Inventory of Federal Population Research" and "The Extramural Program of Research on Aging." In addition, the Branch developed a variety of narrative and tabular reports indicating Institute support in important research areas, such as Sudden Infant Death Syndrome, perinatal physiology, pediatric neurology, and maternal nutrition and its effects on fetal and postnatal development. A Branch member has served on the Interagency Committee on Early Childhood Research and Development and the Interagency Committee on Adolescence.

Despite the abolishment of the Scientific Conference Branch, its functions have been continued, in part, by a Conference Assistant and a Committee Management Assistant. The Conference Assistant participated in the planning and arranging for twenty-one conferences and workshops directly sponsored by the Institute. The Committee Management Assistant provides member processing services for ten Institute advisory committees, including the National Advisory Child Health and Human Development Council. As part of the "Federal Advisory Committee Act" it became necessary for the Institute to conduct a comprehensive review of the activities and responsibilities of each advisory committee. The Committee Management Assistant coordinated the preparation of material for this review.

The Office of Program Services continued to stress the area of staff development by participating in various training programs at all general schedule levels. We plan to hold one half-day session on EEO issues in which all Office of Program Services' members will be expected to be involved.



NICHD ANNUAL REPORT

July 1, 1972 through June 30, 1973
Grants and Contracts Management Branch

SUMMARY

During Fiscal Year 1973, the Institute should support about 1,327 research and research training grants, research fellowships, research career and research development awards and research contracts at approximately \$89.9 million. This would represent a decrease of about 10% in numbers of projects (155) and a decrease of 5% in dollars (\$4.4 million) from the Fiscal Year 1972 portfolio.

The Institute used the DRG system of normalized scores to determine a funding order for regular research grants. Program projects were ranked according to an Institute-developed system for normalizing scores based upon the DRG formula.

Training grants were funded on a priority score basis during Fiscal Year 1973. The priority score was essentially the basis for funding the research fellowship career award, and research contract programs although selected adjustments in funding order were permitted because of program relevance.

Negotiation of Research and Training Grants

Noncompeting research grants with start dates of July 1 through February 1 were not negotiated during Fiscal Year 1973. These noncompeting grants with March 1 through June 1 start dates were negotiated at an average rate of 10% with program projects negotiated at a higher rate of 15 to 20%. Competing grants with start dates of July 1 through February 1 were negotiated at an average rate of 6%. Those competing grants with March 1 through June 1 start dates were negotiated at an average rate of 10% with program projects negotiated at a higher rate of 15 to 20%. Through these negotiations we expect to acquire approximately \$2.7 million which will enable us to fund about 45 additional grants.

The phasing out of training programs was begun during Fiscal Year 1973 under the guidelines set by the NIH policy. In order to stay within the amount of funds available to NICHD, all training grants were negotiated at a minimum rate of 18%.

Research Grants

During Fiscal Year 1973, we expect to award 859 regular research grants in the amount of \$54.4 million, a decrease of 49 grants and \$0.2 million from Fiscal Year 1972. The total consists of approximately 615 noncompeting grants for \$39.6 million and 244 competing awards for \$14.8 million. About \$0.2 million of the \$14.8 million will be obligated for scientific evaluation grants since there is no separate line item in the Fiscal Year 1973 budget for these awards. In our Special Research Program we will award 11 Mental Retardation Center grants for \$5.6 million. We transferred \$1.4 million to

the Division of Research and Resources for the General Research Support Grant Program.

Training Grants

During Fiscal Year 1973, we expect to award 93 training grants for \$7.7 million, a decrease of 18 grants and \$2.4 million from Fiscal Year 1972. The total consists of 85 noncompeting grants for \$6.9 million and 8 competing grants for \$0.8 million. No separate allotment was available for scientific evaluation grants in Fiscal Year 1973. These grants were obligated from the \$0.2 million in regular research funds referred to in the paragraph on research grants.

Fellowships

We expect to award approximately 34 postdoctoral and special fellowships in the amount of \$0.4 million. This is a decrease of 55 awards and \$0.5 million from Fiscal Year 1972.

Research Career Awards and Research Career Development Awards

During Fiscal Year 1973, we expect to award 105 research career and research career development awards in the amount of \$2.5 million. The total consists of 7 research career awards for \$0.2 million and 98 research career development awards for \$2.3 million. This represents a decrease of 12 awards and \$0.3 million from Fiscal Year 1972.

Research Contracts

During Fiscal Year 1973, we expect to process 225 contracts and reimbursable agreements for approximately \$19.3 million. This will consist of approximately 175 research contracts and reimbursable agreements in the amount of \$14.7 million for the Center for Population Research Program, about 12 contracts in the amount of \$0.7 million in the Adult Development and Aging Program, and approximately 38 research contracts in the amount of \$3.9 million for the Child Health Program areas.

The Fiscal Year 1973 contract program will represent a net decrease of 21 contracts and reimbursable agreements and \$1.0 million from the Fiscal Year 1972 program.

SUMMARY OF INSTITUTE SUPPORTED PROJECTS
(Dollars in Millions)

	<u>1972</u>		<u>1973</u>		<u>Change</u>	
	<u>No.</u>	<u>Amount</u>	<u>No.</u>	<u>Amount</u>	<u>No.</u>	<u>Amount</u>
<u>Research Grants</u>						
Noncompeting	521	\$35.9	622	\$41.3	+101	+\$5.4
Competing	398	24.3	248	18.7	-150	-\$5.6
	<u>919</u>	<u>60.2</u>	<u>870</u>	<u>60.0</u>	<u>- 49</u>	<u>-0.2</u>
 <u>Training Grants</u>						
Noncompeting	81	7.5	85	6.9	+ 4	-0.6
Competing	30	2.5	8	.8	- 22	-1.7
Scientific Eva. ^{1/}		.1				-.1
	<u>111</u>	<u>10.1</u>	<u>93</u>	<u>7.7</u>	<u>- 18</u>	<u>-2.4</u>
 <u>Research Fellowships</u>	89	0.9	34	.4	- 55	- .5
 <u>Research Career and Research Career Development Awards</u>	117	2.8	105	2.5	- 12	- .3
 <u>Contracts</u>	<u>246</u>	<u>20.3</u>	<u>225</u>	<u>19.3</u>	<u>- 21</u>	<u>-1.0</u>
 GRAND TOTAL	1,482	\$94.3	1,327	\$89.9	-155	-\$4.4

^{1/} No separate allotment was available for these grants in FY 1973. The costs were paid from the regular research line item.



NICHD Annual Report
July 1, 1972 through June 30, 1973
Program Statistics and Analysis Branch

SUMMARY

During this fiscal year the Branch underwent an expansion of activities and a reorganization. Expansion took place in providing two new recurring reports on research grants and contracts, the development of a data base for application information, and extended coverage in the inventory of population research.

The reorganization within the Branch involved the dissolution of the Research Analysis Section. That Section was concerned with the summarization of progress in research supported by NICHD. More pressing Institute work priorities brought about the reallocation of the Section's personnel to other areas in the Branch to meet these needs.

This year, in answer to demands for support information, the Branch developed a triannual report for the NICHD Director in the area of Sudden Infant Death Syndrome, and a semiannual status report on research contracts for Institute staff. In both instances, Branch staff worked closely with the requesting offices to provide reports which exactly answered their needs.

Once again the Branch worked hand in hand with staff of the Center for Population Research and the Interagency Committee on Population Research (ICPR) to provide better information communication. The Branch Chief continued to serve on subcommittees and task forces of ICPR to help improve the information process.

The Branch continued to service the NACHHD Council and Program staff through the preparation of studies and reports and again produced two publications for the scientific community at large: "Inventory of Federal Population Research" and "The Extramural Program of Research on Aging."

Staff of the Branch have been active in improving their job effectiveness and in other areas of self improvement. Many members of the Branch have participated in varied training activities. Courses taken included statistics, programming and data processing, secretarial practices and other office skills. Some staff members are also attending the Upward Mobility College.

Active participation in the EEO program has always characterized this Branch and this year the scope of activity has been broadened to include leadership in the Women's Task Force of NICHD. Among the participating staff, one staff member is the Task Force representative to the NICHD EEO Committee and another is an NIH EEO Counselor and member of the NIH EEO Council.

Program Analysis Section

Major functions of the Program Analysis Section continue to include the assignment of grant applications to appropriate program areas in the Institute and to categories within these program areas, the scientific classification

of grants and contracts, and the preparation of analytical reports in research areas pertinent to the interests of NICHD.

Partly due to the increase in staff resulting from another reorganization, the Section was able for the first time to keep current on the scientific classification. Information on grants and contracts was entered on the computer within a few days of the time the award statement was received.

Preparation of the Inventory of federally supported population research continued to occupy a large portion of the Section's time. For the first time, information was collected in a systematic basis on the scientific discipline of the research, the organism studied, and the anatomical sites and body systems involved. Data generation activities were tabulated and analyzed separately from research activities, making it easier to evaluate trends in substantive research. The number of tables was increased, and the statistical analysis expanded.

Section staff were also involved in exploring the feasibility of developing an inventory of population research sponsored by the major private organizations. Tentative plans have been outlined for obtaining the information necessary for this purpose.

The Section also has responsibility for a publication describing the extramural program of the Adult Development and Aging Branch. Summaries were prepared for each of the FY 1972 projects, and these were expanded this year in order to provide more detailed information on program projects.

Congressional interest in various areas of research generated several requests for information from the Section. One of these resulted from the continuing concern over the Sudden Infant Death Syndrome. Staff were asked to identify all projects ever supported by the Institute in this area, and also to identify applications submitted to the NICHD to determine whether these were disapproved, approved, and/or funded.

Another Congressional inquiry pertained to the testimony on the authorization of a new Institute on Gerontology. For this purpose an identification was needed of all research concerned with nutrition in the aged. In another context we were asked for a description of any NICHD research projects in which a comparison was being made of the differential effects of structured versus nonstructured educational intervention programs for preschool children. Congressional interest was also responsible for requests for information on research on human fertilization and implantation, on male versus female contraceptives, and on congenital nephritis. Hearings related to a proposed bill concerned with responsibility for research on autism led to a request for information on support of NICHD projects relevant to this disorder.

Staff of DHEW or of other agencies were responsible for other requests. As background information for a Department audit of Mental Retardation Centers, the Section was asked to provide an identification of all NIH projects at three institutions having such Centers. NIH coverage was also needed for grants and contracts on iron deficiency, erythropoiesis, and iron transport, metabolism, and storage. The Office of Education and the National Education

Institute both requested information on our grants and contracts concerned with educational research; the Office of Child Development was interested in projects dealing with societal and parental expectations of children; and the National Institute for Mental Health asked for the identification of all projects dealing with children.

As in previous years, the Interagency Committee on Early Childhood Research and Development needed a listing of all grants relevant to its interest. This year, a parallel committee was established for adolescence, and information on relevant projects was also provided to this group. It is hoped that between the two committees, information will now be available on all government research involving human subjects from the time of conception through age 21.

Program staff within the Institute were also responsible for initiating requests to the Section. Several of these were to provide background information for presentations at meetings. One of these was in the area of perinatal physiology. Our staff was involved in establishing the criteria to be used, in identifying the relevant projects, and in providing a narrative description of the grants in one of the sub-sections -- dealing with physiological development in the early postnatal period.

A request was received concerning pediatric neurology. Although much of the research in clinical neurology is supported by another Institute, NICHD does support a number of projects in basic areas relevant to child neurology, including neuropsychology, neurophysiology, neurochemistry, and neuroanatomy. Section staff identified and categorized these projects and provided a narrative description of the research.

As background for a meeting, a narrative was provided on NICHD projects in the area of maternal nutrition and its effects on fetal and postnatal development.

Several requests from program staff were of a recurring nature, and involved information needed on an annual basis. Included were projects on nutrition, primary and subsidiary for both extramural and intramural research, and projects in reading, communication, and related areas. Other information required by program staff included the following topics: EEG and the evoked response, prostaglandins, abortion, race relations, the use of specific populations in research, such as the mentally retarded and American Indians, aggression in children, lead poisoning, language deficiency and self-concept, and environmental noise effects on human populations. The identification and categorization of grants in pharmacology was also requested by Institute staff.

A member of the Section staff served, during this past year on various committees, both within the Institute and in conjunction with representatives from other agencies. These included the NICHD Committees on the Man-Made Environment and on Program Interrelatedness, the Interagency Committee on Early Childhood Research and Development, and the Interagency Committee on Adolescence.

Statistical Analysis Section

New responsibilities assumed by the Statistical Analysis Section during the year included two new recurring reports. The first was a semiannual report on research contracts designed for Institute staff. The second was a tri-annual report to the NICHD Director on the status of research grants and contracts, as well as actions on applications, relevant to the Sudden Infant Death Syndrome. Additionally, the Section participated in the establishment of new clearance procedures for research grant applications and contract proposals related to abortion and has responsibility for one portion of the procedure.

The Section again this year was responsible for furnishing to the Division of Research Grants NICHD data for inclusion in the NIH annual report to the National Science Foundation. Recurring reports on NICHD grant and contract holdings, covering awards from FY 1972 funds as well as the Institute's active portfolio, were issued by the Section as in previous years.

Our machine data bank was expanded during the year by the addition of items concerned with the review of research grant applications. The new additions to the system were limited for the present to data required in the computation of funding rates, but, for trend purposes, the new file includes all years from FY 1964 through FY 1972. The file will be updated periodically as required to keep it current.

Special studies prepared in the Section included an update of a 1967 report entitled "How Does the Young Investigator Fare in Study Section Review?" The new study provided comparisons of funding success not only between young versus senior investigators but also between M.D.'s and Ph.D.'s, men and women, biomedical and behavioral scientists, and by NICHD program.

Because of special concerns regarding research training programs, a great deal of work was done in the Section throughout the year to supply both actual and estimated data on NICHD trainees, fellows, and RCP awardees to offices at various levels. Included in these numerous jobs was a study entitled "Trends in NICHD Trainees" prepared for the National Advisory Child Health and Human Development Council.

Another special study divided program projects into component sub-projects and examined approval rates and other characteristics.

Cost estimates of individual intramural projects were developed. The Section also furnished in a number of instances statistical information and visual aids for use in speeches to be delivered both by Institute staff and by scientists outside NIH.

Other special job requests handled by the Section sought the following information:

- (1) Awards to institutions serving minorities;
- (2) Grants and contracts in specific local geographic areas;

- (3) Data on women over 50 from the 1970 census;
- (4) Distribution of grants and contracts by program subcategories within programs;
- (5) Vital statistics, such as infant and perinatal mortality rates, birth rates, fertility rates, death rates by cause, etc.;
- (6) Sex identification of all NICHD investigators since FY 1964;
- (7) Research contracts awarded to medical schools;
- (8) Grants and contracts awarded to educational institutions;
- (9) Research grants by type of application;
- (10) Training grants by the department providing training;
- (11) Review actions of certain study sections on HD applications;
- (12) Fellowship awards for study in foreign countries;
- (13) Center (core) grants and Center-related grants by program;
- (14) Future dollar commitments on research grants;
- (15) Approval and funding rates on applications in the Adult Development and Aging program.

Information Systems Section

Procedural controls was an item of major emphasis in the Information Systems Section this past year.

At the outset of the year a "request form" for data processing work was put into effect. This was done to establish positive work commitments, and to allow for scheduling of work activities within the Information Systems Section.

A general cost accounting system was also instituted. This involved the establishment of designated account numbers within the section for job groups. This year's experience has indicated the need for more specific cost controls to be instituted.

In conjunction with the Statistical Analysis Section, this Section developed the programs to provide for two new recurring reports. An active status report of research grants and contracts relative to Sudden Infant Death Syndrome is provided three times a year, and a semiannual report on research contracts was developed for the Institute.

A special study assignment in the Statistical Analysis Section on funding success comparisons among young and senior investigators, and M.D.'s, Ph.D.'s, sexes and biomedical and behavioral scientists provided the Information

Section an opportunity to show its ability to provide data for analysis in tabular form. The Section was able to provide all comparison tables called for in a timely fashion.

The "investigator" study enabled the Section to take advantage of the developed data file and convert it into a "Research Grant Applications" file. The file will enable machine computing of funding rates.

Last year we were engaged in a trial procedure to put out the monthly activity reports on a more timely basis. This year the procedure is part of our regular routine and we have negotiated a considerable savings in time and money with the new process.

Two major changes in the Computer Section of the Division of Computer Research and Technology caused this Section to alter its operational procedures.

The first change was the announcement that the NIH Language Libraries were to be discontinued. This meant that DCRT was no longer going to provide the maintenance of a program library service for all of NIH. The Section, going through a two and a half month file conversion to meet a March 1 deadline, set up a private program library for the Branch.

Following the library change, in March DCRT installed a Time Sharing Option in its center and ISS suddenly faced a rash of illegal condition codes in its regular runs. ISS was forced to invoke a regeneration procedure of ANS Cobol (version 4) routines to offset the condition and allow normal processing to resume.

A substantial amount of time was devoted to the processing of the Inventory of Federal Population Research in conjunction with the Program Analysis Section. A number of alterations and additions were incorporated into the Inventory System to facilitate product improvement. Programmatically the Section provided:

1. Allowance in the record for additional data to be incorporated into the file, i.e., disciplines, anatomical site, organism.
2. The routine to dump to the WYLBUR disc file was rewritten in COBOL to facilitate changes in routines.
3. All routines, in areas dealing with funds, were recoded to preclude errors being introduced into the file.
4. The routine providing the data in supporting agency order was reorganized to separate out the data generation items.
5. New edit techniques were incorporated into the system through the terminal to insure proper alignment, justification and format of the information.
6. New routines were added to the system to produce tables for Statistical Analysis.

Currently under development in the Section are a pair of specialized quick retrieval programs called QUERYGEN and GOPHER. Also we are investigating the advantages and disadvantages of using direct access files (disc storage). Also being formulated is a redesign of the Current Master File to provide greater dexterity and efficiency of use.

NICHD Grants and Contracts Active During March 1973
(including scientific evaluation grants)

Mechanism of Support	Funds (thousands)					
	Total	PR	ADA	Child Health		
				Total	PBIM	GD
Research Grants	\$ 66,337	\$18,845	\$6,465	\$12,120	\$13,279	\$15,338
Training Grants	11,512	3,539	2,177	2,392	2,816	1,589
Fellowships	682	140	50	249	197	45
RCP Awards	2,649	672	143	600	871	362
Total Grants	81,180	22,197	8,834	15,362	17,163	17,533
Contracts (by related program)	25,490	19,324	933	1,207	1,473	2,038
Total Grants and Contracts	106,670	42,021	9,766	16,569	18,637	19,372

Mechanism of Support	Number of Projects					
	Total	PR	ADA	Child Health		
				Total	PBIM	GD
Research Grants	1,001	347	69	244	241	98
Training Grants	129	28	19	30	35	17
Fellowships	70	16	4	23	22	5
RCP Awards	111	30	5	25	36	15
Total Grants	1,311	421	97	322	334	135
Contracts (by related program)	264	211	17	8	11	16
Total Grants and Contracts	1,575	632	114	330	345	151

NICHD Grants and Contracts Funded by Other Institutes

Source of Funds	Number						Funds (thousands)					
	PR	PBIM	GD	ADA	NR	PR	PBIN	GD	ADA	NR		
											PR	PBIM
Total	3	1	11	2	3	\$91	\$45	\$549	\$185	\$113		
U.S.-Japan Cooperative Medical Science Program (NIAID)	-	-	4	-	-	-	-	133	-	-		
Research Grants	-	-	3	-	-	-	-	309	-	-		
Contracts	3	1	4	2	3	91	45	107	185	113		

Special Genetics Program (NIGMS)--Research Grants

Notes: (1) Research grant totals include two scientific evaluation grants totaling \$290,776 not assigned to a program.
 (2) Contract totals include one contract for \$15,029 not assigned to a program.
 (3) Dollar figures may not add to totals due to rounding.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973

Center for Population Research
Office of the Director

Even though Fiscal Year 1973 was the first year in the brief history of the Center for Population Research for which there has been no increase in appropriations, there is much positive achievement to report. The progress of each area of the Center's responsibility forms an impressive display in the pages that follow.

Unlike previous years, there have been no major new research programs initiated. However, the completion of research in some of the earlier supported projects has dictated development of additional methods for communicating research findings and reporting in depth the methodology and techniques of population sciences. Specifically, a number of CPR Monographs are planned to be published by the GPO and several manuscripts are currently in preparation. The first publication is expected in early FY 1974. Additional information about communication and liaison efforts of the CPR appear later in this summary.

Budget: In a year of no appropriation for the Department, and a variety of expectations throughout the appropriation process regarding the Center's ultimate funding, the following table compares budgetary levels for program activities in FY 1973 with the previous year. (Dollars in Millions)

CPR Component - Program Activity	FY 1972 (Actual)	FY 1973 (Estimate)
1. Population and Reproduction Grants Branch	(20.3)	(20.1)
A. Fundamental Biomedical Research	13.4	13.1
B. Fundamental Social Research	2.5	2.6
C. Population Centers	1.6	2.0
D. Research Training	2.8	2.4
2. Contraceptive Development Branch	(7.4)	(7.0)
A. Directed Biomedical Research	3.8	3.5
B. Product Development	3.6	3.5
3. Fertility Regulating Methods Evaluation Branch	(5.2)	(5.0)
Evaluation of Contraceptives in Use	5.2	5.0
4. Behavioral Sciences Branch	(2.7)	(2.7)
Directed Social Research	2.7	2.7
5. Office of the Director	(2.2)	(2.6)
A. Scientific Information Services	0.2	0.3
B. Population Program Management	2.0	2.3
6. NICHD Intramural Research	(2.2)	(2.4)
TOTAL - Population Research Program	40.0	39.8

In October 1972, in fulfillment of requirements of Public Law 91-572, the Family Planning Services and Population Research Act of 1970, the Center prepared and submitted to the Deputy Assistant Secretary for Population Affairs the second annual progress report of population research programs covering calendar year 1972. As of mid-May 1973, this report and its companion segments from the National Center for Family Planning Services, HSMHA, had not been presented to the Congress and had not been published. That report, as does this one, reported activities and accomplishments in the program categories itemized in the table above.

You will find in considerable detail, in the subsequent segments of this report of CPR activities, description of research relating to new contraceptive agents (Contraceptive Development Branch, evaluation of current contraceptive methods (Fertility Regulating Methods Evaluation Branch), and analysis of social factors in population problems (Behavioral Sciences Branch). Also, description of fundamental research in reproductive biology and the social sciences, and description of institutional support programs follows (Population and Reproduction Grants Branch).

Staffing: By the end of June 1973, the Center filled its full complement of 23 professional and 14 full-time clerical staff. Staff changes during the year included the following:

Contraceptive Development Branch - Kenneth Savard, Sc.D., assumed full-time the duties of Branch Chief in November 1972. Dr. Savard had served as a Consultant to the Branch during the previous year while completing his responsibilities as Professor of Biochemistry and Director of the Endocrine Laboratory at the University of Miami School of Medicine. Also, joining the Branch as a Biologist in November 1972, Dolores Patanelli, Ph.D., was recruited from the Merck Institute for Therapeutic Research. Dr. Patanelli is a specialist in biology of the male reproductive system and has particular responsibilities for research to develop male contraceptives. A third addition to the Branch is Hyun K. Kim, Ph.D. who was appointed as a Senior Staff Fellow in 1972. Dr. Kim is a medicinal organic chemist with responsibilities in the growing program of synthesis and testing of potential antifertility compounds for the "new drug development" program of the Branch. He was previously employed by Bristol Laboratories in Syracuse.

Population and Reproduction Grants Branch - William A. Sadler, Ph.D., who joined the Branch staff in May 1972 was selected to succeed Everett Wilson as Branch Chief in December 1972. Dr. Wilson resigned to return to his former position as Dean, School of Science, Sam Houston State University in Huntsville, Texas. Dr. Sadler, who is a former Dean, Department Head, and reproductive physiologist at Texas Southern University, has already proved his effectiveness with direction of the varied CPR grants program. William Spillane, Ph.D., transferred into the Branch in September 1972 from his former position with the contract program of the Behavioral Sciences Branch of the Center. Dr. Spillane, a social scientist with training and experience in public health, adds essential manpower to management of the rapidly expanding number of grants in fundamental social research. Allyn Waterman, Ph.D., retired from the Branch in August 1972. Fortunately, Dr. Waterman has remained

available to the Branch and the Center as an intermittent Consultant since his retirement. As a former Branch Chief, and senior Health Scientist Administrator in terms of Branch service, Dr. Waterman's knowledge and experience has been invaluable.

Behavioral Sciences Branch - Wendy Baldwin, Ph.D., was appointed as a Staff Fellow in August 1972 following receipt of her doctorate in sociology from the University of Kentucky. Dr. Baldwin's experience includes 10 months in Bogota, Colombia working on the 1969 National Fertility Survey with the Association of Medical Faculties and the Population Council there. Her interests include sex role identification, husband-wife interaction in setting fertility goals, and the influence of women's labor force participation on fertility. Aaron Botbyl, Ph.D., was appointed to the Branch in November 1972 following his graduate work in psychology at the University of Tennessee and a year's internship in clinical psychology at the University of North Carolina Memorial Hospital. Dr. Botbyl has also been associated for several years with the work of Planned Parenthood.

Fertility Regulating Methods Evaluation Branch - John J. Schrogie, M.D. resigned the position of Branch Chief in May 1973 to assume the post of Director of Clinical Research with the Schering Corporation of Bloomfield, N.J. Named as his successor was Heinz W. Berendes, M.D. former Chief of the Perinatal Research Branch, NINDS. Dr. Berendes' extensive experience in organization and administration of national research programs and his capabilities in rationalizing massive data will be most important as results are received from current Branch studies.

Liaison and Scientific Information: The Center continues to carry out its responsibility for coordinating the population research activities of the various Federal agencies through the Interagency Committee on Population Research (ICPR). A primary function of this Committee is to facilitate the exchange of information and ideas and coordinate the efforts of the Federal Government concerned with research related to human population problems. The ICPR, which has representatives from 20 Federal agencies with the Chairman and Executive Secretary from the Center, has been extended by the Secretary of HEW through June 30, 1974.

One of the important products of the Committee is the Inventory of Federal Population Research, prepared by staff of the Institute's Program Statistics and Analysis Branch. The fourth in a series of such annual inventories is being published. The Inventory represents a cooperative effort on the part of each of the reporting Federal agencies funding projects in population research. It includes all projects with a primary emphasis on population research, classified according to the principal focus of the research. Efforts are underway to produce a complementary publication containing the population research projects funded by the major private organizations.

The ICPR also annually prepares the Analysis of Federal Population Research. This report of the Committee is intended to be used to inform and guide Federal Agencies regarding activities and issues of interest and concern to them in population research. The Analysis contains a summary and eval-

uation of current Federally supported population research with recommendations, utilizing the Inventory as a data base.

The communication of population research information is also being enhanced by the Center through various means. Two experimental issues of Population Sciences: Index of Biomedical Research have been published and sent to a sample of scientists in the field for evaluation. This bibliographic citation journal was planned and developed by staff of the Center and the National Library of Medicine (NLM) based on the information contained in the Library's Medical Literature Analysis and Retrieval System (MEDLARS). The responses to the initial experimental issues of Population Sciences have been predominantly favorable and indicate that it provides a useful service. Approval is being requested to go into regular publication on a monthly basis.

In order to provide more comprehensive scope and coverage of the scientific literature dealing with the biomedical aspects of population research, a contract was let in June 1972 with the Karolinska Institute in Stockholm to produce a Reproduction Thesaurus which will potentially increase the data base and retrieval capability of NLM's bibliographic services in the field of reproductive biology and related areas. This will be accomplished by developing an expanded terminology and improved vocabulary, and by greatly increasing the number of journals reviewed and thus the amount of literature indexed and included in the data base. The Thesaurus will be modeled after NLM's Medical Subject Headings (MeSH), their list of technical terms used for indexing biomedical journal articles, and it will be compatible with MEDLARS, the Library's computerized indexing system. The results of the work to be performed under this contract are expected to significantly enrich the data base and provide for more effective dissemination of biomedical research information in the population sciences.

The Annual Summary Progress Reports of Population Research Centers and Program Projects, was compiled and edited by Dr. William Sadler, Chief of the Center's Population and Reproduction Grants Branch. This publication was initiated at the July 1972 meeting of center and program project Directors as a means of furthering communication among the scientists in these programs regarding significant developments in the biomedical and behavioral aspects of population research. The Directors meeting was followed by a useful and interesting Conference on Receptors for Reproductive Hormones. This scientific meeting which dealt with the role of receptors in the mechanism of action of both sex steroid and gonadotropic hormones was organized by Dr. Bert O'Malley, then Director of the Center at Vanderbilt, and will be published by Plenum Press of New York City.

As stated earlier, the Center is also publishing monographs on an occasional basis in order to further enhance the communication of population research information. The monographs will provide a review and evaluation of the state-of-the-art in a specialized substantive area of the biomedical or social science aspects of population research. They will be published as individual books on a non-periodic basis by the Government Printing Office, and will usually contain an evaluative review of the field in a specific

area of population research, annotated citations to the scientific literature, and an analysis of the next steps for achieving progress with regard to meeting research gaps.

In July 1972, the book Human Sterilization, edited by Richart and Prager, was published by Charles C Thomas. This volume reported a 1969 conference sponsored by the International Institute for the Study of Human Reproduction of Columbia University and the Center for Population Research. In January 1973, the Regulation of Mammalian Reproduction, a book edited by Segal, Crozier, Corfman and Condliffe was published also by Charles C Thomas. It reported proceedings of an international conference held in 1970 at NIH under the sponsorship of the CPR and the Fogarty International Center.

Numerous conferences, workshops, symposia and seminars were held on a wide ranging number of topics in both the biomedical and behavioral sciences. The most important of these various meetings are reported in the program summaries of the responsible Branch. Notable for the CPR was the annual meeting of the Population Association of America (PAA) held in April 1973 in New Orleans. At the meeting, Arthur Campbell, Deputy Director, succeeded to the Presidency of the Association for the 1973-1974 term. Mr. Campbell will preside over the affairs of PAA until its 1974 meeting to be held in New York City. Also in New Orleans just before the PAA meeting, Rolf Versteeg, Program Liaison Officer, presided at the Sixth Annual Conference of the Association for Population/Family Planning Libraries and Information Centers (APLIC). This meeting completed Mr. Versteeg's tenure as President of this Association.

This summary has not attempted to recapitulate the accomplishments of the four Branches of the CPR. Each Branch encompasses a diverse and complex research segment of the Center's program. In the following pages, each has presented commendable summaries of its programs.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Center for Population Research

Research relating to population examines the interrelationships between population size, growth and distribution and economic development and other social and psychological factors, and analyzes ways in which individuals are motivated to reduce the size of their families. It is, therefore, concerned with a great variety of processes. It takes into account individual, interactional, familial, institutional, and social processes of great complexity involved in decisions relating to family size and the effective implementation of population policies and their consequences. Research in this area has been greatly expanded in line with NICHD's designation as the focal point for DHEW research in population.

Ad hoc advisory panels were established in FY 69 to guide the development of a program of research and to evaluate research proposals which are developed in the behavioral sciences in four areas:

- (1) The first of these areas of population research is concerned with the antecedents, processes and consequences of population structure, distribution, and change, and the interrelationships between social and population change. Special importance is attached to the interrelationships between economic factors and population growth, structure and distribution for this and other nations and for particular subgroups of the nation. Of increasing concern is the ecological impact of population growth and of related urban technological and industrial development, information about which is needed to guide the formulation of policies with reference to environmental quality. Attention is also being given to migration as a major vehicle of social change.
- (2) Major concern focuses on trends in fertility and related variables, such as marriage rates, age at marriage, unintended pregnancies, the incidence of abortion, trends in divorce, and changes in attitudes toward childbearing, family size, methods of fertility control, and other topics, including the failure to prevent unwanted births despite verbal commitment to family planning. Considerable effort is being made to develop a broader data base for more rapid determination of trends and the factors affecting them.
- (3) Ultimately the factors affecting trends in fertility will express themselves mainly within the framework of the family, so that a major area of research on population must concentrate on the family, on differences in this institution among various population groups, and on changes which are occurring in its structure, particularly as they affect fertility. Emphasis must also be

placed on sexual behavior, both in and out of marriage, insofar as this bears on fertility. Research is also sought on the process of socialization for marriage and parenthood, including the social pressures exerted by the immediate family, kin networks, peers, and the broader society to have--or refrain from having--children. More needs to be known about the factors influencing decisions that lead to the number and spacing of births, and including perceptions of alternatives to child-bearing. Needed, too, is research on the relationship between family structure, childbearing patterns, and child development.

- (4) Concern with problems of population growth leads to questions of public policy. Research on all aspects of growth is thus relevant to policy but is also needed specifically on the ways by which population growth or movement are influenced by public policy, and the effects upon population of policies already adopted regarding family planning programs, welfare payments, parity payments, tax exemptions and allowances, direct incentives, etc. As to the relationship with family planning programs, the Center for Population Research is concerned with research on the assumptions under which programs are organized and the evaluation of these programs in terms of those assumptions; operational research is considered the primary responsibility of other agencies.

About one-in-five of the proposals submitted under the contract program in the behavioral and social sciences have been approved by peer review committees and funded. Funding of approved projects totalled \$0.7 million in fiscal 1969, doubled to almost \$1.6 million in fiscal 1970 and redoubled to approximately \$3.9 million in fiscal 1971, dropped off slightly to \$2.9 million in fiscal 1972, and was approximately \$2.7 million in 1973. Since the initiation of this research program in early 1969 through fiscal year 1972 a total of 66 contracts have been funded. During the current fiscal year a total of 15 contracts have been approved for funding, most of which will be funded during the current fiscal year. The remaining contracts will be funded in early fiscal year 1974. Investigators in a wide variety of institutions, mainly universities, but also in governmental and private research organizations, have become involved in the research program. Fields of specialization represented are sociology, psychology, economics, anthropology, medicine, and statistics.

Since the beginning of the Behavioral Sciences Research programs, several studies have been successfully terminated. However, most are still in the active stage. As those now underway and others to be initiated are completed, however, it is hoped that our knowledge of the influences upon the birth rate and completed family size will be greatly expanded.

Much of the discussion about "the population problem" assumes that population plays a large role in many of our other social problems, but to what extent, and in what ways, are not sufficiently well known or agreed upon by competent scholars. Better knowledge concerning the effects of population growth, structure, and distribution are essential if informed and acceptable public policies

regarding population are to be formulated and adopted. These studies, dealing with a variety of interrelated factors not easily disentangled, require that a program be structured in long-range terms, and it will be at least several years before results highly useful to policy makers will emerge.

Emphasis here is on projects which were continued (including those for which a final report was received) or funded and initiated in fiscal 1973.

During this fiscal year 62 contracts were active including 3 reimbursable agreements with other Federal agencies), 2 of which are extensions (with additional funds) of projects negotiated in previous years.

Fertility, Abortion, Illegitimacy and Family Planning

Center for Population Research contracts indicate both the concentration of interest in fertility and family planning and the range of problems dealt with.

Under a contract with the University of North Carolina, (DA-66)* a study has been undertaken in Tamil Nadu, India, to investigate whether there is a threshold of social and economic development after which family planning action programs will make more rapid progress. A study at the University of Colorado (DA-49) is looking into the relationship between social mobility within urban areas and the acceptance of family planning services.

A study on the consequences of illegitimacy, and methodological problems associated with studying the phenomenon, has been undertaken at the University of California (DA-20). This study is being followed up by one at the same institution looking at the interrelationship of illegitimacy, abortion and family structure variables, including patterns of sexual relationships (DA-48).

A series of Current Population Surveys by the Bureau of the Census (DA-21), has revealed a decline in the number of children expected by young married women.

A study being carried out by the Center for Family Planning Program Development (DA-72) is attempting to determine the effects of family planning programs on the fertility of women throughout the United States.

An essentially methodological study, arranged under contract with the University of North Carolina (DA-12), seeks to ascertain through computer simulation the effects on fertility of various childspacing patterns in an effort to improve the analysis of current trends in fertility. A Temple University study (DA-33) is designed to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients. Calspan Corporation (DA-27) is developing refined techniques for measuring desired family size and the values which are attributed to different numbers of children.

*Number of page on which greater detail is given in the summaries of individual projects that follow this general summary.

A contract with DUALabs (DA-55) has developed a support technique to facilitate the wider use of the 1960 and 1970 Census Public Use Samples (PUS). The project emphasis is on the handling of very large data files by writing programs to merge different types of records, create smaller work tapes and provide programs for cross tabulations and analysis of the data. These computer programs will make the Public Use Samples more accessible to researchers in the field. The proposal also includes the writing of users manuals and provision of classroom instruction in the use of these programs which will be available in the public domain at low cost.

Socio-economic Correlates of Fertility

Six studies deal with socio-economic correlates of fertility in various historical and cultural settings. Rand Corporation research (DA-38) is attempting to identify the principal determinants--e.g., income, time of marriage, female labor force participation, and migration characteristics--of family size and current fertility patterns in the United States. The influence of socio-economic factors on fertility should provide the basis for estimating the probable impact on the birth rate of: (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in tax deductions for dependents, (4) housing and rent subsidies, and so forth.

One study being conducted by the Research Institute for the Study of Man (DA-67) will use data from standard and special 1970 and earlier decennial census tabulations to examine the fertility and its socio-economic correlates of the major Spanish-American population groups in the U.S.: Mexican, Puerto Rican, Cuban, Central and South American and the descendants of the original (17th and 18th Century) Spanish group.

A contract with Princeton University (DA-10), a continuation of a study underway for several years, deals with the correlates of fertility reduction in European countries since the 19th century. The objective of research being undertaken at Western Washington State College (DA-23) is an investigation of social, political, and economic influences bearing on fertility, including rural differentials by size of land holding and abortion and fertility differentials by employment status of women in Poland, and the socio-economic correlates of fertility and birth control in the U.S.S.R.

A contract with the Food Research Institute, Stanford University (DA-19), is investigating the socio-economic correlates of fertility reduction in developing countries. Peasant communities in Indonesia, India, and Mexico and a farming community in the United States will be studied in the field by Columbia University (DA-36) investigators to assess the costs and benefits associated with different family sizes in each community.

Sex Roles and Socio-Psychological Correlates of Fertility

Other studies directly related to fertility approach its analysis from several different standpoints. The 1970 National Fertility Survey, being conducted by Princeton University (DA-9), is studying the control of fertility and fertility intentions of American women in terms of a variety of social and

economic characteristics. In addition, several studies investigate the relation of fertility to marriage and family structure. A contract with the Institute for Survey Research, Temple University, (DA-81) is studying the role of husband-wife communication and decision-making regarding fertility-related activities. A contract with Indiana University (DA-18) studies this relationship in terms of the definition of sex roles, emphasizing particularly the effect of employment of women. The University of California at Los Angeles (DA-57) has been awarded a contract to elucidate the behaviors and motivations of 120 employed career women relating to fertility and family planning and to specify the patterns of temporal ordering of family and work. The Center for Policy Research (DA-63) has undertaken a study of occupational experience as a determinant of fertility among American women. The principal investigator intends to examine an important aspect of the relationship between women's participation in the labor force and their fertility behavior, namely, the characteristics of the occupations they work in. This inquiry is particularly directed toward determining what effect on fertility is resulting from the increasing number of women who are taking or attempting to take a more active role in the labor market, especially in professional occupations.

A study based on a complex model of the relationship between fertility and women's labor force activity being initiated in Research Triangle Institute, N.C. (DA-44), suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly. An analysis of the 1960 and 1970 Census Public Use Samples, under contract with Emory University (DA-82), will focus on the influence of childbearing on women's labor force participation. Based on data collected in the Greater Athens Metropolitan Area in 1966-67, a Wayne State University study (DA-28) is examining the antecedent factors relating the employment of women to fertility and birth control to explain how the level of urban Greek fertility can be low in the absence of (a) organized family planning and other governmental programs, and (b) economic industrial development.

The University of Kansas (DA-61) is investigating the dynamics of social norms concerning large and small families--reactions to explicit and implicit social pressures in relation to normatively defined family size ideals. A contract with Calspan Corporation (DA-73) will produce a compendium of extant research regarding the various consequences of different size families. This research will include development of the theoretical framework for studying the consequences of family size and also a critique of research methods used in projects reviewed. Another contract with the Wright Institute in California (DA-60) is analyzing data that are available on 3,180 Vassar College women during the period 1953 through 1960. This study may reveal many of the current revolutionary themes which may have been latent in the 1950's. For example, women's lib, sexual freedom, decline of the number of children desired, etc., may have been in their embryonic form.

The Center for Policy Research (DA-50) also has underway a research project to examine the possible relationship between ideology and fertility-related behavior. Specifically, the study includes the development of a quantitative model which will examine the relationships between: (1) family background,

(2) family ideology (the attitudes and values of the respondent's mother and father), (3) the respondent's ideology, (4) the respondent's career and family role orientations, (5) sexual attitudes, (6) sexual behavior, and (7) preferred family size. A contract with Illinois State University (DA-83) will investigate the influence of attitudes towards sexual behavior held during teenage years on fertility attitudes and behavior during the early years of family formation.

Another contract, with the University of Wisconsin (DA-11), analyzes trends in marriage, divorce, remarriage, and their relationships to size of family, using data from the Survey of Economic Opportunity in 1967. Research underway at Bowling Green State University (DA-46) involves a re-interview of mothers interviewed in Toledo in 1963 coupled with a sample of married women in the Toledo SMSA to test for possible associations, within broad religious and socio-economic categories, between selected social-psychological and demographic variables. Under a reimbursable agreement with the Census Bureau (DA-77), a study of the relationship between age at marriage, birth intervals, and total fertility is being made based on the Current Population Survey.

Demographic and Attitudinal Factors Affecting Family Formation

Research underway in Massachusetts by the Worcester Foundation for Experimental Biology (DA-32) is testing attitudinal factors affecting family size. Other research, being undertaken in Louisville, Kentucky, by the Human Resources Research Organization (DA-42), is exploring the factors involved in "moral judgment" with respect to marital status and family size. This study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts.

Another study, being undertaken at the University of Southern California (DA-26), looks to the kin network in the United States as the locus of influence for its members with respect to fertility and migration decisions. A University of Kansas (DA-40) study is questioning a group of first-time parents to find factors--"purposive-rational" goal oriented, traditional, or economic--that are affecting their decision-making as to the number and spacing of births.

Migration, Social Mobility, and Residential Patterns

Ten contracts deal with migration. The University of California (DA-58) has under investigation an effort to study the causal structure and decision-making process underlying the migration behavior of the rural poor. This will explore the potential for various public policies which could influence migration from rural and small areas to big city ghettos. The study will test a series of alternative causal models for explaining migration while investigating in depth a model of individual adjustment to economic and social displacement. The principal investigator will conduct a secondary analysis of data previously collected by ABT Associates and funded by OEO. To date, the analysis of these data have been only cursory. Data for 1800 lower socio-economic respondents have been collected.

The University of Texas (DA-79) is studying the demography of the black population. The purpose of this study is to describe and analyze trends in the demographic characteristics of the black population, and compare these with trends in the white population. The basic analytical approach will be cohort analysis by sex and color.

Battelle Columbia Laboratories (DA-53) is conducting an analysis of migratory responses to employment opportunities. The aim of this research is to clarify the relationship between migration and certain demographic and economic variables and in particular potential employment, i.e., differential migration in response to economic conditions.

A University of Georgia study (DA-39) analyzing the trends and patterns of population change for the total white and non-white populations of all counties in the United States from 1950 to 1960 is concentrating on fertility and migration, as did another study undertaken at Louisiana State University, completed last year.

A Brown University study (DA-31), based on a probability sample of women residing in Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955, is designed to investigate: (1) how spacing of pregnancy and of fertility varies; (2) the life conditions under which women are likely to resort to terminations and sterilizations; and (3) how the size of the family of origin and the position on a traditionalism scale are related to social mobility, present socio-economic status, aspirations for children, and fertility. Upward social mobility, given the economic means and a lack of social barriers, frequently has meant in the United States and Canada a change to different housing in another neighborhood--and perhaps in another city--for, among other purposes, childbearing and childrearing.

The U.S. Bureau of the Census (DA-34), under a reimbursable agreement, will combine data from the March Current Population Survey taken by the Bureau for the years 1966-1970 to study migration during that period by single years of age in order to assess the joint influence of various socio-economic and familial statuses on the probability of making different types of moves during an individual's lifetime if he moved according to present age-specific rates of residential mobility.

A University of Michigan (DA-22) study is analyzing 1970 U.S. census data on tapes for 20-25 SMSAs (Standard Metropolitan Statistical Areas) and is comparing these with census materials for four Canadian metropolitan areas to determine: (a) variations in population distribution in different metropolitan areas; (b) the degree of segregation of families in different stages of life cycle; (c) the degree of residential segregation of different socio-economic groups; (d) the pattern of segregation of housing of different types and ages; and (e) the relation to housing type to socio-economic characteristics of occupants.

Another study deals with the economic costs and benefits of migration to areas which lose and areas which gain from migration. The study, being undertaken at Colorado State University (DA-30), is concerned with the population changes that have occurred during the period 1950-1970--about half of the

counties have been losing population--in the Mountain Region stretching from Montana to Arizona and is considering the consequences of those changes to public finances, retail activity, housing utilization, and agricultural activity. The University of Kentucky (DA-15) is conducting an economic analysis of migration from rural Eastern Kentucky to selected urban centers in Kentucky and Ohio to measure the impact of migration in terms of the provision of selected public services by individual donor and recipient local governments and the Federal government.

TRACOR, Inc. (DA-65) has a contract to review research findings on rural-urban migration and develop a complete annotated bibliography.

Research Oriented Toward the Possible Development of Population Policies

A University of Illinois project (DA-25) is exploring the effect of income on fertility in two different studies: (1) by asking hypothetical questions in a sample survey administered to a regional sample of the U.S. population concerning the effect on personal fertility of government payments for more or fewer children; and (2) by studying the relationship between income and fertility using the 1-in-a-1,000 tape from the 1960 census and demand theory.

The Pennsylvania State University (DA-64) has been awarded a contract to investigate in detail, historically and contemporaneously, the non-metropolitan population changes in the State of Pennsylvania. That State is a large mature area with a highly diversified non-metropolitan sector. The investigators propose to create a data bank of relevant historical-statistical information and to create a typology for the non-metropolitan areas in order to analyze the most important processes shaping change in those areas. Data will be derived primarily from the census but several other sources will be used and field studies will be conducted. The studies will focus on transport, technology, changing labor force roles of females, attitudes toward metropolitan living, and others - which have shaped the non-metropolitan sectors of the population.

A study at the University of Virginia (DA-62) is examining the relationship between population growth in the United States and economic measures of quality of life.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : National Fertility Survey, 1970-1975
Contractor : Princeton University
Money Allocated: \$377,238 (FY 1965); \$107,531 (FY 1967); \$222,260 (FY 1970);
\$519,349 (FY 1971); \$390,871 (FY 1972)

Objectives: The objective is to survey approximately 6,500 women representing a national sample of all ever-married women under age 45 in the conterminous U.S. to determine fertility behavior and fertility expectations of American women. The study is a continuation of studies begun as the Growth of American Families Study, 1955, repeated in 1960, and of a similar National Fertility Survey conducted by the present investigators in 1965. The study will compare fertility behavior and expectations by age, marital duration, race, education, income and religion, and will supply data on the use of various contraceptive methods, and the influence of a broad range of factors on their use. It will include a sample of women previously married but not currently in the married, husband-present category.

Major Findings: This survey has documented a substantial decline (36 percent) in the rate at which women were having unwanted children between the period 1961-65 and 1966-70. This improvement in the control of fertility resulted from increases in the proportions of couples using contraception and, particularly, in the proportions using the more effective methods. A time series on the use of oral contraceptives shows that the proportion of married women under age 35 using this method rose from about 2 percent in early 1961 and 30 percent in 1967 and remained close to this level through September 1970. The study also shows that the use of sterilization for the prevention of conception has become much more common than it was. Among older couples of childbearing age (wife aged 30-44) using any method to prevent conception, 25 percent report sterilization (12 percent of the husbands and 13 percent of the wives). In 1965 the comparable proportion was 16 percent. Sterilization has become the most common method of fertility control at the later child-bearing ages and oral contraception is the next most common (21 percent).

Significance to Biomedical Research and Program of the Institute: This study will continue a unique series of studies on fertility, birth expectations and contraceptive practices among women in the U.S., and will provide a base of reference for prospective studies on contraceptive practices and the influences upon the health of women and children. It is an essential part of the public health aspect of the study of contraceptives as well as of attitudes toward family size.

Proposed Course: This contract, initiated in 1970, was continued into fiscal 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Analysis of the Decline of Fertility in Europe,
by Province
Contractor : Office of Population Research, Princeton University
Money Allocated: \$68,670 (FY 1970); \$87,160 (FY 1971); \$21,977 (FY 1972);
\$113,384 (FY 1973)

Objectives: The propose is to analyze the decline in fertility in the more than 700 provinces in Europe from the time in the 19th Century that fertility was essentially constant until the present, or until it reached a minimum and began to rise. Its aim is to determine the circumstances, in terms of the education, religion, occupational structure, residence patterns, etc., of the population, under which fertility declined. This study will make a definitive contribution to the social history of Europe and will substantially improve our understanding of the factors relating to the decline of fertility.

Major Findings: So far, two books have been published as a result of this project: A Century of Portuguese Fertility, by Massimo Livi Bacci, and The Decline of Fertility in Germany After 1870, by John Knodel (in press). In addition, a great deal of methodological development has occurred as a result of this study--particularly a new procedure for reconstructing age distributions on the basis of time series of births and deaths.

A recent finding of general interest relates to the effects of declines in infant mortality on the fertility rate. It has generally been supposed that a decline in infant mortality is a pre-condition for reductions in fertility--that is, reductions in infant mortality tend to increase average number of surviving children, and parents compensate for this rise by having fewer births. Knodel's manuscript on the decline of fertility in Germany shows that the decline in marital fertility and the decline in infant mortality proceeded at about the same time, but in almost half of the provinces the decline in fertility came before the decline in infant mortality.

Other findings suggest that the diffusion of knowledge about methods of fertility control may operate to reduce fertility independently of some of the social and economic factors often thought to be essential in accounting for reductions in fertility.

Significance to Biomedical Research and Program of the Institute: Fertility has been designated as an area of the highest priority by the Center for Population Research. This study will furnish information in far greater detail than has previously been available on the social and the economic factors associated with the decline in fertility in many countries of Europe.

Proposed Course: This project, underway for several years, was extended into fiscal 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : A Study of Differentials and Trends in Marital Disruption,
Remarriage, and the Fertility of Remarriage

Contractor : University of Wisconsin

Money Allocated: \$34,773 (FY 1970); \$42,178 (FY 1971)

Objectives: The purpose is to examine patterns of marital disruption, remarriage, and the fertility of remarriages in the United States from data obtained in the 1967 Survey of Economic Opportunity, which involved a large sample and covered the national population. The study will be concerned with (a) differentials among socio-economic groups and racial groups, (b) patterns of divorce and widowhood in relation to age, marriage duration, color, education, age at marriage, order of marriage, sex, presence of children, place of residence, and the length of the interval between marriage and the birth of the first child, and with patterns of remarriage in relation to the above; and (c) how these patterns have changed through time. It will also investigate the fertility patterns of second and subsequent marriages and how differential fertility and changes in fertility are influenced by differential marital disruption.

Significance to Biomedical Research and Program of the Institute: This study will contribute to the understanding of the effects of marital disruption and remarriage on fertility.

Proposed Course: This contract, initiated in FY 1970, is expected to be completed during FY 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : New Estimation Techniques for Demographic Analysis
Contractor : University of North Carolina
Money Allocated: \$75,579 (FY 1970); \$172,876 (FY 1971)

Objectives: The purpose is to develop sensitive indicators of change in fertility patterns. Its specific objectives are to develop and assess appropriate measures for the analysis of the lengths of "closed" intervals (between births) and "open" intervals (since the most recent birth). Such data are assuming increasing prominence as predictors of natality change and indicators of the effects of family planning programs. The contract will develop new measures, assess their accuracy, reliability, and sensitivity, and compare various procedures for collecting the necessary data. To evaluate the measures, an existing microsimulation model will be modified and other special purpose models utilized as necessary. The project should contribute to more general methods analyzing current natality data and to the choice of appropriate indices of change.

Under this contract, experiments using augmented simulation models will be devised to (a) simulate reproductive performance, postulating different behavior patterns reflecting different levels and rates of population change, (b) simulate different schemes of sampling in data collection in real populations, (c) calculate from such simulated data the values of the measures derived above, and (d) assess the behavior of these measures in response to the conditions of the experiments.

Major Findings: Efforts to date have focused on modifications of existing microsimulation models of fertility behavior to use in carrying out the purposes of this study. Various experiments with these models have dealt with a number of aspects of fertility measurement, among them measures of "fecundability," or the monthly probability of conception, which generally varies between .15 and .20 among women not using contraception.

Significance to Biomedical Research and Program of the Institute: The development of more sensitive indicators of current changes in fertility has important implications for both the collection of vital statistics and the evaluation of the effectiveness of public health programs, including those relating to family planning.

Proposed Course: The contract, initiated in FY 1970, has continued through FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Urbanization, Migration and Fertility in Thailand

Contractor: Brown University

Money Allocated: \$28,647 (1970); \$92,475 (1971)

Objectives: The purpose is to analyze census data and data from a national longitudinal survey of Thailand to determine the relationship between urbanization, migration, and fertility. The fertility data available from this national survey, cross-tabulated by residence and household type, permit their evaluation in terms of a rural-urban continuum, and their control for age differentials provide the opportunity for comparisons of fertility levels across the urban-rural continuum for differentials by religion, literacy, education, economic status, migration, and marital status.

More specifically, the study will analyze: (1) cross-sectional and trend data on urbanization and on the relationship between fertility and socio-economic variables based on extensive data collected in the 1960 Thai Census and preliminary data from the 1970 Census; (2) rural and urban data from the National Longitudinal Survey of Social, Economic, and Demographic Change in Thailand collected 1968-1970 to ascertain: (a) total migration experience of the population; (b) the role of population and economic pressures on movement away from the village of birth; and (c) the relation between migration and fertility; (3) rural-urban labor force, occupation, and industrial differentials and data on literacy, education, and religion; and (4) data for integrating the material from the rural and urban survey operations.

Significance to Biomedical Research and Program of the Institute: The study will add to available knowledge of rural, urban and other socio-economic differentials in fertility in an area for which data have previously been non-existent, and for a culture which is quite different from that in the United States. Hence, it will add another perspective to the understanding of influences upon fertility behavior.

Major Findings: The high fertility and low mortality characterizing Thailand place its population among the fastest growing in the world. Yet within Thailand there are considerable fertility differentials by residence, education, occupation, and religion. Fertility in Bangkok was 25% below that of most of rural Thailand, corroborating the significant impact of urban residence on fertility. Education is also inversely related to fertility, regardless of residence status (urban or rural). For older women the sharper differential is between those with no schooling and those with primary schooling; for younger women achievement of more than a primary education has the greatest effect on fertility. The relationship between labor force participation and fertility in Thailand based on analysis of the 1960 census is less clear,

because unpaid family workers were included in the labor force, but for the Kingdom as a whole, women engaged in farming have by far the highest fertility. There are also significant fertility differentials among religious groups. Controlling for both age and urban-rural residence, Confucian women have slightly more children than do Buddhists; Moslem women have the fewest children. Community surveys indicate that more frequent divorce and re-marriage may reduce Moslem rates but do not explain the lower Moslem rate in the youngest age group, since Moslems tend to marry early. Moslem women limit the number of their children to a greater extent than do either Buddhist or Confucian women. Greater reliance on older traditional methods of birth control or higher rates of sterility due to poorer health may be the explanatory factors.

Proposed Course: The contract, initiated in 1970, has been extended to FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Economic Analysis of Migration from Rural Eastern Kentucky to Selected Urban Centers

Contractor: University of Kentucky
Money Allocated: \$71,600 (1970); \$61,000 (1971)

Objectives: The purpose is to measure the impact of migration in economic terms on individual donor and recipient local governments and the Federal Government. It will seek to measure and analyze the cost and returns of population relocations through migration from eastern Kentucky to recipient metropolitan areas in Kentucky and Ohio and to isolate the major socio-economic variables giving impetus to this movement. It will include analyses of both initial migration and return migration and the marginal cost of providing selected public services in high density neighborhoods in the cities studied.

Significance to Biomedical Research and Program of the Institute: The evaluation of the consequences of migration is especially relevant to the program of the Center for Population Research, which has as one of its emphases the study of the consequences of population growth and change. It will provide information of concern to the Public Health Service in that it will document the cost to both donor and recipient communities of migration within the United States.

Major Findings: At this writing, analysis of the data has not been completed, but preliminary analysis indicates that the migrants studied improved their personal economic position by moving and did not represent a net cost to the receiving community in terms of services rendered.

Proposed Course: This contract, initiated in FY 1970, will be completed in FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Processes of Demographic Development in Imperial Russia and the Soviet Union

Contractor: The Ohio State University
Money Allocated: \$52,700 (1970); \$107,155 (1971)

Objectives: The purpose is to study the mutual relationships between population growth, economic and social change in Imperial Russia and the Soviet Union. In order to identify the long-term factors that determine and influence demographic changes, the study will explore the determinants of Soviet population growth from a long-run point of view, which also indicates the early stages of change during the Imperial Russian period, and to appraise the Soviet efforts to design and implement population policies to influence fertility patterns and other demographic variables. The principal themes of the proposed research will be those concerned with the implications of the developmental process for population growth, with major attention given to the question of fertility.

Significance to Biomedical Research and Program of the Institute: The study of demographic development during a process of economic development and social change during the 18th, 19th, and 20th centuries in a society which shows both European and Asiatic influences is of the highest relevance in understanding the process of population change in the world at large. The findings will increase knowledge of the factors associated with declining fertility, the use of contraceptives and of abortion, and will provide some insight into the effectiveness of a variety of population policies.

Major Findings: A general decline in fertility over much of the Soviet Union has become evident since the early 1960s. Net Reproduction Rates--less than unity in all but Central Asia, the Caucasus and scattered parts of the RSFSR--imply ultimately declining growth of the total population rather than the long-run moderate rates of increase that most Soviet demographers and planners regard as desirable. Changes have been dramatic, and regional differences, very great. In European Russia the downward trend in fertility was persistent. Fertility declined from 1900 to 1959 in both urban and rural areas, with the rural level being about 25% above the urban. It declined sharply in the rural areas of European Russia, in the 1930s, which were also affected by exceedingly high out-migration as a result of agricultural collectivization and industrialization. However, Soviet Central Asia and the Caucasus, except for the Georgian and Uzbek Soviet Socialist Republics, evidenced little or no decline in fertility. Implications of this research are: (1) the recently declining fertility will result in a decline in the population of working ages beginning in the 1980s; (2) the crisis in labor allocation and distri-

bution will be aggravated if present regional fertility differentials are maintained and if certain national groups in areas of relatively high fertility in Central Asia/Caucasus continue to be unresponsive to economic or other incentives to move; and (3) Soviet demographers are becoming increasingly concerned that non-Slavic groups through their higher fertility may overwhelm the Slavic groups in the long run.

Proposed Course: The original contract, initiated in 1970, was renewed with funding for FY 1971 and FY 1972. Completion is expected in FY 1973 but may be given an extension.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Sex Role, Family Structure, and Fertility Control

Contractor: Indiana University

Money Allocated: \$207,260 (1970)

\$37,120 (1971)

Objectives: The purpose is to study the relationships between definitions and perceptions of male and female roles within the family and achieved and anticipated fertility. The specific concern of the study is with those decisions relating to childbearing and with the antecedent conditions that structure them, with emphasis upon the husband-wife interaction and, particularly, with reference to the alternative sets of roles and rewards which are perceived as valued and open to the female. Emphasis will be placed upon the effect of wife's employment and career orientation upon decisions with respect to the size of family. The contract involves the interviewing of a regional probability sample of 3,000 persons, 15 to 45 years of age, drawn from the East North Central Region of the United States.

Significance to Biomedical Research and Program of the Institute: This contract bears on many aspects of the Institute's concern with contraceptive practice and family planning: age at marriage, decisions affecting fertility, alternatives to childbearing, such as employment of the woman, probability of divorce, and socialization for marriage and parenthood.

Proposed Course: This contract was initiated in FY 1970 at a funding level of \$207,260. It was renewed in FY 71 with \$37,120 in funds. The progress of the project has been excellent, but unavoidable delays were experienced with the survey. As a result, it was extended until 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Socio-economic Factors in the Reduction of Natality
in the Less Developed Areas

Contractor: Food Research Institute
Stanford University

Money Allocated: \$60,902 (1970)
\$65,698 (1971)
\$84,402 (1972)

Objectives: The objective is to examine the usefulness of the notion of the demographic transition, or modifications thereof, as a model for fertility reduction in non-Western areas, and to determine the context of socio-economic conditions that appear to be the basis for the onset of a decline in fertility in the underdeveloped or developing countries of the world. Measures of natality and of socio-economic development are being studied in each of the several major regions of the world to determine their accuracy and comparability, and to analyze their interdependence. The study will also seek to provide a possible measuring stick or projective device for estimating the effects of efforts to reduce fertility in the future.

Significance to Biomedical Research and Program of the Institute: This study will provide insight into the process of fertility decline in the developing and underdeveloped regions of the world and the extent to which the socio-economic conditions associated with natality decline in the West are having, or are likely to have, the same effect in the non-Western world, as well as indicating what additional factors may influence fertility decline in these areas.

Proposed Course: This contract, begun in FY 1970, was renewed with funding for FY 1971 and was continued into FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Illegitimacy - Demographic and Sociological Studies

Contractor: University of California
Money Allocated: \$86,969 (1970); \$111,076 (1971)

Objectives: The purpose is to develop new information about illegitimate births and expand the methodology available for further study of illegitimacy and related phenomenon. The study, dependent in large part upon available vital and health records, will include the evaluation of California State data on illegitimate births and an analysis of the incidence data for the years 1966 and 1967. The study also includes record linkage cohort studies and an evaluation of interview studies relating to the phenomenon. On the basis of the data to be accumulated, the study will: (1) evaluate State data on illegitimate births; (2) estimate the incidence and distribution of illegitimate births; (3) study the antecedents and consequences of out-of-wedlock pregnancies, as shown by linked vital records; and (4) investigate the feasibility of surveys of attitudes and behaviors in relation to non-marital unions and illegitimate births.

Significance to Biomedical Research and Program of the Institute: This study represents a much-needed basic investigation concerning information which is difficult to get but bears upon a persistent social problem with great significance to the health of both women and children. It will also contribute to the methodology of studying these phenomena.

Major Findings: In 1967 an estimated 10.5 percent of all births in California were illegitimate as compared with 9.0 percent for the United States. For black women, the rates in California in 1967 were four to five times the size of the rates for white women. Compared to mothers of legitimate births, both white and black mothers of illegitimate babies average much younger and are more likely to be bearing a first child. Some 42.5 percent of illegitimate births occurred to mothers under age 20 while only 14.7 percent of legitimate births occurred to mothers under age 20. Illegitimate rates, however, are highest for women in their twenties. Illegitimacy is much more frequent in lower income and social class groups. Illegitimate births are by far the most common in poor minority groups but the social meaning and social consequences of illegitimate births are not adequately understood. An increasing number of children face serious and overwhelming handicaps in their growth. With some exceptions illegitimate children are not wanted in the first place by mothers who themselves are immature and most illegitimate children lack a father substitute.

Proposed Course: The contract initiated in FY 1970 was completed during FY 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Relationship between Age at Marriage, Birth Intervals and Total Fertility
Contractor : U.S. Bureau of the Census (reimbursable agreement)
Money Allocated: \$140,000 (FY 1971); \$165,000 (FY 1972)

Objectives: This project has enabled the Census Bureau to collect, tabulate, and analyze data on fertility from the Current Population Surveys of June, 1971, 1972, and 1973. The agreement permitted the addition of questions on children ever born, childspacing, birth order, birth expectations, and marital history to the June 1971 Current Population Survey. In June 1972 and 1973, the project permitted the addition of questions on children ever born, birth expectations, and date of last live birth. The general objectives of the project are to provide data for the analysis and interpretation of current fertility trends and to develop better methods of projecting fertility rates into the future.

Major Findings: The most notable finding of this project has been the decline in the number of births expected by married women aged 18-24. In 1972, the average number of births expected by such women was only 2.3, compared with 2.9 in 1967. The proportion of young married women expecting a total of two children increased from 37 percent in 1967 to 57 percent in 1972, while the proportion expecting three or more children declined from 56 percent to 30 percent. The number of children expected by young women is now consistent with the replacement level of fertility--that is just enough births to replace the number of people in the parent generation. If such low rates continue indefinitely, the population of the United States will stop increasing, except for immigration, in about 65 years.

Significance to Biomedical Research and Program of the Institute: This study will make an important contribution to the analysis of trends in fertility, by analyzing age at marriage and timing of births within marriage in relation to levels of fertility. It will also provide basic information for an understanding of fertility behavior and its changes in the United States over time. The project thus bears directly upon a primary concern of the Institute's program.

Proposed Course: This project has been continued through FY 1974 to permit the tabulation and analysis of the results of the June 1973 Current Population Survey.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Distribution and Differentiation of Population Within Metropolitan Areas

Contractor: University of Michigan
Money Allocated: \$110,936 (1971)

Objectives: This study analyzes 1970 U.S. census tape data for 25 SMSAs and compares these with census materials for four Canadian metropolitan areas to determine: (1) the degree of residential segregation of different socio-economic groups and of families in different stages of the life cycle; (2) the pattern of segregated housing of different types and ages; (3) the relation of housing type to socio-economic characteristics of occupants; and (4) variations in population distribution in different metropolitan areas.

Significance to Biomedical Research and Program of the Institute: The distribution and differentiation of population in cities is of major importance, but there have been few empirically based studies on this topic. This study will provide a framework for considering the similarities and dissimilarities of a sufficient number of metropolitan areas in the U.S. and Canada to give meaningful results as to population dynamics and will develop generalized analytical tools for the study of other cities. This research goes beyond a study of segregation patterns to look at associated factors. It is thus highly relevant to NICHD goals and should be useful to urban planners as well.

Major Findings: In most large urbanized areas the suburban population has a higher average socio-economic status than has the central city population, and there has been a slight increase in city-suburban differentiation as determined by occupational and educational indices. The disparity in racial composition increased from 1950 to 1970, and even where there was suburbanization of blacks around seven large cities, the vast majority of blacks were heavily concentrated in a few suburbs. Economic criteria account for very little of the observed distribution of blacks. In the suburban ring as well as the central city, blacks at all educational attainment levels are highly segregated from whites of similar attainment. The average level of school segregation among the 60 largest cities in 1967, as measured by the index of dissimilarity, was 79 (87 in the South and 74 in the North).

Proposed Course: This project, begun in FY 1971, is expected to be completed in FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Human Fertility in Poland and the U.S.S.R.

Contractor: Western Washington State College

Money Allocated: \$47,169 (1971)

Objectives: The primary objective is an investigation of social, political, and economic influences bearing on fertility in Poland and the U.S.S.R. and including rural fertility differentials by size of land holding, and abortion and fertility differentials by employment status of women in Poland, and the social and economic correlates of fertility and birth control in the U.S.S.R.

Since the mid-1950's the Soviet Union and the Eastern European countries within its sphere have published more demographic and economic data and have liberalized abortion laws (partly because of limited housing and the need to increase the number and proportion of females in the labor force).

The demographic processes in the Communist countries need to be studied for the light they may shed upon relationships of population to socio-economic-political conditions which are both similar to and different from those in other regions. Fertility differentials between the more developed parts of the RSFSR on the one hand and the five Union Republics of Central Asia and of the Caucasus, and of the autonomous republics on the other hand should throw additional light on basic demographic processes in various cultural contexts. In addition, the effects of sharp and contradictory policy changes need exploring both to assess the effects of such shifts and to avoid similar problems in the U.S.

Significance to Biomedical Research and Program of the Institute: All aspects of the research are program relevant, but particularly the impact of abortion, of urban-rural, religious and other ethnic differentials and, more generally, of "modernization," on fertility trends. Moreover, the investigator is one of a very small number of persons in the U.S. with both the language and technical skills to pursue studies in the Slavic countries.

Major Findings: The inverse relationship between human fertility and education is fully substantiated with data for the 36 major ethnic groups in the U.S.S.R. Variation in fertility attributable to the traditionally religious values can be explained in terms of the age-specific marriage and educational differentials known to have existed in the past and still present in the multi-national population of the Soviet Union.

A number of social and demographic variables indicate that fertility bears no relation to the proportion of economically dependent women in the political and social context of the Soviet Union. Non-fertility variables (dis-

proportion between married women and married men and the percentage of urban population, and other components identified with modernization, such as education, divorce rates, etc.) bear more significantly on female employment. The decrease in the marriage differential for men and women and the rise in urbanization between 1959 and 1970 suggests a substantial rise in the proportion of females not entering into, or retiring from, the labor force.

Proposed Course: This study initiated in 1970, has been extended to early FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effect of Income Payments to Reduce or Increase
Natality

Contractor: University of Illinois
Money Allocated: \$34,843 (1971), \$9,253 (1972)

Objectives: This study will explore the effect of income on fertility in two different studies: (1) by asking supposititious questions in a sample survey administered to a regional sample of the U.S. population concerning the effect on personal fertility of government payments for more or fewer children; and (2) by studying the relationship between income and fertility using the 1-in-1,000 tape from the 1960 census, using multiple regression techniques within fine sub-classifications and interpreting results with the aid of demand theory.

Significance to Biomedical Research and Program of the Institute: The relationship between income and fertility comprises a critical area of research and thus is highly relevant to the Institute's program.

Major Findings: The data suggest that money incentives could have a significant effect in inducing people--particularly low income people--to have more or fewer children. Moreover, money incentives would seem to be more effective for reducing family size than for increasing it. Respondents feel that money incentives would have more of an effect upon their neighbors than upon themselves.

Proposed Course: The contract, initiated in fiscal year 1971, was completed in fiscal 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Kin Network and Population Dynamics

Contractor: University of Southern California

Money Allocated: \$17,124 (1971); \$20,086 (1972); \$8,561 (1973)

Objectives: This study will collect data on the extent and relevance of kin networks in the United States to determine how they affect fertility and migration. It is hypothesized that the greater the intensity of the kin network (frequency of contact, etc.) and of the interchange therein, the higher the fertility. The kin network may also influence the amount and direction of migration. A conference of persons experienced in the family and fertility will be convened to approve and amend the trial questionnaire and another to evaluate the results. The pretest will be conducted by National Analysts, Inc.

Significance to Biomedical Research and Program of the Institute: Research on the relationship of the kin network to fertility has high program relevance. Very little work in this area has been done.

Proposed Course: This project was initiated in FY 1971 and has been extended through FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Explorations in Fertility Values

Contractor: Calspan, Inc.

Money Allocated: \$139,916 (1971)

Objectives: The purpose of this project is to develop and demonstrate methods to ascertain how fertility values are related to family size preferences. The principal investigator is currently developing ways in which family size preferences and the desired number of children under various economic, social, and psychological conditions can be quantified. Once the methodologies for these two tasks are refined, it will enable other investigators to measure accurately the number of children persons wish to have under various situations. Moreover, it should give insight into how people come to desire different numbers of children at different points in time.

The study entails two surveys of 300 white, married women in the 15-44 age range. For each major religious group (Protestant, Roman Catholic, and Jewish) there will be 100 women represented.

Dr. Terhune has, in his first study, isolated the following values associated with family size desires: (1) economic concerns in general, providing for children in particular; (2) parental attention to each child; (3) companionship of siblings; (4) the mental strains and worries of parenthood; (5) physical work and energy requirements of parenthood; and (6) over population concerns. Furthermore, he has been able to measure relatives preferences among the family sizes. A psychometric method (expanded pair comparisons) gave useful and internally consistent results as a measure of family size preference. In the second year Dr. Terhune will measure the degree of satisfaction expressed for each of the fertility values when different sized families are considered. A single model of overall satisfaction will be developed to predict family size preferences.

Significance to Biomedical Research and Program of the Institute: This project examines the socio-psychological factors which influence individuals' desired family size under different situations and in different points in time. This type of study has great potential in assisting family counselors in helping individuals plan their family and in assisting individuals who may be experiencing familial problems as a result of too many or too few children.

Proposed Course: This is a two-year contract. The first survey has been completed and the data analyzed. The second survey will be done during the contract's second year.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Correlates of Low Fertility in a Developing Country

Contractor: Wayne State University
Money Allocated: \$21,545 (1971); \$16,639 (1972)

Objectives: This study will examine the antecedent factors to explain how the level of urban Greek fertility can be low in the absence of (a) organized birth control and other governmental programs, and (b) economic industrial development.

Data already collected in the Greater Athens Metropolitan Area in 1966-67 will be analyzed to study: the relationship of the employment of women to fertility and birth control, examining three aspects of female employment: (a) the degree of compatibility between the working and mother roles, (b) the type of occupation, and (c) the degree of work commitment.

Significance to Biomedical Research and Program of the Institute: The central problem of trying to account for the low birth rate in Greece without much industrialization or family planning programs is of considerable theoretical and possibly practical significance. The project is highly relevant to the Institute's interests in the determinants of fertility.

Major Findings: The fertility pattern in Greece follows a U-shaped distribution according to socio-economic status--lower and working class women and wealthy aristocratic women have larger families than do middle class women. Couples who contribute financially to their parents, and who in turn expect future help from their children, have relatively large families, partly because mother substitutes are readily or cheaply available. Conversely, the higher the level of aspirations that a couple has for its children, the fewer the number of children it will bear. Couples in which the husbands are occupationally stable or upwardly mobile have significantly fewer children. In general, with middle class people, intervening variables such as income, degree of social mobility, and socio-psychological factors such as wife's degree of work commitment play a more determining role with respect to fertility.

Two categories of women--those of very traditional values and little education who begin work before age 18 in the fields or in low paying urban jobs and those who start work in their thirties because they need the money--have significantly higher fertility than those who commence work between 18 and 24 years of age (lesser professional, clerical, and sales women) and those who start in their later twenties (college educated, professional women). Work commitment is also a significant factor, for those women who have a high level of commitment to their work rather than working merely to supplement the family income, have fewer children than do non-working women and working women with

low work commitment. Indeed, unpaid or low salary working women have as many children as do non-working women.

Proposed Course: It is expected that the work on this contract, begun in 1971, will be completed during fiscal 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Rates and Consequences of Population Change: The Growth and Decline of Counties in the Mountain Region

Contractor: Colorado State University (Fort Collins)
Money Allocated: \$85,480 (1971)

Objectives: This study will describe the population changes that have occurred in the Mountain Region (8 states, 279 counties stretching from Montana to Arizona) during the period 1950-1970. Almost one-half of these counties lost population between 1950 and 1960 and more than one-half declined in population during the decade 1960-1970. The main interest centers on the consequences of population change--consequences to public finances, retail activity, housing utilization, and agricultural activity.

Censuses of population, governments, businesses, housing and agriculture, and other published and unpublished pieces of information (available from state and local sources) will be used. Components of change as well as indices of social welfare, such as doctors per capita, use of health services or school retardation, and grade continuation rates will also be explored to clarify some of the relationships and problems being investigated.

Significance to Biomedical Research and Program of the Institute: Depopulation is one of the more important, yet neglected, aspects of the population problem in the United States. Most of this nation's counties either lost population or experienced net out-migration in the last two decades, and very large geographic areas are coming to have sparse populations. Few studies have investigated the process or the consequences of such population losses.

Proposed Course: This study, funded in 1971, is expected to be completed in FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Analysis of the Spacing of Pregnancies, Births, and Completed Fertility in Relation to Socio-economic Status, Intergenerational Mobility and Aspirations for Children, Aberdeen, Scotland

Contractor: Brown University
Money Allocated: \$48,469 (1971)

Objectives: The study will investigate: (1) how spacing of pregnancy varies with the type of terminations of previous pregnancies, the stage in the family life cycle, age at marriage, age at first pregnancy, and socio-economic status; (2) how the spacing of live births, the number of children ever born, and the length of fertility span vary by age, age at marriage, age at first pregnancy, socio-economic status, social mobility, and mobility aspirations for children; (3) the life conditions under which women are likely to resort to terminations and sterilizations; and (4) how the size of the family of origin, and position on a traditionalism scale are related to social mobility, present socio-economic status, aspiration for children and fertility.

The data are based on a probability sample of resident women of Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955. The pregnancy histories of these women up to 1964 have been copied from hospital records and will be updated to 1970; the other required information has been obtained by means of an interview survey (2,000 cases). Preliminary tabulations of the survey data indicate that a "significant test of intergenerational mobility hypothesis" is possible with the data at hand and that the "data on mobility aspirations for children are particularly exciting and promising."

Significance to Biomedical Research and Program of the Institute: The Aberdeen data provide a rich source of information on how the outcome of one pregnancy influences subsequent pregnancies, how family origin variables relate to childspacing, and how fertility relates to social mobility, and thus contributes significantly to an understanding of factors influencing child-bearing as well as the consequences of family size.

Proposed Course: This study, initiated in 1970, will be completed in FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Attitudinal Factors Affecting Family Size

Contractor: Worcester Foundation for Experimental Biology
Money Allocated: \$40,089 (1971); \$42,397 (1972); \$39,036 (1973)

Objectives: This research (1) tests the hypothesis that women who perceive little difference between sex roles and who reject (for themselves) the stereotype of women as less assertive, ambitious, intelligent, etc., than men are the ones most likely to accept employment and to desire and achieve relatively small family size; and (2) the "perceived sex-role differences and self-concepts" will be related to "antecedent biographical characteristics and family backgrounds." The second goal of the study will provide information for a later longitudinal study. The sample will include 1,000 persons--375 married men, their 375 wives, 125 single men and 125 single women of evenly distributed ages (17 to 55) and socio-economic levels (based on husband's occupation or, for younger men, education). The subjects will be obtained from local civic, professional, religious, and labor union groups and will be selected to be representative of the community.

Subjects are being asked to fill out the questionnaires in group sessions. One questionnaire calls for rating 122 tested items three different ways, to diagnose male and female "sex-roles" as perceived for men, for women, and for themselves. The other questionnaire covers background data, employment and occupational history--including attitudes toward employment, achieved and desired family size, and contraceptive attitudes and practices. There are also questions about plans for working at the time of marriage, the factors that determined entry into the labor force or withdrawal from it, ideal family size and family planning orientation.

Significance to Biomedical Research and Program of the Institute: This study of several aspects of the relationship between achieved or planned fertility and the perception of the sex role of women has importance to the understanding of fertility behavior, which is crucial to the Institute's mission.

Proposed Course: This project is expected to be completed in fiscal 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Physicians' Attitudes Toward Abortion

Contractor: Temple University

Money Allocated: \$208,053 (1971); \$44,000 (1973)

Objectives: The major purpose of this study is to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients. The doctors' attitudes concerning criminal as well as legal (therapeutic) abortions and relevant general attitudes toward fertility intervention and birth control will also be examined. Some 1500 interviews will be given to a national probability sample of M.D.'s and osteopaths whose practice makes the subject of fertility control a reasonable occurrence. The interview schedule will be developed by video and audio taping a series of "In-group" discussions with physicians from four regions of the United States: the Northeast, South, Midwest, and West. The tapes will be analyzed and questions concerning how best to approach the subject of birth control and abortion will be determined. The questionnaire will include the following areas: definition of pregnancy and of abortion; perception of peers and of the public mood toward abortion; individual perceptions of patients and their circumstances toward abortion; etiology in the correlates of attitudes toward abortion and specific objections to abortion; and the actual behavior of the physician regarding prescriptions of birth control methods and when appropriate, the performance of abortion.

Significance to Biomedical Research and Program of the Institute: Since there is a trend toward the liberalization of abortion statutes and the physician is becoming increasingly involved, it is crucial to determine how the medical profession views abortion. This study accordingly has a great deal of relevance to the Institute's program.

Proposed Course: This project, initiated in 1971, was originally funded for 2 years and has been extended for eight more months.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Socioeconomic Status and Family Factors in Residential
Mobility

Contractor: U.S. Bureau of the Census (reimbursable agreement)
Money Allocated: \$4,983 (1971)

Objectives: This study will combine data from the March Current Population Survey taken by the Bureau of the Census for the years 1966-1970 to study annual migration during that period by single years of age. It is designed to provide new perspectives on the joint influence of various socioeconomic and familial statuses on the probability of making different types of moves. It will also produce statistics allowing more precise estimates to be made of the number of times an individual would move in his lifetime if he moved according to currently existing age-specific rates of residential mobility. The study will also examine the effects of number and ages of children (simultaneously considered) on the probability of moving. In these comparisons, age of family head will always be controlled, and so will occupation in some cases. Other questions to be investigated include the effect of the wife's labor force status (whether or not she has a job) on the family's geographic mobility when age and occupation of husband are held constant. A related question is the overall effect of occupation on geographic mobility for men and women, when age and marital status are held constant. In most cases the effects of the various socioeconomic and family statuses will be examined for the Negro and white populations separately. Comparison of the annual data on moves with data from the 1970 census based on residence in 1965 and 1970 will be possible.

Significance to Biomedical Research and Program of the Institute: This research will produce information on characteristics of migrants not previously assembled, making possible more refined estimates of probabilities of moving each year, by occupation, education, race, and family characteristics. It is a carefully planned project by a competent investigator, at marginal cost. Study of migration differentials is relevant to the Institute's program, especially as these relate to family status and size of family.

Major Findings: This study documents certain aspects of the high mobility of the American population. The principal investigator has calculated that the U.S. population moves an average of 13 times in his life. Half of these moves occur before the 26th birthday, one-fourth before age 18. The college-educated are more likely to be involved in long-distance moves; the poorly educated predominate in short distance moves. Childless couples are more mobile than couples with children, and size of family decreases the probability of long-distance moves between counties and states. Most mobility of couples with children occurs when children are under 6 years of age.

Proposed Course: Work on this contract was completed in fiscal 1972.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Economic Cost and Value of Children in Four Societies

Contractor: Columbia University

Money Allocated: \$215,320 (1971)

Objectives: This research is directly aimed at the assessment of the importance of economic costs of, and returns from, children as possible factors in fertility decisions. The secondary aim will be the development of refinement of research methodology for such studies.

Peasant communities in Indonesia, Nepal and a Latin American country and a farming community in the United States will be studied using anthropological techniques of intensive observation together with extensive questionnaire surveys. Field investigation and data analysis will be carried out by four research assistants familiar with the particular communities in which they will be doing the research. Intensive observation of domestic and productive activities will be used to assess the costs and benefits associated with different family sizes in each community. Census and fertility surveys of the four populations will be carried out to determine actual fertility and attitudes regarding fertility. Data analysis in the third year will provide comparable data for all societies.

An accurate census will be taken with respect to demographic information, socio-economic status, land holdings, etc. The communities studies will each comprise about 400 households of which a sample will be selected for intense study to include: (a) households in different stages in the developmental cycle, etc.; and (b) the organization and productivity of labor, and the economic cost of children, both direct and indirect. A third sample will be comprised of all married women of reproductive age or a randomly drawn sample of such women with interviews to be based on the short list of variables for comparative fertility studies from the International Union for the Scientific Study of Population.

Significance to Biomedical Research and Program of the Institute: This study deals with a crucial area of population research that has definite relevance to the Institute's program. Very little information has been available on the economic cost or benefit of children and how these are perceived, in either industrial or non-industrial societies.

Proposed Course: This study, initiated in 1971, is a three-year project scheduled for completion in FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of the Growth of Mexican-American Families

Contractor: University of California at Los Angeles

Money Allocated: \$71,811 (1971); \$33,599 (1973)

Objectives: The objective of this research is to design and carry out an initial and exploratory analysis of the determinants of fertility and family planning among Mexican-Americans in Los Angeles county. Next to Blacks, Mexican-Americans constitute the most important minority in the United States and have the highest fertility of any major cultural or ethnic minority. The study emphasizes the effects of social structure and social norms on fertility and family planning but also considers socio-psychological variables. A multistage probability sample of 1200 Spanish-surname women of childbearing ages is being interviewed to collect the following data: (1) reproductive experience, including a history of births and pregnancies as well as number of children expected or desired; (2) past and anticipated contraceptive usage; and (3) determinants of both reproductive and contraceptive experience. More specifically, these determinants include knowledge of, and attitudes about, contraceptive practices and family size, extent of orientation toward or involvement in Mexican-American society, socio-economic status and social mobility, family structure, characteristics of community residence, migration history and religious practices. Various multi-variate techniques will be used to analyze the data.

Significance to Biomedical Research and Program of the Institute: The Mexican American minority is of particular interest because it comprises one of the minority groups and enclaves of high fertility in a nation with generally declining fertility.

Major Findings: The principal investigator and the Survey Research Center at UCLA have found that they must take into account the wishes and concerns of Mexican American women and organizations in designing the sample, using women interviewers suggested by their organizations, etc. This points up the increasing sensitivities of respondent groups in undertaking social sciences research.

Proposed Course: This contract funded in June 1971, will be completed in FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Determinants of Fertility
in the United States

Contractor: The Rand Corporation
Money Allocated: \$160,000 (1971)

Objectives: This study attempts to identify the principal social determinants of family size and current fertility patterns in the United States. Using three sets of data (1) Survey of Economic Opportunity 1967, (2) Longitudinal Survey of Family Economics (1967-1970, and (3) a Time Budget Survey taken in 1964, the investigator is concentrating on the effects of such factors as income, time of marriage, female labor force participation, and the timing and frequency of migration upon size of family. The method of analysis consists of multivariate analysis of data from the three surveys.

Significance to Biomedical Research and Program of the Institute: The study of the determinants of fertility and the development of methodologies related to them are one of the major concerns of the program in the Behavioral Sciences of the Center for Population Research. Through the process of multivariate analysis this study will clarify the effect of the various factors already known to affect fertility and will describe with greater precision the behavioral and biological mechanisms affecting fertility and how these differ for various segments of the U.S. population. The study should provide a basis for estimating the probable impact on the birth rate of (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in female earnings relative to those of males, (4) change in tax deductions for dependents, and (5) housing and rent subsidies.

Proposed Course: This contract was funded in 1971 for a period of 18 months and will be completed during fiscal 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Determinants of Fertility, Migration, and Population
Change of Counties in the United States, 1950 to 1960

Contractor: University of Georgia
Money Allocated: \$81, 781 (1971)

Objectives: This study will analyze the trends and patterns of population change for the total white and nonwhite populations of all counties in the United States from 1950 to 1960. Changes in this period will be explained in terms of fertility, mortality, and net migration, the major emphasis being on fertility and migration and upon the white and nonwhite differentials. The study also investigates the socio-economic and demographic determinants of each of the three components of population change among both white and nonwhites. County fertility rates and differentials, for example, will be examined by age (and age standardized), sex, color composition, and by levels of urbanization, industrialization, income, occupation, education, and related factors. Finally, tabulation and organization of the data by color, not previously compiled on a uniform basis will provide benchmark data for all counties in the U.S. for the 1950-1960 period for comparison with similar trends in the 1960-1970 decade. Plans have already been made for future computation of the 1960-1970 components of county population change by color. Maps will be prepared showing county birth, death, and net migration rates, and natural increase rates, by color. Birth rates of counties which gained and lost population will be systematically examined, and constellations of counties with similar fertility and net migration patterns will be identified.

Significance to Biomedical Research and Program of the Institute: This efficient utilization of massive data already processed and coupled with further data from the censuses and the county and city data books, for the 1950's is a useful approach to the analysis of population change in clusters of counties and regions of the United States, and is basic to a projected analysis of the dynamics of the 1960's. This study is exceedingly relevant to the study of population processes in the United States and, therefore, to the Institute's program, both from a descriptive and analytical point of view.

Proposed Course: This project is expected to be completed during fiscal 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effects of Purposive-Rationality, Traditionalism, and Economic Aspirations on the Process of Family Formation

Contractor: University of Kansas
Money Allocated: \$44,000 (1971)

Objectives: This research specifies a unique data collection procedure involving hand delivering to the household questionnaires which are then self-administered and returned by mail. It would study 1,000 first-time parents to find factors affecting decision making as to the number and spacing of births. The independent variables are: (1) purposive-rationality, (2) traditionalism, and (3) economic aspirations as orientations affecting fertility (dependent variable). These independent variables should provide a parsimonious system with a high degree of explanatory power (e.g., as contrasted with the status variables of SES, education, and religion).

Significance to Biomedical Research and Program of the Institute: This study of how persons make decisions concerning family formation is definitely relevant to the Institute's program. The "training of respondents to use a self-administered questionnaire facilitates followup over time and imparts to this study a methodological as well as substantive importance, since one of the problems with longitudinal studies is their very great expense. If this methodology works, it can serve as a model for other longitudinal studies at more reasonable cost.

The project should make contributions to the sociology of the family and be particularly helpful in understanding how and why persons decide to have different numbers of children. As a result, it should prove useful for persons in the area of family planning.

Major Findings During Year: Of the variables tested, economic aspirations and dimensions of traditionalism tend to be related to fertility intentions and desires as expected. However, on the criterion of instrumentalism (the belief that one can influence one's future and not simply be subjected to fate), it was found in preliminary analysis that wives with the highest instrumental orientation want and expect slightly more children rather than the fewest, but the more instrumental wives do favor a greater number of years for spacing between children. This finding may reflect a Middle Western approach to family size consistent with certain other studies in which people said they wanted 3 or more children but were still concerned with environmental questions.

Proposed Course: This project, initiated in 1972 with three years' funding is expected to be completed in FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Factors Involved in the Moral Judgment of Marital Status and Family Size

Contractor: Human Resources Research Organization, Fort Knox, Kentucky
Money Allocated: \$32,000 (1971)

Objectives: Some 500 middle class persons (100 of 16-17 year olds, 100 persons 18-20 years of age, and 300 adults divided among younger and older groups) drawn from ten organizations (PTS's, unions, alumni associations, Catholic groups, etc.) will be asked to respond to situation descriptions presenting marital status (single, married) and family sizes (0, 2, 6, children) under varied conditions of choice or choicelessness: personal desire, ability (power), and environmental circumstances. Respondents will describe their family status and how they achieved it. Various characteristics of the judges will be studied for their impact on judgments. Perceived attractiveness of the subjects judged will be examined (by bi-polar scale) to determine effects of moral judgment on this dimension. The study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts. Analysis of variance will be performed to determine the effects of each of the three factors (from Heider) on moral judgment.

Significance to Biomedical Research and Program of the Institute: An understanding of population dynamics relative to future changes in the U.S. population requires a knowledge of attitudes toward family formation. This involves not only the couple but also the social context in which they live. Historically, parents, relatives, and friends have brought pressures on the couple to marry and to start childbearing early and to have large families. Thus it is highly relevant to the Institute's program to explore moral judgments as a framework for evaluating the effectiveness of programs designed to alter attitudes toward marriage or family size. The findings should be useful in counseling situations, family planning clinics, in wider educational programs dealing with marriage and family size, and in welfare programs. They should also contribute to improved maternal and infant health to the degree that they would foster changed attitudes and action programs designed to encourage the spacing of children at longer intervals.

Proposed Course: It is anticipated that this project will be completed in fiscal 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Support of Publication of Population Index

Contractor: Princeton University
Money Allocated: \$98,288 (1971); \$72,984 (1973)

Objectives: The purposes of this support are to permit the continuation of the publication of Population Index as a quarterly journal containing bibliographic information and special articles in the population field; to computerize the preparation of final copy for photo-off-set reproduction of the Index; and to develop a computerized retrieval service for the production of special bibliographies.

Significance to Biomedical Research and Program of the Institute: The need for knowledge about changes in population size and composition and the way in which these are related to socio-economic factors is well recognized by policy makers in the field of public health. For these and other users of demographic resources, an accurate and comprehensive guide is essential. NICHD funding permits the maintenance of a bibliographic tool of tested utility and the broadening of its usefulness for searching and finding. The Index over the past 36 years has also carried a broad range of articles on current developments in demography.

Major Findings: With this financial support and the acceptance of NICHD guidelines, the Population Index staff (1) has increased office efficiency, (2) has improved the quality of the publication and its timeliness, and (3) is developing a new computer program for the Author Index. Increasing circulation (up 7% in the past 15 months to about 4,300) and inclusion of advertising (beginning January 1973) should help to offset the costs of producing and distributing the Index. Sale of G.K. Hall & Co.'s Population Index's Master Cumulative Files for 1936-1968 indicates the continuing usefulness of bibliographic information covering the three past decades.

Proposed Course: This contract, initially funded in 1971, will be considered for a one-year renewal beginning in July 1973.

NICHD Annual Report
July 1, 1971 through June 30, 1972

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Women's Labor Force Participation and Fertility

Contractor: Research Triangle Institute

Money Allocated: \$109,692 (1971)

Objectives: This two-phase study is based on a complex causal model of the relationship between women's fertility and their labor force activity. The model suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly.

Phase I entailed the secondary analysis of two previously collected data sets which report on the fertility behavior of American women of childbearing ages: (1) Growth of American Families Survey, 1960; (2) Survey of Work Experience, 1967-70.

Phase II will develop and administer an interview schedule to a sample of 400 women and sub-sample of 40 of their husbands. If these two phases of the study are completed satisfactorily, the principal investigator plans to submit a proposal entailing a national sample.

Significance to Biomedical Research and Program of the Institute: The proposal deals with a very crucial area of population well within the Institute's labor force participation and its influence on fertility behavior, but there is still much confusion about this relationship. This proposal approaches the problem in a sophisticated, fresh manner, and even if the causal model does not work, the descriptive data amassed will allow researchers to construct new conceptual and methodological approaches.

Major Findings: In the analysis of the interrelationships of work participation and fertility carried out in Phase I, it was found that early labor force experience is significantly related to family size among both most blacks and whites even with controls for marital duration or educational attainment. To the extent that early work experience measures women's work commitment this finding suggests that such commitment can influence the course of fertility. Secondly among the poorly educated women, the socio-economic status of longest held jobs both before marriage and after the formation of the family is significantly related to family size, those women with better jobs having fewer children. Job SES may be a measure either of personal work commitment or economic motives for working and therefore suggests further study on how motives may influence fertility. Finally, wife's potential earnings (as measured by educational attainment) are significantly related to family size primarily among women from low-income groups.

This pattern suggests that economic motives for wife's work can influence fertility decisions. The strong and significant relationship between the duration of work experience since the first child and family size is as suggestive of constraints on work imposed by family responsibility as it is of a reverse process. Motivation for working may reduce fertility but also, responsibilities which occur when children are born are an important factor in determining subsequent labor force participation. This secondary analysis of data suggests that women's labor force participation has complex inter-relationship with fertility. The second state of this analysis, the original data collection and analysis, will hopefully shed light on this complex process.

Proposed Course: This project, funded for a total of two years, is expected to be completed in FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Social-Psychological Correlates of Urban Fertility

Contractor: Bowling Green State University

Money Allocated: \$77,424 (1971); \$34,367 (1972); \$29,027 (1973)

Objectives: This research, involving a re-interview of 754 mothers interviewed in Toledo in 1963 and a sample of 3,000 married women in the Toledo SMSA, has several interdependent objectives designed to test for possible associations, psychological and demographic variables: (1) to determine by means of longitudinal study the degree to which present family size can be predicted from alienation scores obtained from the sample of mothers in 1963; (2) to determine the extent to which the obtained differentials in fertility by alienation variables in previous research would be accentuated and refined in a community-wide sample that would include mothers who are continuing their childbearing as well as mothers who have successfully terminated their fertility; (3) to identify some of the social and demographic factors associated with differential kinds and degrees of alienation among mothers at given age, parity, and marital duration levels; (4) to determine the extent to which selected husband-wife interaction patterns are associated with differential alienation-fertility relationships; and (5) to derive a conceptual model of differential family planning and fertility behavior by combining stratification, religion, mobility, alienation, family, and information variables.

It will concentrate on attempts to understand better fertility and family planning differentials within social class and religious categories. Alienation variables will be considered as intervening between social structures and fertility, and the primary emphasis will be on specifying the conditions--of education, religion, age, etc.--under which particular patterns of alienation dimensions may be causally related to the dependent variables.

Significance to Biomedical Research and Program of the Institute: A study of the social psychological factors affecting fertility will make an important contribution to our understanding of fertility behavior, the sine qua non of the Institute's program in population research.

Major Findings: A positive association was found between high alienation scores and early marriage and high incidence of premarital and early pregnancies. High alienation scores in 1963 were associated with higher levels of fertility over the next eight years and with higher proportions of women who wanted no additional children but who had additional children nonetheless. Alienation scores remained relatively constant over the eight-year time interval.

Proposed Course: This study was funded in 1971, and extended with additional funds in 1972. Completion of the project is expected in FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Study of Family Formation and Fertility - Key Trends
and Patterns

Contractor: University of California

Money Allocated: \$171,088 (1972)

Objective: The purpose of this study is to develop a data base to facilitate the study of the relationship between illegitimacy and social changes in the family and sexual behavior, including abortions and venereal disease. The investigator will continue work on data linkage of marriage records and birth certificates to determine legitimacy status of mothers delivering children in 1967, other birth records and divorce records, to develop comparative data on patterns of marriage, divorce and separation of a sample of married and unmarried mothers in 1967. In addition, investigator will develop a questionnaire for a sample of California households to study the process of family formation and fertility. And he will do a comparative study of trends in illegitimate and legitimate fertility in California 1966-1971, including abortion and reported V.D. cases and do a comparative analysis for approximately 20 other cooperating states having varying abortion statutes, marital patterns, and V.D. experience.

Significance to Biomedical Research and Program of the Institute: This study will contribute to knowledge concerning illegitimacy, abortion, and V.D. in relation to sexual and family formation patterns and prepare the groundwork for a more extensive study of how social patterns relating to sex and marriage affect the incidence of illegitimacy, abortion and the current rise in V.D. rates. It therefore has immediate relevance to both health and population related behavior.

Proposed Course: The current contract is for 1 year, but it is expected to be renewed to complete the comprehensive study envisioned.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Social Mobility and Fertility Control

Contractor: University of Colorado

Money Allocated: \$116,263 (1972)

Objective: The purpose of this study is to test an hypothesis concerning the urban mobility process and its relation to the utilization of family planning practices. The investigator hypothesizes that as residents of central city slums in the Philippines improve their earning capacity they move to squatter settlements on the periphery of the city and in the process both the economic value of children decline and their aspirations for their children increase, with the result that the squatter settlement residents develop desires to limit their family-size and adopt family planning. He is testing the hypothesis in Davao City through demographic surveys of central city slums and squatter settlements and intensive interviews of a sample of households in those areas and examination of family planning records. Ethnographic interviews with key informants in each area studied will be conducted for the history of the settlement. The study is expected to contribute substantial reinforcement to the mobility theory in respect to adoption of small family values and birth control practices. The investigators are familiar with the area in which the study is being conducted.

Significance to Biomedical Research and Program of the Institute: This study will contribute substantially to knowledge regarding factors influencing reduction of fertility generally and in an Asian setting particularly, and thus contribute to the improvement of theory of fertility. It will also contribute to the improvement of family planning program policy in suggesting strategic locations for program efforts in underdeveloped countries.

Proposed Course: The study is scheduled to run for two years and will be completed in FY 1974 or early FY 1975.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Family, Career and Sexuality

Contractor: Center for Policy Research

Money Allocated: \$41,946 (1972)

Objectives: The purpose of this research is to examine the possible relationship between ideology and fertility-related behavior. Specifically, the study includes the development of a quantitative model which will examine the relationships between: (1) family background, (2) family ideology (the attitudes and values of the respondent's mother and father), (3) the respondent's ideology, (4) the respondent's career and family role orientations, (5) sexual attitudes, (6) sexual behavior and (7) preferred family size. The model will be developed through the analysis of data already available, in 4 data sources: First, an Ideology Study of 668 students attending the University of California, Berkeley, in 1970. Second, a similar survey of Berkeley students' parents, entitled Intergenerational Study. Third, a National Student Study sponsored by the Carnegie Commission involving approximately 71,000 college students. Fourth, a National Adult Study which has data similar to that collected in the Intergenerational Study.

Significance to Biomedical Research and Program of the Institute: This project would deal with a new way of analyzing fertility behavior. The model resulting from this project should encourage other researchers to examine population dynamics from a new perspective.

Major Findings: Preliminary analysis shows that family background and father's ideology have a strong influence on the child's social ideology. For instance, the father's general liberalism or conservatism and his religious values affect his child's sexual permissiveness and not the father's specific views on sexual permissiveness. Permissiveness in turn has a major influence on attitudes toward abortion.

Proposed Course: This study will be continued during fiscal 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Social and Psychological Factors Affecting Fertility,
Family Planning and Clinic Utilization

Contractor: Wake Forest University
Money Allocated: \$95,936 (1972)

Objectives: The purpose is to re-analyze in-depth data collected in three KAP-type interview surveys concerned with black males and females in Winston-Salem, North Carolina, and Hackensack, New Jersey, and with Spanish females in Costa Rica. Emphasis will be placed on psychological and social-psychological variables as they relate to fertility, family planning and clinic utilization. For women with a high risk of high fertility various social-psychological measures will be devised and related to different sets of explanatory variables. Twelve different analyses will be undertaken to test out hypotheses.

Six analyses will pertain to black women in the United States regarding: (1) definition and indexing of the context of "risk" (biological, societal, and medical); (2) early sexual experience and attitude toward sex; (3) comparison of male- and female-headed black households as to differences with respect to such factors as education, parity, number of current sex partners, and attitude toward abortion; (4) poverty as a "culture of poverty" vs. the relationship to intervening variables, such as education, age at first marriage, legitimacy of first conception, and age first learned to prevent conception; (5) religion, social class and abortion; and (6) consequences of being born out of wedlock.

The six analyses pertaining to Costa Rican women will be concerned with such aspects as: (1) modernization and modernism; (2) correlates of fertility; (3) trends in childspacing; (4) credibility of sources of family planning; (5) urban-rural differences in fetal and infant mortality; and (6) comparison of consensual and legal marriages.

Significance to Biomedical Research and Program of the Institute: This project is relevant to the NICHD program relating to the interface between population and family planning in that it goes beyond the clinical approaches taken by such governmental agencies as AID, and the National Center for Family Planning Services. It also brings in a cross-cultural aspect, which relatively few studies have done.

Major Findings: The studies undertaken among blacks in New Jersey and North Carolina communities and among women in Costa Rica indicate the following: (1) blacks have accepted the national family size ideal but evidence a wide-range of acceptable number of children around that ideal point; (2) in Costa Rica, education, age at first conception, nature of emotional relationship

with father of first child and age when woman learned of contraception were determining factors in a woman's life situation; (3) consensual unions, contrary to the literature, do not depress fertility; (4) women in consensual unions differ from those in legal unions primarily in terms of social class and related KAP (Knowledge, Attitude and Practice) factors; (5) psychological factors may be of greater importance in determining family planning clinic attendance than previously reported in the literature, i.e., fears and untoward experiences at a family planning clinic and with contraceptives are more important than a woman's demographic characteristics in predicting whether or not she will continue attending a clinic or drop out; and (6) the most important factor in communication about family planning in Costa Rica is the positive or negative reinforcement of friends, relatives and neighbors rather than the quality of the information transmitted. Findings have been disseminated through professional journals. In addition, authorities responsible for family planning both in the United States and in Costa Rica have used these findings in planning their objectives and programs.

Proposed Course: This contract is funded for two years, beginning June 1972.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Analysis of Migratory Responses to Employment Opportunities

Contractor: Battelle Columbia Laboratories

Money Allocated: \$53,500 (1972)

Objectives: The aim of this research is to clarify the relationship between migration and certain demographic and economic variables, particularly potential employment. For a sample of 100 counties, drawn from a 14-state area, in-migration rates for 1955-1960 can be obtained from a special tabulation of the 1960 census. Rates of net-migration by area, sex, and color, will be obtained from the Bowles-Tarver series and, after adjusting for a five-year period (instead of the decade over which they were computed), rates of out-migration will be obtained by subtracting rates of in-migration from adjusted rates of net-migration. With age, sex and education controlled, a clearer relationship between migration and economic opportunity should emerge. Migration rates for each group will be regressed on variables measuring aggregate employment opportunities and group specific employment opportunities in county labor market areas. The 1955-1960 data will be used to predict 1965-1970 migration and will be checked against cross-tabulated data from the 1/100 sample for 1960 and 1970.

Significance to Biomedical Research and Program of the Institute: The proposed research has high program relevance, fitting into a mosaic of our existing contract migration studies relating to: (1) the analysis of the Social Security file covering 90% of employment in the United States to obtain data on inter-regional, metropolitan, and non-metropolitan migration patterns by sex, color and age, (2) migration from Appalachia, and (3) the impact of depopulation upon economic and social institutions in the Rocky Mountain area. The present research proposal relating migration and economic opportunities should make a real contribution to the understanding of the determinants of migration, a subject of increasing policy interest.

Major Findings: (1) The hypothesis that migration is determined in part by the existence of job opportunities is tentatively proved; (2) education and age have a combined relationship upon migration--education makes one more mobile and the relationship becomes stronger and clearer with increasing level of education. Individuals with 16 or more years of education have high average rates of in-migration and responsiveness to employment opportunities, since they are likely to be aware of alternative job opportunities in various areas, have been relocated by their companies, or have contracted for employment in a new community upon completing college. High average in-migration rates for females in the 20-24 age group are probably due to education and marriage effects. For females with 13-15 years of education, the additional education may have opened new job opportunities or resulted in marrying a more

educated and mobile spouse. Males over 40 years of age do migrate, but primarily in response to specific job opportunities rather to generalized opportunities.

Proposed Course: This project should be completed by FY 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Methods and Techniques for Using the 1960-1970 Census
One Percent Public Use Samples

Contractor: National Data Use and Access Laboratories, Inc.
Money Allocated: \$235,403 (1972)

Objectives: The purpose of this proposal is to develop support techniques to facilitate the wider use of the 1970 Census Public Use Samples (PUS). The project emphasis is on the handling of very large files by writing programs using COBOL and FORTRAN program to merge different types of records, create smaller work tapes, and provide programs for cross-tabulations and analysis of data. The proposal also includes the writing of users' manuals and provision of classroom instruction in the use of these programs which will be available in the public domain at low cost.

Significance to Biomedical Research and Program of the Institute: The U.S. Census is an important source of data for demographers and other social scientists, as are other censuses which are made available from time to time. The sheer volume of the 1970 output, however, requires special effort. The needs of most researchers could be satisfied with select variables and records on a single manageable reel tape but the problem is to produce that reel or tape for individuals. DUALabs is a unique facility, incorporating previous experience with census, and several years' analysts lacking this experience.

Major Findings: The principal investigator has completed work on the PUSH software system, which will enable researchers in the field to use commonly available statistical methods (such as the Statistical Package for the Social Sciences, SPSS) with the Public Use Sample. This is a major advance in making the Public Use Sample tapes available to a large number of people. The tape and manual will be distributed in the Spring of 1973. The manual is being published through GPO and the tape is being distributed by DUALabs. In the second year of the project the principal investigator will be developing a CENTS-AID program to allow investigators to use the public use sample with the very fast cross-tabulation program (CENTS) developed by the Census Bureau. DUALabs will also be offering seminars and instruction in the use of both PUSH and CENT-AID.

Proposed Course: This project is scheduled to run for a period of two years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Correlates of Family Size and Childspacing in the United States and Puerto Rico

Contractor: Boston College
Money Allocated: \$150,612 (1972)

Objectives: The purpose of this two-year study of 500 families of high school students in a middle class suburb of Boston is to show the effects of family size and the spacing of children on the "quality of life" of the parents and of the children. Variables on the parental side include: age, socio-economic status, religiosity, education, work history, childbearing attitudes, recreational patterns, financial conditions, membership in voluntary organizations, degree of integration into the community, and rationale for birth of each child. Variables on the children's side include: academic achievement, intellectual capacity, vocational and educational aspirations, personality, social relations, work history and expected age at marriage, family planning attitudes and subsequent life style. Interviews and questionnaires in addition to peer ratings and school records of the children are being used as sources of the information. This project replicates the key aspects of another NICHD project conducted in a middle class suburb of San Juan, Puerto Rico, by the same co-investigators. New variables are being added in this study, including childspacing and the impact of family size on the lives of parents.

Significance to Biomedical Research and Program of the Institute: The analysis of how family size and birth spacing affects the welfare of both parents and children is a major focus of the program of the Center for Population Research, NICHD.

Proposed Course: This contract is funded for two years, November 1971 to November 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Employed Woman: Family Planning and Careers

Contractor: University of California at Los Angeles

Money Allocated: \$38,361 (1972)

Objectives: The purpose of this proposal is to elucidate the behaviors and motivations of 120 employed career women relating to fertility and family planning and to specify the patterns of temporal ordering of family and work. Psychological motivations for having children will be delineated and the intensity of desire for children will be compared for each group of women with the intensity of desire for various alternatives to childbearing. One important facet of the research will consist of an attempt to quantify the perceived rewards and cost of both the motherhood role and the career role. Data analysis will be structured to relate reward cost data directly to fertility variables such as actual number of children and expected number of children and to career variables such as length of full-time employment, personality and developmental factors, that may influence the differences in employment history, fertility and desire for children.

Significance to Biomedical Research and Program of the Institute: This study represents an attempt to gain insight into the relation of family limitation and childbearing and work experience and aspirations of women.

Proposed Course: The contract initiated in 1972 to run for one year will require an extension in time into FY 1974 to offset delays in obtaining OMB final clearance. After the first year's work has been completed, the contract may be continued to allow the investigator to conduct a longitudinal study of a subsample of 40 subjects.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Analysis of the Causes and Patterns of Rural to Urban Migration of the Poor

Contractor: University of California (Berkeley)
Money Allocated: \$17,684 (1972)

Objectives: This study will investigate the causes and decision-making process underlying the migration behavior of the rural poor and explore the potential for various public policies which could influence migration from rural and small areas to big city ghettos. The study will test a series of alternative causal models for explaining migration while investigating in depth a model of individual adjustment to economic and social displacement. The principal investigator will conduct a secondary analysis of data previously collected by ABT Associates and funded by OEO. Data for 1800 lower socio-economic respondents have been collected but the analysis of the data has been only cursory. Approximately half of the study population were migrants from rural to urban areas, the others were current residents of rural areas. Three migrant streams were involved: Southeastern Blacks, Appalachian Whites and Southwestern Spanish Americans. The data collected include information on perceptions, experiences, and attitudes as well as migration behavior.

Significance to Biomedical Research and Program of the Institute: The study population is important in terms of explaining the causes of migration patterns of the poor. Economic and other pressures which may force poor families to migrate to urban areas have implications for the planning and implementation of public health programs.

Proposed Course: The contract, initiated in fiscal year 1972 will be completed early in fiscal year 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Role in Fertility Patterns of Urban Mothers

Contractor: Columbia University
Money Allocated: \$88,828 (1972); \$9,687 (1973)

Objectives: This project is exploring the relationship between female roles and fertility. The major hypothesis of the study is that timing of the first birth greatly influences the number and timing of subsequent births, the manner in which a woman views herself in relationship to familial roles (i.e., mother and wife), and how she perceives herself in non-familial roles, (e.g., student and worker). The principal investigator is conducting an exploratory study of women in the New York area between the ages of 15 and 29 who have recently born their first child. The research instrument concentrates on the determinants and timing of the first birth. Specific areas to be explored are the socialization process the subjects underwent in terms of their family of origin, friends, social economic status, religion, race, and their perception of their mother and father. This project is innovative in the sense that most studies which deal with fertility and, especially those that try to project future fertility patterns, have concentrated on the most recent birth which may or may not be the first birth. It is also a methodological study which will develop a research strategy and instrument which, if successful, will be utilized in a larger study.

Significance to Biomedical Research and Program of the Institute: This project has great potential for increasing our knowledge of the factors which determine fertility at the individual's level. As a result, the information which may ultimately come from the project will be valuable for the medical and allied professions in advising persons how to better plan their families.

Proposed Course: This project was initiated on March 15, 1972 and extended to June 30, 1973. During the contract period, the research instruments and findings of the pilot study will be evaluated to determine if further research in this area will be fruitful.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: College Women in the 1950's: Attitudes and Behavior With Respect to Marriage, Children, Divorce, and Sexual Practice

Contractor: The Wright Institute
Money Allocated: \$15,466 (1972)

Objectives: The purpose of the proposal is to analyze data that are available on 3,180 Vassar College women during the period 1953 through 1960. These women participated in the Minnesota Multiphasic Personality Inventory, the California Psychological Inventory, the Vassar Attitude Inventory, and responded to several other standard questionnaires relating to such topics as marriage, children, career plans, sexual behavior, etc. Based on these data the following hypotheses will be examined: (1) in the years 1953-60 almost every Vassar student aspired to marriage; (2) almost every student aspired to motherhood; (3) almost no student expressed interest in adoption unless a physical condition hampered pregnancy; (4) almost no student expressed concern with problems of overpopulation. The above represents four of 15 marriage and fertility-related issues which will be examined in this secondary analysis.

Significance to Biomedical Research and Program of the Institute: This study may reveal many of the current revolutionary themes which may have been latent in the 1950's. For example, women's lib, sexual freedom, decline of the number of children desired, etc., may have been in their embryonic form.

Major Findings: The four hypotheses mentioned above were confirmed. In addition, it was found that two thirds of the women would like careers, which they expected to interrupt for childrearing and then to resume. Most women were content with the status of educated women in the 1950's. Seniors desired a mean of 3.4 children. Sexual attitudes and behavior became considerably more liberal during the college years.

Proposed Course: This investigation of secondary data was completed in February 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Psychological Studies of Social Norms Influencing Family Size

Contractor: University of Kansas

Money Allocated: \$83,802 (1972)

Objectives: This study will investigate the dynamics of social norms concerning large and small families--reactions to explicit and implicit social pressures in relation to normatively defined family size ideals. The following laboratory experiment is proposed: positive and negative evaluations of women as a function of the number of children (0-5) they have in relation to their age (25, 35, and 45) and career types (none, child-related career, non-child-related career, and non-child-related career with high career commitment). In the laboratory situation a sample of white, middle class, married women will evaluate women based on these variables in a design in which they will not be aware that these are the crucial variables in the experiment.

Significance to Biomedical Research Program of the Institute: The study of the dynamics of social norms concerning large and small families is highly relevant to an understanding of the determinants of fertility.

Proposed Course: This study will be extended into fiscal 1974 because of delays in obtaining clearance.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Optimum Population Levels

Contractor: University of Virginia

Money Allocated: \$58,275 (1972); \$95,000 (1973)

Objectives: This study is exploring different approaches to, and a methodology allowing the calculation of, an "optimum level of population" in terms of increases or decreases in an index of quality of life (IQL). The major effort is toward a modification of the Gross National Product (GNP) to adjust for the amount of the national income accounts which are directed to the dis-economies produced by population growth. The study will attempt to project changes in the so-called IQL as population increases and as population distribution changes under the assumption of various technologies relating to mineral consumption, water use, energy, and other natural resources.

Significance to Biomedical Research and Program of the Institute: This is one of the very few studies in the Behavioral Sciences program of the Center which addresses itself to the problem of measuring the consequences of population growth and change. Much of the current concern over the effects of increasing population size has been based on data and on assumptions which have not always met with general acceptance. This study is attempting to develop a methodology which will make the consequences of population growth and change much more specific and identifiable, at least in economic terms, and therefore should be of great benefit to policy makers in the determination of what population goals should be pursued.

Proposed Course: This study was funded for one year in fiscal 1972 and was renewed for an additional year in 1973. Additional funding is anticipated if progress under the current contract justifies it.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Occupational Experience as a Determinant of Fertility Among American Women

Contractor: Center for Policy Research
Money Allocated: \$104,800 (1972)

Objectives: The negative relationship between women's participation in the labor force and their fertility is well established. Previous studies, however, have analyzed the relationship between women's working and fertility in terms of participation and non-participation. The proposed study will take a more sophisticated strategy by considering the characteristics of the work women do and the relationship between these occupational characteristics and fertility. The study's main goal is to determine whether differences in occupational opportunities and rewards result in variations in the fertility behavior of women.

The study will use published data from the 1940, 1950, 1960 and 1970 censuses. The bulk of the analysis will not be based on published material, but rather will be based on the one percent Public Use Sample tapes of the 1960 and 1970 censuses. The 1970 one percent tapes will be especially useful since the interview schedule contained a number of retrospective questions concerning the occupation of each household member in 1965. Moreover, there are a number of questions on the subject's marital history and number and age of children. Lastly, the tapes will have an occupational code which has a high level of specificity that will allow the researcher to categorize occupations in the most suitable theoretical and methodological manner.

Significance to Biomedical Research and Program of the Institute: This project will make valuable contribution to the population field. The principal investigator intends to examine an important aspect of the relationship between women's participation in the labor force and their fertility behavior, namely, the characteristics of the occupations they work in. Recently there are indications that a large number of women are taking or attempting to take a more active role in the labor market, especially with professional occupations. Currently, it is not known how the differential participation in the labor market or different sectors of the labor market influences fertility and social institutions, such as the family. This project would do much to better understand contemporary and future changes.

Proposed Course: This contract was initiated in FY 1971 and will run for a two year period.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Population Redistribution and Depopulation in Non-Metropolitan Pennsylvania: Social and Economic Correlates and Policy Implications

Contractor: The Pennsylvania State University

Money Allocated: \$294,056 (1972)

Objectives: This is a complex undertaking which will investigate in detail, historically and contemporaneously, the non-metropolitan population changes in the State of Pennsylvania. That State is a large, well-established area with a long history and highly diversified non-metropolitan sector. The investigators propose to create a data bank of relevant historical-statistical information and to create a typology for the non-metropolitan areas in order to analyze the most important processes shaping change in those areas. Data will be derived primarily from the census but several other sources will be used and field studies will be conducted. The studies will focus on transport, technology, changing labor force roles of females, attitudes toward metropolitan living, and others - which have shaped the non-metropolitan sectors of the population.

Significance to Biomedical Research and Program of the Institute: This proposal will differ from earlier research on related topics in demography and related fields by its strong focus on a great range of non-metropolitan characteristics and trends in a highly advanced region. The issue of population dynamics in non-metropolitan areas affecting population distribution has been a very neglected area for research. Several proposals are currently pending in Congress and elsewhere for increasing the attractiveness of non-metropolitan areas. Whether or not these are well advised depends upon the understanding of the forces and processes affecting such areas and upon projected distributions of population and economic opportunities.

Proposed Course: This multi-faceted research program will extend over a period of two years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Review of Research Findings on Rural-Urban Migration
with Annotated Bibliography

Contractor: TRACOR, Inc.
Money Allocated: \$30,818 (1972)

Objectives: This project, originally funded by OEO, was continued by NICHD when OEO decided to terminate support of projects on migration.

Work has continued on the development of an annotated bibliography and review of all research on rural-urban migration. The principal investigator proposes to annotate items in the existing bibliography, add more items, expand the summary of research findings, and suggest needed research.

Significance to Biomedical Research and Program of the Institute: The development of annotated bibliographies such as this are of tremendous benefit to both established investigators who may have missed significant publications and to students entering this field of research.

Proposed Course: The scientific aspects of this project should be completed in FY 1973. A resultant publication in the CPR monograph series is planned to appear in FY 1974.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Determinants of Fertility Change in Tamil Nadu, India

Contractor: University of North Carolina

Money Allocated: \$180,000 (1972)

Objectives: This study aims to investigate whether there is a threshold of social and economic development after which family planning action programs will make more rapid progress. The approach proposed is "observational" rather than experimental. The existing geographic differences in social and economic development and in family planning action program inputs in one state (Tamil Nadu) in India will be used to select an appropriate sample of areal units (Community Development Blocks and Hamlets), and the fertility and family planning behavior of the populations in these units would be studied.

The total project has two parts. In Part 1, the available data for all 375 Community Development Blocks in Tamil Nadu would be compiled and analyzed to assess the variation in fertility or contraceptive acceptance attributable to: (a) differences in family planning program inputs, (b) differences in socio-economic level of development. These data would also be used in selecting a sample of Blocks for Part 2. Within each of the 54 sample Blocks 12 Hamlets (of 1,000 population each) would be selected. All the sample units would be drawn so as to represent all combinations of program inputs and levels of socio-economic development to permit repeating the analysis in Part 2 with more accurate data on program outputs.

Significance to Biomedical Research and Program of the Institute: This study affords an excellent opportunity to investigate whether there is a threshold of social and economic development above which family planning action programs may make more rapid progress.

Proposed Course: This proposed research, initiated in 1972, has been scheduled to run three years.

Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Fertility of Spanish-American Populations in the United States and their Socio-Economic Correlates

Contractor: Research Institute for the Study of Man, New York

Money Allocated: \$254,315 (1972)

Objectives: Using data from standard and special 1970 and earlier decennial census tabulations, the investigator is examining the fertility (and its socio-economic correlates) of the major Spanish-American population groups in the U.S.: Mexican, Puerto Rican, Cuban, Central and South American and the descendants of the original (17th and 18th Century) Spanish group. The 10 million Spanish Americans living in the United States comprise about 5% of the total U.S. population. Information on country of birth of the foreign born and the foreign-born parents of the native born, on mother tongue, and Spanish surname will be used with data on children ever born and such standard demographic items as age, sex, marital status, educational attainment, labor force and employment status, family income, etc. to ascertain fertility trends and differentials among these groups for as long a period as the data permit.

While encompassing an historical analysis, this study emphasizes the testing of several specific hypotheses: 1) factors associated with fertility differentials generally (urban-rural residence, educational attainment, labor force and employment status, occupations of the husband and wife, and family income) pertain in kind, if not in degree, among the Spanish-American groups; (2) residence in ethnic concentrations results in higher fertility, and conversely dispersed living results in fertility approximating that of the general population; and (3) cultural continuity carries over from the country of origin but is diminished with each success of generation born in the U.S.

Significance to Biomedical Research and Program of the Institute: Diverse information on the Spanish-American population groups in the United States has suggested much higher fertility, somewhat higher mortality, and generally poorer health conditions than among the general population. This study is the first to attempt to look at the groups as a totality and at the similarities and dissimilarities among them. The most important groups are the Puerto Ricans concentrated in the New York City area, Mexican-Americans in the Southwest, and the Cubans primarily on the Eastern seaboard. From a policy point of view for family planning programs, etc. it is desirable to have baseline data as to the level and trends of fertility among the various Spanish-American population groups.

Proposed Course: This contract, begun in 1972 is for a three-year period.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Age at Marriage and Time to First Birth: A Longitudinal Study of Parental Attitudes and Children's Personality

Contractor: College of William and Mary

Money Allocated: \$111,868 (1973)

Objectives: The proposed research consists of an intensive longitudinal study of the effects of age at marriage and time to first birth on parental attitudes, children's personality development, and family interrelationships. The major focus of the research is upon first-born children within self-selected families. The sample consists of 80 two-child families, 20 in each of the four categories; early marriage and short interval to first birth, early marriage and long interval to first birth, late marriage and short interval to first birth, and late marriage and long interval to first birth. The four groups will be matched to control for socio-economic factors and will be limited to whites. Various measures of selected attitudes and personality attributes will be made along with direct observations of family interaction several times during the three years of the project. During each visit 180 minutes of observation will be completed in 5 minute segments. The categories from Patterson's Manual for Coding Family Interactions will be used to classify the behavior observed during each segment. Data will also be obtained concerning characteristics of the parents, characteristics of the second-born children, and the interactions between parents and children within the families.

The subjects will be self-selected respondents to advertisements placed in newspapers of Williamsburg, Virginia, and surrounding metropolitan areas.

Significance to Biomedical Research and Program of the Institute: This study will expand our knowledge of the consequences of age at marriage and time to first birth for parental attitudes and the children's personalities. It is relevant to the Institute's interests in the consequences of childbearing patterns.

Proposed Course: This study is to extend for three years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effect of Birth Order on Mother-Child Relationship

Contractor: Educational Testing Service

Money Allocated: \$331,410 (1973)

Objectives: The Principal Investigator will study the effects of birth order, sex of infant, and socioeconomic status upon mother-infant interaction and upon the cognitive-intellectual and emotional development of the infant up to 2 years of age.

This study focuses on infants at each of four orders of birth; first, second, third, and fourth or higher. The sample will be selected so that each of these birth-order categories will contain 60 cases. Each category will be further divided into four (15 cases each) according to sex and socioeconomic status. The subjects will be visited at ages 3 months, 1 year, and 2 years.

The independent variables recorded at the beginning of the study are sex of infant, birth order, class (middle and lower), mother's age at first birth, number of siblings, birth interval and sex of siblings. The dependent variables, 4 standardized tests (1 socioeconomic and 3 cognitive) and 26 structured observations, will be recorded at the time of observation. Analysis will compare children at each of the observation periods as well as their development over the period of the study.

Significance to Biomedical Research and Program of the Institute: This study will expand our knowledge of the consequences of family size, childspacing, age of mother at birth, birth order and socioeconomic status for mother-infant interaction upon the cognitive-intellectual and emotional development of the infant. Hence it is relevant to the Institute's interest in the consequences of various childbearing patterns.

Proposed Course: This study will extend for three years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Research on the Consequences of Childbearing and Child-Spacing Patterns

Contractor: American Institute for Research
Money Allocated: \$67,108 (1973)

Objectives: The Principal Investigator will conduct a secondary analysis of the project TALENT longitudinal data bank at the American Institutes for Research. The data was collected at four different times. In 1960 a national sample of over 4000,000 high school students were given two-day battery of tests and questionnaires dealing with information on the student's personalities, abilities, interests, aspirations, and background (e.g., age of father when respondent was born, sibship position of the respondent, number of brothers and sisters). Follow-up interviews and questionnaires, one, five and eleven years later, yielded information on schooling, career choice, marriage, timing of the birth of the first child, and subsequent family development.

These data allow the examination of three kinds of relationships: (1) The childbearing patterns of the students' parents will be examined for their consequences to the students' personalities, abilities, interests, and aspirations. (2) Relationships between the characteristics in 1960 and subsequent schooling, career, marriage and childbearing patterns of the students, (3) Consequences of childbearing and child-spacing patterns of TALENT marriage partners on career aspirations, socioeconomic statuses, etc.

Significance to Biomedical Research and Program of the Institute: This research is relevant to the Institute's interests in both the antecedents and the consequences of marriage and childbearing patterns.

Proposed Course: This study will extend for eighteen months.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title : Development of Methodology for Evaluating the Demographic Impact of Organized Family Planning Programs in the United States

Contractor : Center for Family Planning Program Development, New York
Money Allocated: \$112,302 (FY 1973)

Objectives: The major purpose of this project is to estimate the effects on fertility of the provision of subsidized family planning services. Subsidiary purposes are to identify the factors accounting for geographic differences in fertility and the factors associated with varying levels of family planning program input. The dependent variables will be various measures of fertility based on 1970 Census data on reported numbers of children ever born and numbers present in households. These fertility indicators will be computed for groups classified by age, color, and poverty status for single counties or groups of counties. The major independent variable is the level of family planning services provided (reported patient loads averaged for fiscal year 1968 and calendar year 1969 divided by an estimate of the population of low income women in need of contraception). In addition, there will be several intervening variables: proportions of women in intact marriages, average number of years of school completed, female labor force participation at ages 15-44, percent urban, and estimate of the 1965 general fertility rate.

Significance to Biomedical Research and Program of the Institute: This is the only national study of the impact of family planning service levels on the fertility of the low-income population. It should provide answers to questions that people have raised about the effects of voluntary family planning programs. As such, it will have direct relevance to the formulation of policies regarding population and family planning in the United States.

Proposed Course: The contract, initiated in FY 73, is expected to be completed in FY 75.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Review of Actual and Accepted Consequences of Family Size

Contractor: Calspan Corporation

Money Allocated: \$35,396 (1973)

Objectives: The purpose of this study is to perform a critical review of the existing literature on (1) the actual consequences of family size, parents, children and general family relations, and (2) the expected consequences of family size as perceived by parents and prospective parents. The review of the consequences will give special attention to the consequences of specific family size and consequent variables. Considerations of possible confounding factors and other aspects of research designs and gaps in our knowledge will be central to the review. Special attention will be given to a comparison of "real" and expected consequences of specific family sizes. In addition to the reviews themselves, planned outlooks of the project are: (a) theoretical development of the mechanisms by which family size has certain consequences, (b) recommendations from proved research designs in substantive areas for research, and (c) implications and applications both by Government and elsewhere.

Significance of Biomedical Research and Program of the Institute: The Institute has made explicit its interest in the consequences of family size and currently there is no one source book dealing with what is already known in this area or what gaps there are in our knowledge about the consequences of family size. This research will be valuable both as a resource for others as well as an original work. The analysis of the research designs employed by other investigators will help to guide future researchers toward the use of better designs.

Proposed Course: This project will require one year.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Fertility Dynamics of Cuban Refugees

Contractor: University of Miami

Money Allocated: \$176,584 (1973)

Objectives: The purpose is to investigate the relationship between fertility, migration, cultural and socio-economic changes among a population of 300,000 Cuban refugees--or half of the U.S. total--living in the Miami-Dade County area of Florida. The study will test a number of hypotheses relating fertility to minority status, religiosity, migration and acculturation within an overall theoretical framework of "convergence theory," namely, that fertility as well as social and other values and behavioral patterns of the Cuban refugee population will converge over time with those of the general population. A combination of questionnaires administered to 800 women, family histories obtained from in-depth interviews of members of a sub-sample of 25 households and ancillary data from the Cuban Refugee Office and the Research Institute for Cuba and the Caribbean will be used to compare fertility (children ever born to married women 20-44) in Cuba and the United States and changes during the period of adjustment to a new environment.

Significance to Biomedical Research and Program of the Institute: In an effort to understand the fertility behavior of the U.S. population, considerable interest has been focused on the Spanish-American population. The Cuban refugee population represents an important segment of this population. While sharing with other segments the Spanish-American culture, it also exhibits striking differences with respect to socio-economic status and conditions under which migration has occurred. Research on this group complements several other NICHD-supported studies on Spanish-American populations in the United States, all of which emphasize the search for influences on fertility behavior.

Proposed Course: This research is scheduled to run for two years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Work and Childcare Patterns of Women After the Birth of Their First Child

Contractor: University of Kansas
Money Allocated: \$31,622 (1973)

Objectives: The purpose is to expand on an ongoing study to obtain data regarding the rationale for women working or not working outside the home and characteristics of their work patterns since the birth of their first child. Socio-economic background, attitudinal and aspirational variables will be analyzed for their effect upon the extent to which women work after the birth of their first child. Special attention will be given to how the kind and availability of childcare facilities or arrangements effect the mother's work activity.

The data will be obtained in a second questionnaire to be administered in a longitudinal study in three Kansas counties of the process of family formation among 1,000 young couples who had their first child in 1971. Each couple completed a first questionnaire in 1972 under an NICHD contract designed to find how sequential decision-making as to the number and spacing of births was affected by three independent variables: purposive rationality, traditionalism, and economic aspirations.

Significance to Biomedical Research and Program of the Institute: This study builds on one already underway and should provide information about the factors that encourage or discourage labor force participation of a woman when her first child is about 2 years old. The subject is of particular cogency at this time when significantly large numbers of women born in the postwar period are entering the prime labor force, marriage, and family-formation age group, many of whom are reported in the labor force. For example, the proportion of mothers of children under six years of age working outside the home doubled from 15.7% in 1952 to 30.4% in 1969--or nearly as much as for mothers of children 6-17 years of age.

Proposed Course: This research is scheduled to run for two years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Patterns of Emotions and Feelings in Sex-Related Behaviors

Contractor: Vanderbilt University

Money Allocated: \$64,625 (1973)

Objectives: The purpose is to test hypotheses concerning the emotional and attitudinal components of experiences in relationships involving love, sex, contraception and abortion. The underlying theory holds that the emotion system is a principal motivational system, with the emotions possessing unique properties in interacting drives (hunger, thirst, pain, avoidance, etc.) in sexual matters. Thus, other emotions interacting with the sexual urge can affect sexual behavior in a variety of ways, and may be extremely important in the effective practice of contraception. For example, a man who holds a woman in contempt may be less likely to use adequate contraceptive methods even though pregnancy may be unwanted. The research will be conducted among (a) 1,000 college students at Vanderbilt University and Middle State Tennessee University; and (b) 150 each unmarried pregnant women (at a Florence Crittenton home) and 150 married women pregnant for the first time.

Significance to Biomedical Research and Program of the Institute: The interaction between emotions, sexual and contraceptive behavior may be a crucial factor in explaining unplanned and unwanted pregnancies. A woman's pregnancy and childbearing may result from feelings of insecurity and inadequacy or by guilt in having sex for pleasure or may represent an effort to fight off the emotional distress of loneliness or rejection. With more adequate information on how discrete emotions influence the sexual urge, sexual behavior, and reproduction, more effective methods can be developed for re-enforcing or changing attitudes consonant with the well-being of the individual family and of the broader society.

Proposed Course: This research is scheduled to run for 2 years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Childspacing and Current Fertility: 1970 Census

Contractor: U.S. Bureau of the Census (Reimbursable Agreement)

Money Allocated: \$350,000-\$400,000 (3 fiscal years, 1973-1975)

Objectives: From the 1970 census a special magnetic tape file will be derived dealing with women aged 14-54, including information where applicable on husbands, children, and other household or family characteristics. The project will provide data by socio-economic characteristics for several population groups on: (1) age at marriage, intervals between marriage and first birth, and birth intervals for later births by cohort to determine any changes in childspacing patterns from cohort to cohort; (2) the effect on fertility of separation and of marital dissolution and remarriage; (3) unintended childbearing (pre- or immediately post-marriage or after age 35 following a long interval without bearing children; and (4) social and work roles of women. Data on current fertility, as measured by births and birthrates, 1960-1969, will replace less precise 1960 census data on children under five years of age. A brief report on major results will be published early, followed by a special 1970 census Volume II report of some 400 pages on Childspacing and Current Fertility, and the magnetic tape file will be made available for other researchers to use.

Significance to Biomedical Research and Program of the Institute: The potential fruitfulness of this project for the study of fertility correlates is inestimable. The report will provide basic data on childspacing of the U.S. population, and the data file will permit detailed fertility analyses heretofore impossible to make, facilitating the study, for example, of the consequences of alternative childbearing and childspacing patterns. A further major value of this research lies in its usefulness in relating fertility process to such factors as employment, occupation, income, housing conditions, etc.

Proposed Course: This research is scheduled for a period of two years, covering parts of three fiscal years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: On the Consequences of Reproduction: A Utility Model of Reproductive Behavior

Contractor: University of North Carolina

Money Allocated: \$448,000 (1973)

Objectives: The purpose of this study is to test a utility model of fertility designed to predict the probability of various reproductive events to couples. The basic premise is that individuals regulate their reproductive behavior in light of their expectations and perceptions of the consequences of reproduction for them. Variables will include perceived consequences in terms of costs and rewards of facilitating or preventing a subsequent birth. The study will also be concerned with the congruence between expected and actual consequences and the effects of the congruence or lack thereof on subsequent expectations. Completed family size is viewed as resulting from constantly changing fertility behavior in the face of changing life circumstances and the changing perception of the consequences of reproduction. It takes into account changes relating to the anticipation of a child, the birth of a child, the impact that this has on future childbearing, etc. Each of these events affect the perception of subsequent anticipated costs and benefits--the utility functions--and alter behavior accordingly.

A sample of 3,200 women living in selected low income areas in 16 U.S. cities interviewed previously under an existing NICHD contract will be asked various questions concerning the perceived assets and liabilities of adding children to their families. A sub-sample of 1,200-1,500 women under 30 years of age, married and husband present, will constitute a group to be re-interviewed at least twice. In addition, 500 women and their husbands will be interviewed in suburban areas in the cities.

Significance to Biomedical Research and Program of the Institute: Testing of the important hypothesis that fertility behavior is "rational" within the perceptual framework of the actors--low income and middle income alike--will, if verified, have important implications for policy at a variety of levels.

Proposed Course: This research is scheduled to run for 3 years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Demography of the Black Population

Contractor: University of Texas

Money Allocated: \$87,792 (1973)

Objectives: The purpose of this study is to describe and analyze trends in the demographic characteristics of the black population, and compare these with trends in the white population. The basic analytical approach will be a cohort analysis by sex and color. The principal investigator did a related study for the 1960 census, Changing Characteristics of the Negro Population: A 1960 Census Monograph, U.S.G.P.O., 1969. Part of this project will be to check the projections outlined in the earlier work, seek to explain changes from 1960 and 1970, and to update the earlier monograph. The principal investigator will focus on how changes in social policies, e.g., the Civil Rights Act of 1964, may have influenced changes. Projections will be made for future trends. Although the period from 1960 to 1970 will be emphasized, the 1930, 1940, and 1950 censuses will also be utilized. Topics to be dealt with are: income, education, home ownership, place of residence by region by region, urban and rural, relationship to head of household, employment status, and the beginning of a series on region of residence by state of birth, migration, population distribution, occupational structure and change, and marital patterns and household composition.

Significance to Biomedical Research and Program of the Institute: The dynamics of the black population are not well understood and, until relatively recently have not been systematically analyzed for their own value. Rather, the demographic changes of the black population were considered in a secondary manner, as something to compare the white population with. This project will take the opposite tack and analyze the black population, considering the white population only as a reference point for differential change. This project will consolidate what is known about the dynamics of the black population. There is sizeable literature on blacks and a number of reports on the demographic aspects of the blacks. They usually concentrate, however, on a particular subject and/or region, e.g., black migration to the North and their levels levels of income. This report would take a holistic approach to the black population.

Proposed Course: This is the first of two years' funding.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Demographic Study of the Mexican-American Population
(Conference)

Contractor: University of Texas
Money Allocated: \$12,075 (1973)

Objectives: The objectives of this 3-day workshop to be held at the University of Texas at Austin in the Spring of 1973 are to bring together established and potential scholars with an interest in Spanish heritage groups; (1) to discuss the conceptual problems in classifying ethnic groupings, to explain the census definitions presently in use, and the relative advantages of each; (2) to indicate what is known regarding the demographic composition and processes of the Mexican-American population; (3) to expose the scholar to Mexican-American community needs for demographic data through an interchange between scholars and community leaders; and (4) to identify high priority research areas. This workshop should stimulate significant research on the demographic dynamics of the Mexican-American population.

Significance to Biomedical Research and the Program of the Institute: Spanish-American population groups in the United States are of particular interest because they feature generally lower health status and higher fertility enclaves in the total population. This contract fits in with other NICHD-funded research underway at Columbia University on the "Fertility of the Spanish-American Populations in the U.S. and their Socio-economic Correlates;" (2) a UCLA "Study of the Growth of the Mexican-American Families" in the Los Angeles area; and (3) a University of Miami Study of "Fertility Dynamics Among Cuban Refugees."

Proposed Course: It is expected the workshop will result in a publication of proceedings and a series of recommendations for needed research on the subject population.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Study of Fertility-Related Decision-Making Among Married Couples

Contractor: Institute for Survey Research, Temple University
Money Allocated: \$110,405 (1973)

Objectives: The main objective of this study is to investigate the nature and etiology of decision-making and implementation processes related to fertility regulation of married couples. This will be accomplished by an interview study of a sample of 450 married couples living in urban, suburban and rural areas. Interviews will be conducted with both marital partners. The study will provide analyses of decision processes and related attitudes focusing first on the couple as a unit and then on both the husband and wife.

The study will investigate: (A) The degree of congruence of knowledge and attitudes among the husbands and wives, (B) the ability of each spouse to accurately perceive the attitudes of the other, (C) the amount and direction of communication between them on topics of fertility and fertility control. Planned analysis will focus special attention on the effects of, and disparity in, partner's attitudes upon the success of fertility control.

Significance to Biomedical Research and Program of the Institute: Fertility decision-making, as an intervening factor between social and psychological attributes and fertility behavior is a topic relevant to the interests of the Center. This study will also make methodological contributions regarding techniques for interviewing men on fertility-related subjects.

Proposed Course: This is a one year contract during which time the survey instrument will be designed, the study carried out, and the data analyzed.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A study of Child-bearing and Labor Force Participation
of Women

Contractor: Emory University
Money Allocated: \$57,309 (1973)

Objectives: The main purpose of this study is to investigate the effect of fertility on labor force participation. The analysis will be based on Census data available through Public Use Samples of 1960 and 1970. The investigator will first seek to determine how the relationship between childbearing, childspacing, and labor force participation changed from 1960 to 1970. A series of control variables will serve to standardize biological and demographic factors and include but are not limited to such variables as age, age at first marriage, parity of the most recent birth, childspacing, family size, migration mobility status, marital status, and number of times married. The second stage of the research will focus on the influence of the other variables on the relationship between childbearing and employment, as for example, how economic need influences a woman's returning to work after the birth of a child. The third stage of the research will elaborate upon the effect of neighborhood characteristics on the relationships of labor force participation and fertility.

Significance to Biomedical Research and Program of the Institute: This proposal deals with an area of considerable interest to the Institute, the relationship between women's labor force participation and fertility. This proposal emphasizes the influences of fertility on labor force participation. While there is undoubtedly a reciprocal relationship this study will focus on consequences of childbearing.

Proposed Course: This project was funded for one year with an expected renewal at the end of that year.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Longitudinal Analyses of variables Predictive of Attitudes
Regarding Fertility and Actual Fertility Patterns

Contractor: Illinois State University
Money Allocated: \$9,639 (1973)

Objectives: The purpose of this study is to isolate and analyze various sexual attitudinal and demographic variables that are predictive of fertility patterns. In 1967 the principal investigator collected fertility-related data from 752 college freshmen. The principal investigator now plans to follow-up the 1967 data base with responses from these same subjects. Study subjects will be between the ages 24 and 25 at the time of follow-up. The data analysis will include sources of sex information, effect of early sexual attitudes on later sexual behavior, changes in reference groups regarding sex, and differences in attitudes and behavior concerning fertility and sex between college graduates and college drop-outs. The general hypotheses presented in this research are that fertility patterns of young adults can be to some extent predicted by knowledge of their pre-marital sexual belief.

Significance to Biomedical Research and Program of the Institute: As part of the Institute's interest in fertility behavior there is concern for the extent to which attitudes towards sexual behavior influence those factors which influence fertility behavior. This study will be an ideal opportunity to take advantage of data already collected to extend our knowledge in this area. The study is exploratory and any conclusions would have to be guarded as far as their generalized ability to a large population. However, this study will provide some unique information not before available.

Proposed Course: This is a one year contract.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Final Household Survey to Complete the Family Planning Evaluation Project

Contractor: University of North Carolina (Chapel Hill)

Money Allocated: \$204,615 (1973)

Objectives: The primary purpose of this project is to complete a large-scale controlled action experiment of an area sample survey of 16 American cities involving a total sample size of about 3200 ever-married women in order to evaluate the effect of family planning programs on the fertility and fertility behavior of communities. This post-survey was made in each city in 1969. Data from the two area sample surveys of ever-married women living in low-income census tracts will be integrated with other data from monthly interviews of newly-delivered mothers, quarterly vital statistics tabulations, operating statistics of the projects, and other data resources (all paid for by other grants) for final interpretation of the impact of family planning programs and the reproductive behavior of the communities they serve. Effects are being measured on four levels: knowledge and attitudes toward family planning, use of services, contraceptive behavior, and reproductive events.

Significance to Biomedical Research and Program of the Institute: Although this project has operational research aspects not described here, it basically examines the premises on which family planning projects operate, and their consequences for the behavior of communities. The initial survey was funded by a grant from Maternal and Child Health Services (MCHS) of the Department of Health Education and Welfare. This project takes an evaluative look at the effect of family planning programs on the fertility of the areas in which the programs are located. It concentrates therefore on demographic impact rather than upon the health of mothers attending, which was the focus of the MCHS grant, or the efficiency of service delivery, which is the usual criteria for family planning programs. It is thus highly critical to the understanding of factors which affect levels of the birth rate.

Proposed Course: This contract is funded for two years, from December through November 1974.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch

Within the scope of the mission of the CDB, namely the development of a number of safe, inexpensive, effective and reversible methods of contraception, a total of 125 individual studies were supported through the contract mechanism during FY 1973. These studies range from research at a fundamental level, where gaps in our knowledge of reproductive physiology exist, to support of clinical testing of known steroidal drugs for their efficacy as contraceptives. Thus a broad spectrum of contract studies with short and long term goals are presently being supported and are described in the following sections.

A. Directed Fundamental Research

The area of basic studies in both female and male reproductive biology is supported because of its promise of providing new points of interference in the reproductive process.

The Oviduct

Considerable progress has been made in analyzing the relationship between oviductal contractility and the transport of gametes within the oviduct. In the rabbit the rate of contractility of the ampullae has a direct relationship to the rate of ovum transport.¹ Agents that interfere with the pattern of oviductal motility not only alter the rate of ovum transport but likewise reduce the fertility rate.

Transducers designed to measure oviductal motility are performing satisfactorily.^{1,2,3} Many of the problems (encapsulation, lead wires) that have plagued the transducer development program have been solved and in the upcoming year their utilization for biological assessment of oviductal and uterine motility should be implemented. Preliminary biological data indicate that, depending on the hormonal state of the animal, the contractile patterns differ drastically. There also appear to be profound species differences.¹ For example, in the rabbit the movement of cumulus masses in the ampulla is accomplished primarily through muscle contractility, whereas in the monkey ampulla transport appears to be accomplished through ciliary activity. An analogous species difference appears to exist with respect to transport of fluids within the isthmus portion of the oviduct. In the rabbit and the pig, estrogen domination produces a current in the direction of the ovary, whereas in the human and monkey oviducts, the current is in the opposite direction. These findings not only stress the hazard of extrapolating from species to species, but also emphasize that the transport of spermatozoa to the site of fertilization in the ampulla may be accomplished by different mechanisms in different species.

Recently special transducers have been developed which are capable of monitoring the passage of ova through the oviduct.⁴ These specialized optical

and ultrasonic transducers not only can track and record the movement of ova through various segments of the oviduct, but likewise can be utilized for the purpose of detecting ovulation. The interval between ovulation and the time cumulus masses arrive into the ampulla is only a few minutes, and thus the time of ovulation can be ascertained with great accuracy. This system has been tested in acute animal preparations and appears to send true signals. Utilization of such transducers in chronically instrumented animals requires further modification. Although it is fully appreciated that utilization of such transducers on large populations is not feasible, it is hoped that the data from this system may lead to more practical methods for ovulation detection. At the present time there are no accurate methods for ascertaining ovulation in the human female and such methods are urgently needed.

Immunopharmacological studies of human oviducts have continued to yield important findings.⁵ Human oviducts develop the anaphylactic reaction much more slowly than guinea pig oviducts and they possess approximately 10% of the histaminolytic activity of guinea pig oviducts. The hormonal effects on histamine content in human oviducts are clear-cut. Oviducts from women receiving progestational drugs have a significantly higher content of histamine than oviducts from women receiving estrogens.

Further studies on oviductal mucins employing radioactive tracers have been continued.⁶ It has been observed that the radioactive tracer appears in oviductal epithelium within 30 seconds, and within 1 hour it can be found in the secreted mucins. This is the first demonstration of the rapidity with which oviductal epithelium can absorb a precursor and secrete a product. Additional studies on the secretory process in the oviduct are presently being initiated.

The Corpus Luteum (CL)

Research on the regulation of corpus luteum function during the past year has yielded a number of important findings. The synthetic corticoid, triamcinolone, was observed to have an effect on ovulation in the baboon.⁷ Parenteral injection of triamcinolone at the time of menstruation or during the early stage of follicle development blocked ovulation and advanced the time of the following menses. Administration of a much larger dose of the natural corticoid, cortisol, did not affect ovulation. It is inferred that a synthetic corticoid can exert important effects on the gonadotropin regulatory mechanism in the baboon. Another unexpected finding deals with the effect of thyrotropin releasing factor (TRF) on ovulation in the baboon.⁷ Administration of TRF for several days prior to and after the anticipated time of ovulation blocked ovulation. The menstrual pattern in these animals remained normal. The mechanism of action of TRF is the subject of ongoing investigation.

As a consequence of TRF administration the circulating levels of the hormone, prolactin, are dramatically increased. In rodents prolactin can stimulate or inhibit corpus luteum function depending on the set of experimental conditions. In sheep it does not appear to have such activity. Administration of a synthetic ergot alkaloid to cycling sheep reduced the circulating level

of prolactin to 1% of normal without affecting cycle length or the levels of circulating gonadotropins.⁸

Since the identification of the structure of the luteinizing hormone releasing factor (LRF) and its subsequent synthesis, this material has been utilized in a number of experiments designed to elucidate the control of ovulation. Studies supported by other funds have clearly indicated that in a number of species including man, injection of this material produces a rapid rise in the blood level of luteinizing hormone. Luteinizing hormone (LH) in turn causes ovulation. In the rhesus monkey, the response to the synthetic LRF is different from that observed in other species.⁹ Intravenous injections of LRF produce only a slight rise in blood levels of LH. Direct application of LRF to the pituitary gland produces a somewhat greater response than intravenous injection. However, these responses are much lower than those observed during the spontaneous midcycle surge of LH which leads to ovulation. Collectively, these data suggest that one reason for the relative insensitivity of the monkey to systemic LRF is rapid inactivation in the peripheral circulation.

Considerable effort has been expended on the study of progesterone synthetic pathways within the corpus luteum. Inhibition of certain enzyme systems results in the lowering of progesterone output.¹⁰ A systematic search for compounds that would inhibit such enzyme systems is presently being carried out. Several prototype compounds have been identified on the basis of in vitro screening assays, and plans are underway to evaluate their biological potential. Since the adrenal gland and the corpus luteum share a common set of enzymes, potential inhibitors must be evaluated rather carefully. Preliminary experiments do suggest that the adrenal enzymes are inhibited to a lesser degree, and as such this type of approach to contraception may be valid.

Further discussion of regulation of CL function can be found under Prostaglandins.

Prostaglandins

During the past year a number of important findings have been made concerning the possible role of prostaglandins in reproductive physiology. Prior research indicated that in the rhesus monkey prostaglandins shortened the menstrual cycle if given late in the luteal phase.¹¹ In the human the luteolytic effect of prostaglandins could not be established.¹² However, when $\text{PGF}_{2\alpha}$ was administered to women prior to ovulation it did delay the ovulatory process.¹² Ongoing research is trying to establish whether the administration of $\text{PGF}_{2\alpha}$ at several day intervals can completely inhibit ovulation; this work is being done in the monkey where serial laparoscopic examinations are possible.

The direct role of prostaglandins in the release of pituitary hormones is being investigated by in vitro experiments.¹³ In such a system, the addition of luteinizing hormone releasing factor (LRF) to pituitary perfusates results in the release of LH. When a prostaglandin inhibitor was perfused together with the LRF, the response in terms of LH output was significantly

inhibited. This research suggests a role for prostaglandins in normal pituitary function.

The relationship of prostaglandins to uterine luteolysis is the subject of several research efforts. Extracts of bovine endometrium from the luteal phase produce a luteolytic effect in the pseudopregnant hamster.¹⁴ However, the luteolytic factor in this extract appears to be a fatty acid rather than a prostaglandin. Administration of arachidonic acid, which is a fatty acid precursor of prostaglandins, to pseudopregnant hamsters has a pronounced luteolytic effect. Sheep endometrium contains prostaglandins, the content of which increases in the pregnant animal¹⁵; uterine venous blood from pregnant animals likewise contains a higher content of prostaglandins. It is of interest that in the pregnant animal this increased content of prostaglandins does not produce luteolysis. A similar increase in prostaglandins in uterine vein blood is observed following estrogen treatment of sheep during mid- to late luteal phase of the sheep's estrous cycle. The relationship of this finding to luteolysis is presently not known. In the monkey, treatment with diethylstilbestrol (DES) during early luteal phase produces a depression in blood progesterone levels which is followed by a rebound.¹⁶ In late luteal phase, estrogen depresses progesterone levels and results in premature menses. These data on DES are very similar to the effect produced by prostaglandins, but the cause and effect relationship has not been established.

Analysis of plasma prostaglandin levels in women during various phases of the menstrual cycle did not reveal any significant fluctuations, nor were the plasma levels significantly higher in women suffering from dysmenorrhea.¹⁷

An important analytical advancement has been made through the use of prostaglandin metabolites rather than the parent molecules as end points.¹¹ Prostaglandins have very short half-lives and increased levels are difficult to measure following their infusion. One readily picks up increased levels of the metabolites because of their greater biological stability.

Studies on Male Reproduction

Spermatozoa achieve their fertilizing ability following passage through the epididymis. This holds true for all mammalian species that have been investigated. The functional integrity of the epididymis is the subject of extensive investigation. Specific receptors for the male sex hormones have been identified in epididymal tissue.¹⁸ The specific receptor for male sex hormones has greater affinity for dihydrotestosterone than for testosterone. Dihydrotestosterone is a metabolic conversion product of the natural hormone testosterone and is a potent androgen. The conversion of testosterone to dihydrotestosterone in the epididymis has been established, and epididymal fluids are rich in this androgen.^{19,20} As to whether this hormone has a direct effect on sperm maturation has not been established at this time.

The responses of the epididymis to castration and hormone therapy show regional differences.²¹ The head of the epididymis, or caput, contains receptors to dihydrotestosterone and responds to castration-induced changes to a greater extent than the other epididymal segments. Replacement therapy utilizing testosterone or dihydrotestosterone fails to restore completely the post-

castration changes observed in the epididymis, while at the same time the other accessory glands are restored.

Hopefully, greater understanding of the hormonal regulation of epididymal function can lead to the development of methods for male fertility regulation based upon interference with normal sperm maturation.

Studies on sperm specific enzymes have been continued.^{22,23} Experimental interference with the activity of these enzymes has resulted in depressed fertility levels. The two approaches that have been utilized are chemical and immunological.^{22,23,24} Both of the approaches require intensive purification of the enzymes and the determination of their biochemical and immunological properties. The development of a specific vaccine designed to interfere with the fertilization process is the subject of ongoing studies. The utilization of this approach for human fertility regulation is not imminent.

The control of mammalian spermatogenesis by sex hormones has been clearly established. Depending on the dose of administered male sex hormones, it is possible to produce either suppression or stimulation of spermatogenesis. It has been postulated that androgens produced within the testes exercise local control over spermatogenesis. It has been recently documented that, in rats in which testicular function has been arrested by removal of the pituitary gland, spermatogenesis can be restored by means of direct intratesticular implantation of androgen. Dose requirements for dihydrotestosterone in these studies was $9\mu\text{g}/\text{testis}/\text{day}$.¹⁹

Mechanism of Hormone Action

The interaction of sex steroids with specific proteins within responsive cells is postulated to represent an initial step in the expression of their biological activity. Estrogen-receptor complexes in the cytoplasm and the nucleus have been shown to bind specifically to the genetic material, DNA. This binding property can be utilized as a method for purifying the cytoplasmic receptor. The specificity of the system has been confirmed by competition studies employing free DNA.²⁵

The presence of progesterone receptors in uteri of mammals has been confirmed.^{25,26,27} These receptors in different species share many common physiochemical properties. One of the interesting differences that has been observed is the difference in the half-life of these receptors. The receptors prepared from rat uteri have a short half-life of about 2 or 3 minutes, whereas receptors from human uteri have a half-life of about 30 to 60 minutes. In all species pretreatment with estrogens increases the number of available progestin receptors.

The fundamental studies on progesterone receptors have been utilized for the design of in vitro screening assays for antiprogestational activity.^{25,27} The aim of these assays is the discovery of compounds which would effectively compete with progesterone for the available receptors while at the same time have no inherent progestational activity. Several prototype compounds

have been identified through these in vitro assays. The interpretation of the results must await determination of biological activity in animals. Because of the known differences among species, it will not be overly surprising if some of the observations utilizing laboratory animal models will not be directly transferable to the human.

Studies with the peptide hormone, oxytocin, indicate that responsive tissues, such as the mammary gland and uterine muscle have receptors for the hormone.²⁸ Receptors for prostaglandins in the uterine muscle have likewise been identified.¹¹ Although both oxytocin and prostaglandins can induce uterine contractility, they appear to do so by activating different sets of receptors. Prostaglandins do not compete with oxytocin for the same receptor.²⁸

Human corpora lutea possess receptors for luteinizing hormone and for human chorionic gonadotropin.²⁹ The uptake of the hormones is related to the functional integrity of the tissue. As was the case with the basic studies dealing with progesterone receptor studies, the gonadotropin receptor studies are of intrinsic scientific interest and furthermore, can lead to the development of screening systems for biologically inactive competitors.

B. Product-Oriented Research

The product-oriented research supported by the Branch entails the following: (1) the synthesis and testing of totally new compounds, as well as the evaluation of existing compounds obtained from other government and non-governmental sources; (2) studies aimed at the development of methods for permanent and reversible sterilization in females and reversible sterilization in males; (3) new drug delivery systems aimed at improving the safety and efficacy of presently available drugs.

Drug Development Program

The advances made in contraceptive technology in the early 1960's (the introduction of orally effective contraceptive steroids and intrauterine devices) held the promise for future improvements and innovations in fertility control. Such breakthroughs have not materialized. Apart from the introduction of several new formulations involving reductions of the estrogen component of the combination pill, no new contraceptive drugs have been introduced on the market. It has become quite clear that we cannot expect the development of a single ideal contraceptive. The oral contraceptives and the IUD certainly do not fulfill necessary criteria, and it is unlikely that any single method will be universally satisfactory. It is for this reason that the proper goal of research in contraception should be the development of a multiplicity of methods which will be suited to a variety of applications. With this objective in mind, the CDB has initiated a long-term program of contract research for the development of a variety of new methods of fertility regulation. This program now includes expanded research efforts in the field of drug development. The drug development program began in June 1971 with support for 9 contracts. It has been expanded and now supports 26 contracts, 11 of which were awarded near the end of FY 73; all involve the synthesis and testing of potential antifertility agents. One of the goals of this drug development program is to find new and efficacious chemical contraceptives which will have fewer side effects than those currently available. An equally important goal is to develop an effective contraceptive that men may use without risking loss of libido.

Of the 26 current contracts in drug development which entail synthesis and biological evaluation, 14 contracts deal with novel steroids, 5 involve unique prostaglandin analogs, 5 embrace LRF and novel analogs of LRF, and 2 can be classified as involving miscellaneous, non-steroidal, chemical entities.

The contracts involving the synthesis of novel steroids have been undertaken with the expectation that these compounds may show antifertility activity with reduced hormonal effects. Those studies which deal with potential anti-progestational agents are either new or are still primarily in the synthetic phases, and preliminary biological results cannot be anticipated until the middle of FY 74. In terms of trying to achieve a separation of antifertility activity from other hormonal effects, one study on silicon-containing steroids³⁰ has given rise to some promising leads. The substitution of certain trialkyl-silylethynyl groups for the ethynyl group of ethynyl estradiol

markedly affects the biological response of these steroids and causes a favorable separation of antifertility activity from estrogenic activity. One compound has shown a 4-6 fold increase in antifertility potency in rats when compared with ethynyl estradiol, but only shows 40%-75% of the estrogenic activity. The same compound also shows a sufficiently favorable separation of activities in rabbits to warrant preliminary studies in monkeys, and this work is currently in progress. The value of a compound with a favorable ratio of antifertility to estrogenic activity would be the availability of a post-coital or antiovolulatory agent with lesser untoward side-effects than those currently attributed to the estrogenic component of "the pill."

Our support for contracts on prostaglandin-like compounds is based upon their potential utility as (a) luteolytic agents, (b) myometrial stimulants, or (c) menses inducers for use as once-a-month pills. Natural and synthetic prostaglandins have been demonstrated to have luteolytic activity in animals but not in humans.¹² The presently available prostaglandins have a number of side-effects which impose a limitation on their utility in fertility regulation. The justification for our modest, but continuing synthetic program in this area is that an agent which is luteolytic in the human would be novel and desirable contraceptive agent, particularly if it were devoid of the undesirable side-effects associated with the known prostaglandins. In addition, one cannot rule out the utility of a prostaglandin-like agent having a greater effect on the uterine smooth muscle than on the gastrointestinal tract. An example of such separation of activities are certain acetylenic analogs of PGF₂ α .³¹ For example, the 13, 14 acetylenic analog of PGF₂ α shows antifertility activity in hamsters ranging from two to five times that of PGF₂ α , depending on the route of administration, subcutaneously or orally, but shows only 1/3 of the activity of PGF₂ α in contracting the colon of the gerbil. More remarkable is the finding that the diastereomer of this compound showed about 1/5 to 1/6 of the antifertility activity of PGF₂ α , but had only 1/500 of the activity of PGF₂ α in contracting the gerbil colon. Both compounds probably act by a luteolytic mechanism in the hamster. Both compounds not only failed to serve as substrates for placental prostaglandin 15-dehydrogenase, but were, in fact, inhibitors of that enzyme. This study opens the possibility of discovering even greater biological selectivity among prostaglandin analogs.³¹ In another study,³² a variety of 11-deoxy-prostaglandin analogs have been synthesized. The best compound was found to have only 1/30 of the antifertility activity of PGF₂ α in hamsters when administered subcutaneously. None of the compounds was significantly active orally in the hamster nor subcutaneously in the rat. These are important observations in terms of the structure-activity requirements for prostaglandin-associated antifertility activity.

Contract support for the testing of LRF and its analogs continues. In early 1972, the Branch awarded a contract³³ for the synthesis of gram quantities of LRF in order to provide ample amounts of the synthetic decapeptide to the scientific community for research purposes and to explore the potential of this gonadotropin-releasing hormone in fertility regulation.

The decapeptide amide, (identified as LH-RH/FSH-RH), is now available as a solution in ampoules containing 100 micrograms per milliliter of solution and is also available as bulk solid material in milligram quantities to qualified investigators for animal experimentation only. Other synthetic efforts are directed mainly towards finding LRF inhibitors. Such compounds, if practically effective, could prevent the midcycle surge of LH and thus lead to a different type of anti-ovulatory contraceptive. It might be necessary, however, to administer small quantities of estrogen and progestogen along with the LRF inhibitor to maintain normal cyclicity. While many LRF analogs have been synthesized, only one has shown modest in vivo LRF antagonist activity.³⁴ This result must be confirmed and then efforts will be made to increase, by structural modification, the potency of this LRF inhibitor.

An interesting observation made in the non-steroidal area is that the feeding of an antibiotic mixture to a large beagle breeding colony prevented pregnancy in 100% of the mated females.³⁵ Conception rates rapidly returned to normal upon discontinuation of the medicated feed. The active antifertility agent has been shown to be not the antibiotic itself, but rather a mixture of quaternary ammonium salts. These are currently being tested in other animal species.

It is anticipated that a modest program for the preparation and testing of plant extracts will become operative in FY 74. Plant species will be selected on the basis of reported antifertility activity in animals and to a certain degree on folklore. The plants will be obtained through the U.S. Department of Agriculture, extracted by commercially available laboratories and tested by our own biological testing facility (see below). Resultant leads will be followed by isolation and identification of the active principle contained in the plant.

Testing Facility

During 1972 a contract was initiated with the Mason Research Institute to establish a biological testing facility for the evaluation of contraceptive drugs and devices submitted by the CDB.³⁶ Baseline studies, including endocrine profiles, antifertility and control data, have been completed on 16 biological standards. This information provides the means for a direct comparison of the activities of a broad spectrum of new compounds. Fifty-four experimental drugs have been submitted for screening, assay, and/or mode of action studies. One promising lead has accrued and this drug is now being evaluated in subhuman primates.³⁰

In addition to the battery of standard endocrine and antifertility assays to which new compounds are subjected, several new test procedures are being developed jointly by the contractor and CDB staff. These will permit a more critical evaluation of mode of action and potential clinical utility.

Sources of compounds for testing include synthetic programs supported by the CDB and numerous private, public and governmental laboratories throughout the world. CDB staff reviews all compounds submitted for evaluation to determine the manner in which each drug will be studied.

Procedures have been developed to safeguard proprietary interests in both compounds and devices. These measures are necessary to protect the rights and identity of compound sources, particularly those involving commercial interests.

Drug Delivery Systems

During this fiscal year the CDB has expanded considerably its limited program dealing with drug delivery systems.³⁷⁻⁴⁰ The aim of this program is to take presently available contraceptive drugs and to develop new means of delivering them to the body or reproductive organs. In a number of instances the drugs will be delivered directly to the uterus or the cervix in an attempt to preclude systemic effects of these drugs. In other instances these systems will incorporate the drugs into a biodegradable matrix where the matrix as well as the drug are slowly absorbed by the body. In view of the fact that the rate of drug delivery can be controlled in advance, such systems require considerably lower drug dosages than the presently available oral contraceptives.

Feasibility studies of the utility of such drug delivery systems in human fertility regulation are currently being conducted by several organizations other than CDB.

Development of Sterilization Techniques (Female)

While procedures for reversible sterilization of the female by means of tubal occlusion continue to present many problems, significant progress has been made in a crucial area. A steerable hysteroscope has been developed under CDB support which permits direct visualization of the utero-tubal junction.⁴¹ The instrument has been used successfully in extirpated human uteri as well as in intact baboons under actual operating-room conditions. Initial human clinical investigations in patients hospitalized for hysterectomy will be undertaken within the next few months. A sliding cervical seal is an integral part of the instrument and permits the expansion or flushing of the uterine cavity through inlet and outlet ports located in the hysteroscope tip. The instrument will facilitate the placement of tubal occlusive devices in the intramural portion of the oviduct and will provide a convenient means of performing sterilization by electrocautery; the transcervical approach eliminating the necessity for abdominal surgery. Occlusive devices are being fabricated with a variety of surface characteristics designed to ensure retention within the oviduct.⁴¹

The development of a reversible tubal occlusive system employing the extrusion of uncured silastic elastomer into the oviducts is progressing satisfactorily. Studies in rabbits are about completed and work in monkeys will be initiated shortly.⁴²

Approaches to intra-uterine contraception are being explored through the development of a hydrogel grafted, copper-containing IUD splint⁴³ and a microcapsule drug delivery system.⁴⁴

Development of Sterilization Techniques (Male)

In the male reversible sterilization program, problems encountered have neces-

sitated reiterations of the prosthesis design, fabrication, and implantation techniques.⁴⁵⁻⁴⁸ Considerable progress has been made in solving the tissue-to-device seal, which is an essential part of a functional occlusive device. Various methods have been developed for denuding the vas mucosa, which appears to be a prerequisite for ingrowth of the underlying connective tissue of the vas wall into the matrix of the device to obtain a firm seal.⁴⁵⁻⁴⁹ While studies in a variety of laboratory animals have demonstrated the effectiveness of the various reversible prostheses to prevent sperm passage and subsequent restoration of the flow of sperm, functional restoration of fertility remains to be demonstrated. Additional animal experiments, however, are required to establish a reasonable statistical base for these initial observations and to provide more complete documentation.^{45,46,48} Of particular interest in this program has been the development of absorbable intravasal stents or catheters for reanastomosis of the vas. These devices are fabricated from biodegradable material, and preliminary results in animals show that they are well-tolerated and absorbed in a few weeks. Evidence of recanalization of the vas lumen was obtained by the presence of sperm in the ejaculate.⁴⁶

It should be recognized that the materials presently being explored for the reversible devices could also be fabricated into stents for reanastomosis of the transected vas; thus providing an excellent backup program, should the reversibility of the devices prove unfeasible.^{45,46,48,49}

Clinical Studies

In an initial clinical trial, Danazol[®] (an isoxazole derivative of ethynyl testosterone having anabolic activity) was administered to normal adult male volunteers alone or in combination with testosterone propionate or testosterone enanthate to determine the effects on sperm output.⁵⁰ The drugs were administered for four months. Danazol[®], orally (p.o.) (600 mg/day), produced a modest reduction in sperm counts, but Danazol[®] (p.o.) plus testosterone propionate, 10 mg. intramuscularly three times weekly, reduced sperm counts to below 2 million/ml in 3 of 4 men. Danazol[®] (600 mg/day, p.o.) plus testosterone enanthate (200 mg intramuscularly once monthly) dropped sperm counts to less than 1 million/ml in 3 of 3 men within eight weeks. The loss of libido and potentia observed with Danazol[®] alone was prevented when the testosterone esters were added to Danazol[®] as a combined regimen. A much more extensive clinical trial employing Danazol[®] together with the orally active androgen, methyl testosterone, is currently in progress.

A clinical trial of a new fluid-filled, soft intrauterine device is presently being initiated.⁵¹ Preliminary clinical data are quite encouraging. It is anticipated that expulsions of the device or removals because of undesirable side effects will be minimal. The device will be tested in 100 women volunteers and its contraceptive efficacy and safety will be evaluated over an initial one-year period.

The CDB will initiate the support of studies dealing with the clinical utility of estrogens, other than DES, as postcoital antifertility agents. This program will be expanded to include several thousand cases during the early part of the next fiscal year.

Hormone Distribution

At the present time the CDB, in cooperation with the NIAMDD, is supplying the scientific community with samples of HCG, HCG subunits⁵² and LRF,³³ all of which were produced through CDB sponsored research contracts. Distribution of ovine LH and its subunits will be initiated as soon as this material is properly characterized.⁵³

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44. Gardner, D. L., 71-2230. The study of intrauterine microcapsules as a prolonged release drug form. Battelle Columbus Laboratories.
45. Brueschke, E., 71-2229, \$244,386. Design and development of implantable contraceptive devices for use in the male. IIT Research Institute
46. Nuwayser, E., 71-2227, \$344,847. Development of new sterilization techniques. Abcor, Inc.
47. Freund, M., 71-2223, \$74,779. The use of Bionyx control as a contraceptive (reversible vasectomy device) in man. New York Medical College
48. Mather, E., 72-2769, \$38,041. Development and evaluation of a reversible vasectomy prosthesis. University of Missouri
49. Robinson, T., 72-2796, \$104,325. Development of a reversible vas-occluding prosthesis utilizing a microporous tissue interface. TECNA Corporation

50. Paulsen, C. A., 71-2234, \$118,056 (2 years). Contraceptive development studies for males. University of Washington
51. Kitrilakis, S., 73-2767, \$51,980. Clinical evaluation of a fluid-filled intrauterine contraceptive device. TECNA Corporation
52. Canfield, R. E., 70-2251, \$194,279 (2 years). Pilot study for the large scale preparation of purified human chorionic gonadotropin. Columbia University
53. Ward, D., 69-2221, \$84,187. Physiological aspect of the attachment of carbohydrate to luteinizing hormone. University of Texas

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Conference on Biorhythms and Human Reproduction
Contractor: Columbia University
Money Allocated: \$35,238 (FY 73)

Objectives: To bring together experts in the fields of chronobiology and the reproductive sciences in a comprehensive review of the fields and to explore new data and methods.

Significance to Biomedical Research and Program of the Institute: The fields of chronobiology and human reproduction have developed in parallel with relatively little overlap between the two. It was the purpose of the CPR in supporting this conference to bring together the investigators from the two disciplines to discuss the statistical, biological and chemical methods used by the investigators in trying to understand the important subject of biological rhythms in reproduction.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Symposium on Mechanism of Ovulation
Contractor: Society for the Study of Reproduction
% University of Illinois
Money Allocated: \$5,000 (FY 73)

Objectives: Arrange and conduct a symposium on the mechanism of ovulation. Specifically relating to the events occurring between hormone impingement and follicular rupture. The symposium will bring together both the old and the new information on this crucial subject. Rapid publication of this symposium will be undertaken.

Significance to Biomedical Research and Program of the Institute: Research on the mechanism of ovulation has been identified by the CPR to be a priority field both by the grant and contract routes, and the periodic assessment of the state of knowledge along with the exploration of new approaches is urgently needed.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Symposium on the Biology and Pathology of Aging Gametes
Contractor: University of Washington
Money Allocated: \$33,678 (FY 73)

Objectives: The objective of this symposium is to explore and evaluate the gamete aging process at the cellular and molecular levels. Furthermore, it is the objective of this symposium to stimulate thinking about the aging process in gametes and to open the way for new directions of research.

Significance to Biomedical Research and Program of the Institute: The aging of gametes can result in abnormal development of the zygote. The study of gamete aging has been identified by the CPR as an important area of research dealing both with the fundamental aspects of the process and its relationship to contraceptive regimens which may result in aging of the gametes.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Estrogen-Receptor Substances of Oviduct Tissue
Contractor: University of Chicago
Money Allocated: \$45,413 (FY 69 Funds), \$29,815 (FY 70 Funds),
\$31,944 (FY 71 Funds), \$36,265 (FY 72 Funds)

Objectives: The investigators are studying the molecular mechanism of estrogen activity in oviduct tissue by biochemical and autoradiographic determination of the presence, intracellular localization, and chemical nature of specific "estrogen receptor" proteins.

Major Findings: Development of a method for large scale purification of the nuclear form of E₂ receptor complex based on a previously developed small scale method was continued. Starting with 1 Kg of calf uteri a 1000-fold purification of the nuclear E₂ receptor complex was achieved by a) exposure of cytoplasmic E₂-receptor complex at 25° to uterine nuclei, b) extraction of nuclei with 0.4MKCL, c) ammonium sulfate precipitation, d) gel filtration over Sephadex G200 in 0.4 M KCL, and e) DEAE-cellulose chromatography (in spite of low yields, the latter step is necessary for a successful subsequent step). Preparative gel electrophoresis did not work. Further purification is therefore achieved by repeated analytical gel electrophoresis. A highly purified product was obtained giving a single band in one electrophoresis system and a single sedimentation peak in a sucrose density gradient. Final yield was 18 μ g.

Nuclear form of E₂-receptor complex has to be obtained by heating at 25° in presence of nuclei. Precipitation in the cold with ammonium sulfate, which also leads to a 4S to 5S transformation was found not to yield qualitatively the same type of 5S compound as the one extracted from nuclei (different gel electrophoresis pattern after extensive purification).

Significance to Biomedical Research and Program of the Institute: This particular study is an important step in understanding hormone and possibly anti-hormone action at the cellular level and is directly relevant to the purposes of the Contraceptive Development Program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Factors Influencing Mammalian Oviductal Secretions
Contractor: Johns Hopkins University
Money Allocated: \$32,090 (FY 69 Funds), \$41,362 (FY 70 Funds),
\$42,686 (FY 71 Funds), \$46,743 (FY 72 Funds)

Objectives: The objective of this research project is to continue the study of the electrical potential difference exhibited across the serosal and mucosal surface of the rabbit oviduct as related to net ion transport. Preliminarily, they have found a net chloride transport across the membrane under short-circuited conditions. Since the net chloride flux is equivalent to the short-circuited current, it has been reasoned that the electrical phenomena can be accounted for by the active transport of chloride. They are now developing an in vitro preparation for the primate oviduct similar to that described for the rabbit, in order to extend their studies of the active transport of ions using the short-circuit technique to this species. Lastly, they wish to determine the effects of drugs and hormones on active transport in the in vitro oviduct system using this as a screening technique for potential contraceptive drugs.

Major Findings: Investigation of stimulation of oviductal adenylyl cyclase activity by catecholamines and prostaglandins has continued. The following catecholamines stimulated adenylyl cyclase activity in the order of potency: isoproterenol > epinephrine > norepinephrine. Their effects could be abolished by beta-, but not by alpha-, adrenergic blockers. These results show that stimulation by catecholamines is mediated by beta-adrenergic receptors. Maximal stimulation of adenylyl cyclase by prostaglandins was obtained at 10^{-5} M. The prostaglandins E₁ and E₂ were more potent (about 10 times) stimulators than the prostaglandins F_{1α} and F_{2α}. Stimulation by prostaglandin E₁ could be prevented by beta-adrenergic blocking agents, which suggests that the effect of prostaglandin E is also through activation of beta-receptors.

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 studies of the mechanisms responsible for the secretion of oviductal fluids and the influence of these fluids on gamete transport and survival.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Bioengineering Approach to the Study of Tubal Activity and Contraception
Contractor: Drexel Institute of Technology
Money Allocated: \$116,456 (FY 69), \$38,437 (FY 70), \$7,688 (FY 71), \$64,893 (FY 72), \$111,742 (FY 73)

Objectives: The objective of the contract is to study tubal contractile activity in the unrestrained animal with respect to the various ovarian cycle phases, and to assess the role such activity plays with regard to ovum transport. The investigator has concentrated on the development of the appropriate transducers and telemetric packages, with initial attempts at animal implantation.

Major Findings: Comparative studies of the silicon strain gage and the silastic strain gage revealed little advantage of one over the other. The problems of transducer encapsulation and lead wires have been successfully resolved. Transducer implants on monkey oviducts have performed satisfactorily for a period of 12 weeks. Data acquisition is in progress. The computer programs are being implemented for the analyses of data.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Oviduct Cilia: Structure, Function, Development
Contractor: University of California
Money Allocated: \$34,343 (FY 70), \$9,097 (FY 71), \$35,038 (FY 72)
\$29,138 (FY 73)

Objectives: The stroke form and fine structure of oviductal cilia is to be explored using techniques previously applied to mussel gill cilia. The aim is to provide information on the beat mechanism of oviductal cilia and to evaluate the influence of various agents on the mechanism. Techniques to be employed in these studies include high-speed cinematography, electron microscopy, and serial-section analysis of stroke form and top structure. An attempt will be made to integrate this information into a unified theory of ciliary beat based on the interaction of various ciliary organelles.

Major Findings: (1) In the untreated normal mouse oviduct, excluding recently ovulated animals, the cilia of the epithelium of the fimbria and ampulla are relatively inactive. This has been demonstrated by phase-contrast and Nomarski optics, transmission and electron microscopy. (2) In gonadotropin treated superovulated mice there is an increase in ciliary activity, both in the fimbria and the ampulla. High speed cinematography indicates a pattern of coordinated activity-metachronism. This aspect could not be substantiated by thin-section and scanning electron microscopy and by these methods metachronism in oviductal cilia is still to be discovered. (3) The investigator has established organ culture of isolated ampulla and fimbria which permits observations on living tissues. Because of the size of the ovum with the adhering follicular cells, the study of the relationship of ciliary beat to the propulsion of the mass is difficult. It is of interest that sea urchin eggs which have a similar diameter as mouse ova are not picked up by oviducts in organ culture. This corroborates other studies which suggest that the presence of follicular cells is required for normal ovum transport into the fimbria and through the ampulla.

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 studies of the mechanism of action of oviductal cilia and their role in gamete transport.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Measurement of Blood Flow in the Mammalian Oviduct
Contractor: Washington State University
Money Allocated: \$14,065 (FY 70), \$14,220 (FY 71), \$14,301 (FY 72)

Objectives: This research project is designed to measure blood flow to the mammalian oviduct and to describe the structural geometry of the oviductal vasculature. For the latter objective, silicone rubber elastomer will be injected, and casts prepared in the Anatomy Department and studied grossly with the dissecting microscope, and microscopically, with 100 to 150 micron sections. For measurement of blood flow volume, two methods are proposed: (1) indicator-diffusion using hydrogen gas; and (2) thermoconductivity. In the first method, blood flow is related to the rate of removal of hydrogen from the tissue, while in the second, the rate of dissipation of heat is the variable related to flow. These methods have been selected because they offer the best opportunity to estimate capillary flow, and appear to be adaptable for use in unanesthetized animals. Two transducer configurations will be tested: (1) a needle type, with the measuring element inserted into the tissue; and (2) a cuff type in which the measuring device is in contact with the surface of the oviduct. During acute experiments the 4-amino-antipyrene indicator-diffusion technique will be utilized for comparison purposes in the measurement of uterine blood flow. This technique will be modified using radioiodinated antipyrene when the oviduct comparison is made. Eventually, it is planned to study the effects of sex hormones on oviductal blood flow in the oophorectomized animal.

Major Findings: Measurement of blood flow by hydrogen gas desaturation in the estrus doe has been reported and the flows during early pregnancy are currently being collected. The major obstruction to progress in this study has been the design of an implantable electrodes with minimal trauma to the tissue.

The conduction of thermal energy from a heated site is the basis of the second technique of blood flow measurement developed under this contract. The circuitry has been modified from published work by previous investigators, and very small electrode probes have been fabricated which are fixed to the organs with cyano-acrylate adhesive. The system has been tested in vitro and found to respond linearly to flow. The system is being compared to hydrogen gas flows and is suitable for chronic in vivo use.

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 studies on the vascular supply and blood flow patterns in the mammalian oviduct.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovariectomized-Hormone Treated Rabbit and Pig-Tailed Monkey, Macaca-Nemestrina
Contractor: Medical College of Virginia
Money Allocated: \$13,195 (FY 70), \$12,764 (FY 71), \$14,402 (FY 72)
\$19,068 (FY 73)

Objectives: This research project is part of an interdisciplinary research program on oviduct structure and function being carried out at the University of Washington. This investigator will carry out at the University of Virginia certain morphological aspects of the work on specimens obtained in Seattle where correlative in vivo observations and physiological measurements are being made on the same material.

A systematic study of the ultrastructure of the fimbria, ampullae, and isthmus of the normal rabbit oviduct in the pre- and post-ovulatory period, in the ovariectomized animal, and in ovariectomized animals receiving estrogens and progesterone singly and in combination will be made. Studies will not be confined to the epithelium but will include fine structural observations on the smooth muscle which may be correlated with changes in oviduct motility. With this base line established on rabbits, similar studies will be made on the oviduct of the pig-tailed macaque.

Major Findings: In vivo observations on transport of ova in cumuli over the surfaces of fimbriae of the rabbit oviduct of ovariectomized and ovariectomized estradiol treated (1-10 days) rabbits were correlated with the cytology of the fimbrial epithelium. If less than 44% of the cells were ciliated egg transport did not take place.

A significant degree of deciliation occurred in most animals after 3-5 days of estradiol treatment. Glycogen bodies appeared most often in ciliated cells of estrous, ovulatory and ovariectomized rabbits treated for 2-6 days. Secretory cell responses to estradiol included variation in size of apical protrusions, number of secretory granules, basophilia and size of nucleus and nucleolus. In smooth muscle associated with the oviduct estradiol treatment resulted in an increase in cellular, nuclear and nucleolar size and in number of ribosomes and cisternae of GER. Glycogen accumulated in large amounts. PAS positive substances in epithelium and smooth muscle of the oviduct and uterus of castrated, estradiol-treated rabbits were studied.

Preliminary light and electron microscopic observations have been made on the fimbrial epithelium of the oviduct of 9 pig-tailed monkeys, Macaca nemestrina. In 7, ciliated cells comprised 48-55% and in 2, 7-25%.

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 studies of the ultrastructure of the mammalian oviduct and its responses to changes in the hormonal environment.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: An Interdisciplinary Program Project on the Biology of the Oviduct and Gamete Transport
Contractor: University of Washington School of Medicine
Money Allocated: \$192,490 (FY 70 Funds), \$177,734 (FY 71 Funds), \$177,734 (FY 72 Funds), \$193,985 (FY 73 Funds)

Objectives: This is a comprehensive interdisciplinary program project on the basic biology, physiology, and endocrinology, of oviductal function in rabbits and monkeys. Seven different specific aims are enumerated: (1) the development of techniques for direct observation of the normal function of the various segments of the oviduct in situ; (2) development of miniature sensing devices and telemetry equipment that will permit study of the normal activity of the oviduct in unanesthetized, unrestrained, rabbits and monkeys; (3) study the electrophysiological characteristics of oviductal musculature; (4) investigation of intrinsic innervation, pharmacological responses, and the possible role of pacemakers in oviduct function; (5) ultrastructural studies of the epithelium and musculature under different physiological conditions; (6) development of techniques of in vitro cultivation of oviductal tissue as a potential bioassay system; and (7) studies of the mechanism of sperm transport through the oviducts.

Major Findings: Analysis of contractility utilizing mutual inductance coils and split image cinematography has shown a correlation coefficient of 0.79. Further studies utilizing improved oscillators will be continued along the same lines. The recording of signals from an open dish preparation was very similar to that obtained from a chronically implanted anesthetized animal. This indicates that the inductance coil system is operating faithfully. The pattern of oviductal activity under anesthesia is different from that observed in the awake animal.

In rabbits injected with HCG the pattern of oviductal activity changes as a function of time. Eight hours after HCG the intensity and frequency of contractions were at their lowest. In animals injected with estrogens activity is greatest some 27 hours after the last injection. This time coincides with the time of rapid egg transport. Increase in oviductal activity is observed following the injection of estrogen antagonist CI628. The fertility of females treated with this drug is significantly reduced.

In the monkey (*Macaca nemestrina*) the transport of cumulus masses over the fimbria is slower than in the rabbit. In post-ovulatory animals the transport time from ostium to the ampullo-isthmic junction is quicker than in the preovulatory animals. Unlike in the rabbit, the transport of cumulus masses through the ampulla appears to be accomplished through ciliary activity rather than through muscular contractility.

Initial studies on the transport of fluids within the isthmic portion of the oviduct indicate that during estrogen dominance (estrus) there was unilateral transport toward the ovary whereas during luteal phase there was segmented transport.

Study of ciliary currents in the rabbit and the pig indicates that in the isthmic portion the current is toward the ovarian end. In the monkey and human oviducts the current is in the opposite direction. These findings emphasize the differences that exist among mammalian species.

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 on the various functions of the oviduct which are responsible for gamete transport and survival, fertilization, and transport of the fertilized egg to the uterus.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Physiology of the Oviduct
Contractor: Stanford University Medical School
Money Allocated: \$91,585 (FY 70), \$10,000 (FY 71) \$141,864 (FY 72)
\$112,458 (FY 73)

Objectives: The objective of this investigation is to develop electrodes, transducers and other instrumentation for study of electrical, mechanical, pressure, and flow phenomena and to use these instruments to investigate the functional properties of the muscle coat of the oviduct (1) as small segments in the tissue bath, (2) in isolated organs and (3) in the intact subject. By determining changes in properties of the oviductal muscles as a function of hormonal environment, knowledge will be sought as to the mechanisms which control oviduct function.

These studies will involve measurements of the electrical and contractile behavior of oviductal tissue using excised oviducts of rabbit and man, at various stages of the reproductive cycle. Studies of pacemaker activity, the interrelationship of electrical activity from one area to another, correlation of electrical and mechanical activities, and intraluminal pressure changes will be conducted. The final phases of the study will involve the measurement of these functions in intact, free-roaming animals, and hopefully, eventually in women. All data will be programmed for computer analysis and a mathematical model of the system will be undertaken similar to one the investigator has developed for intestinal smooth muscle.

Major Findings: During the past contract year the force transducer has been developed to the point of performance testing and appears to be satisfactory for biological studies of tubal contractility. It has been implanted in a number of rabbits and appears to faithfully record the contractility of the organ. Its small size permits multiple transducer implantation. Recordings from two longitudinally implanted transducers indicate similarity of wave form and frequency whereas two transducers implanted transversely, at the same sites as the longitudinal ones, show differences in contractile patterns. The contractor is slowly resolving the problems of leads and encapsulation of these transducers.

During the past year a pressure transducer was likewise developed. Whether the size of the transducer, 1mm, is sufficiently small to be accommodated within the fallopian tube is still a question.

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 encourage scientists from other disciplines to apply their unique skills to studies of reproductive function.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Steroid Hormone Binding in Reproductive Organs
Contractor: Yale University
Money Allocated: \$39,141 (FY 70), \$58,500 (FY 71), \$67,692 (FY 72)

Objectives: The objective of this research project is to study various aspects of the interaction of contraceptive drugs and potential contraceptive agents with steroid binding molecules in organs concerned with reproduction. The investigator will continue studies directed towards in vitro comparisons of estrogen binding molecules from the rat uterus, vagina, anterior pituitary, and hypothalamus. The affinity of estradiol for binding to macromolecules from the supernatant fraction of these organs will be determined after in vitro incubation, and various drugs will be examined for their ability to compete with tritiated estradiol for macromolecular binding. To facilitate these studies, the investigator will attempt to develop a gentle and rapid purification procedure for estrogen binding macromolecules utilizing as the separating medium, estradiol reversibly linked to a standard chromatographic resin.

Major Findings: After attachment of estrogens to the uterine cytoplasmic proteins, the complex appears to undergo some changes and crosses the nuclear membrane and attaches to chromatin. This process is being studied in cell free systems with radioactive estradiol charged uterine supernatant added to purified nuclei. Potentially these subsequent steps may differ between organs with respect to the nature of the estrogen required or factors involved.

To purify the different binding proteins, the investigators have been working toward the development of estrogen affinity chromatography. By studying a variety of estrogen derivatives, they have selected one containing a 17 α -side chain with a triple bond for linking the estrogen to the resin and still retaining high affinity for the binding protein. In another approach, they have synthesized 16-ylideneacetic estradiol which retains partial binding affinity and have coupled the terminal carboxy to aminoethylsepharose.

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 studies of the steroid binding mechanism in reproductive and central nervous system tissues.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunopharmacology of the Human Fallopian Tube
Contractor: Stanford University
Money Allocated: \$40,793 (FY 70), \$79,881 (FY 71), \$91,602 (FY 72),
\$270,622 (FY 73 for 2 years)

Objectives: The work to be carried out under this contract involves two aspects of oviductal physiology: (1) characterization of the permeability and transport characteristics of oviductal epithelium; and (2) characterization and quantitation of the oviductal response to the anaphylactic reaction between an antigen and the specific antibody bound to oviductal cells. Work will be carried out in laboratory animal and human oviductal tissue, in vitro.

Major Findings: Estradiol decreases the activation energy for histamine release, while progesterone increases it. The role of cAMP in the release of histamine was investigated in the isolated atria of guineapigs. Atria were used in these preliminary studies because they release more histamine per gram of muscle than does the oviduct. Thus, if cAMP plays a role in the anaphylactic response it could be seen more clearly in atria than in the oviduct. An increase in tissue cAMP seems to reduce the release of histamine from atria in guinea pigs primed with homologous antibody. This work will now be extended to the oviduct.

The anaphylactic response in human oviducts was studied in samples from 53 donors. The process of sensitization in the human oviduct, to human sera or serum products, is much slower than in the guinea pig and requires a different set of exposure times and temperatures. The response is greatly accentuated if the tissue is stored at low temperatures for several days. In addition to histamine, human oviducts may also release prostaglandins when challenged immunologically. It is of interest also that the human oviduct has approximately 10% of the histaminolytic power observed for the guinea pig oviduct.

The electrical discharge during normal activity in isolated oviducts of the guinea pig and human has been recorded. Histamine seems to enhance this activity while noradrenaline reduces it.

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 innovative studies on the physiological and pharmacological properties of laboratory animal and human oviductal tissue.

Proposed Course: During the forthcoming two years the stated objectives of this contract will be fulfilled.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on Steroid Hormone Receptors and Their Role in Reproductive Biology
Contractor: Vanderbilt University
Money Allocated: \$40,088 (FY 70), \$47,941 (FY 71), \$49,908 (FY 72)

Objectives: The objectives of this research project are: (1) to study further the already established interaction of estrogens with receptor substances of rat uterus in an effort to elucidate the role of the receptors in hormone action; and (2) to investigate estrogen and progesterone localization and binding in chick oviduct, a tissue which recently has been reported to contain a specific binding protein for progesterone.

Major Findings: The contractor has demonstrated that the binding of the cytosol estrogen receptor to DNA can be inhibited by free DNA and suggests the possibility that purification of the cytosol receptor can be accomplished by using DNA in affinity chromatography.

Uterine progesterone receptors from several species were examined. The major species difference among the cytosol receptors is in their rate of dissociation. Thus, the half-life of the receptor-progesterone complex from the rat uterus is only 2-3 minutes, whereas, in the human the half-life is 30 to 60 minutes. This observation may explain the differences in binding constants among the different species.

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 studies of the steroid binding substances in reproductive tract tissues.

Proposed Course: Due to the investigator's transfer to the Mayo Foundation this contract will be continued in that institution. FY 73 funds allocated to the project were \$111,121 for 2 years. 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morphochemical Analysis of Oviductal Mucins
Contractor: University of Minnesota
Money Allocated: \$17,763 (FY 70), \$19,395 (FY 71), \$17,523 (FY 72),
\$29,003 (FY 73)

Objectives: The investigator plans to study the mucosubstances of each of the morphologically distinct regions of the oviduct from cycling and ovariectomized hormonally treated rabbits and from humans during the menstrual cycle by a battery of chemical staining techniques generally known to be specific for the reactive groups in the carbohydrate moieties of the epithelial secretions. The effects of specific chemical and enzymatic alterations of staining patterns will be analyzed to determine the reliability of observed staining alone. The ester sulfate, carboxylate and vicinal hydroxyl groups of the mucosubstances will be stressed in an attempt to correlate their presence or absence with various endocrine states. Various radioactive precursor sugars of epithelial mucins will be used to follow the incorporation of these radioisotopic labels into the oviductal mucins. This will be done under various hormonal states.

Major Findings: The investigator has completed autoradiographic studies of the incorporation and fate of S^{35} in precoital adult rabbit oviducts. It was found that S^{35} , injected intraperitoneally as sodium sulfate- S^{35} in saline, appears over the Golgi region in epithelial cells of the ampulla and isthmus within 30 seconds of administration. Shortly thereafter, the label appears in the secretory granules, and by 1 hour all label is extracellular, especially on the cell surfaces. This is the first demonstration of the rapidity with which the oviductal epithelium can absorb a precursor and secrete a product. The sulfate labeling can be eliminated by treatment of sections with acidified (but not unacidified) methanol at 60°C for 4 hours before applying emulsion. This demonstrates that the S^{35} is present as an ester sulfate in the carbohydrate moiety of the mucin. Ampullary mucins also show alcianophilia at high salt molarities (0.5-0.7 M MgCl_2) which also indicates the acidic nature of the carbohydrate portions.

Isthmus mucins possess carboxylate radicals usually associated with sialic acid (neuraminic acid). Treatment with neuraminidase, as well as mild methylation, blocks isthmus alcianophilia. This strongly suggests that isthmus secretions are rich in sialic acid containing mucins.

In summary S^{35} sulfate can be used to trace mucin formation in the precoital rabbit oviduct. The time course and intracellular location of the label suggests that glycoprotein synthesis and secretion in oviductal cells is similar to that in other more intensively studied cell types.

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 on the oviduct.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Effects of Intrauterine and Embryonic Temperature
Elevation on Pregnancy in Experimental Animals
Contractor: Jefferson Medical College
Money Allocated: \$37,660 (FY 70), \$44,220 (FY 71), \$49,322 (FY 72),
\$67,722 (FY 73)

Objectives: The investigator proposes to develop methods of administering microwave irradiation to the uterus of experimental animals, and to investigate the effects of the microwave energy on the embryos and surrounding tissues in the intact rat and rabbit at various stages of gestation.

Major Findings: The experiments utilizing 2450 MHz non-directed microwave radiation to the exteriorized pregnant rat uterus indicate that microwave radiation can consistently produce fetal resorption utilizing various combinations of exposure time and uterine temperature. Several of these combinations utilize temperatures that are surprisingly low, in that uterine temperatures maintained at 41°C for 30 minutes result in 100% resorption. Even more unexpected was the finding that older embryos maintained this sensitivity to microwave radiation. The same combinations of exposure time and uterine temperature with 10, 12 and 14-day-old embryos resulted in very high resorption rates. This has led to the hypothesis that the embryo is a critical target because of its inability to dissipate heat which is directly related to its separate circulation. Thus, the embryo and eye are the two most susceptible mammalian structures to the thermal effects of microwave radiation. The examination of histological sections of uteri and ovaries from animals that have completely aborted reveals minimal and completely reversible changes. The experiments with directed (microwave) radiation have revealed that irradiation of the intact animal can result in 100% resorption in the rat and rabbit. These studies dealing with the effects of microwave radiation on the developing embryo have demonstrated the great sensitivity of the embryo to microwave radiation. The use of antenna radiation in the intact animal is feasible and, with the proper equipment and shielding, should be able to duplicate the irradiation results of the exteriorized uterus.

Significance to Biomedical Research and Program of the Institute: The work undertaken on this project is important in understanding the interaction of pelvic tissues with radiation and is related to the goal of the program aimed toward the development of new contraceptive technology.

Proposed Course: Terminate

NICHD ANNUAL REPORT
July 1, 1972 through July 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Assay of Progesterone Receptor Activity in the Guinea Pig Uterus
Contractor: Milton S. Hershey Medical Center
Money Allocated: \$20,694 (FY 71), \$23,544 (FY 72) \$35,474 (FY 73)

Objectives: The biological action of progesterone on the uterus may be initiated by the interaction of this steroid with a specific intracellular receptor protein. In the uterus of the castrate estrogen treated guinea pig specific cytoplasmic receptors for progestins have been identified. The purpose of this study will be to characterize these receptors in order to determine the best technique for measuring their binding capacities. A rapid assay technique will be developed for uterine cytosol protein binding activity. This assay will then be used to search for agents which both block progesterone binding to the cytosol receptor and which have relatively little progestational activity. From experience with other steroid receptor systems, it is anticipated that these agents will be antiprogestational and may therefore be useful as contraceptive agents.

Major Findings: The contractor has improved his rapid method for assessing binding of progestational drugs to guinea pig uterine cytosol. He has employed this assay to screen a large number of steroids in order to develop structure-activity relationships. The primary aim being the discovery of compounds which have high binding affinities and low progestational activities. The hope here is that such compounds could prove to be effective antiprogestins. In the pregnane series the compound 5α -pregnane-3,20-dione (allopregnenedione) has a moderately high binding activity but a low progestational activity. This compound could serve as a prototype for the synthesis of antiprogestins.

The cytosol receptor for progesterone is under estrogenic influence in the sense that administration of estrogens increases the number of sites 12-fold. This is accomplished through 4 days of treatment. Progestin receptor activity increases 2 - 3 fold more than total uterine protein during estrogen treatment. The half life of the progesterone receptor is about 4 days.

In similarity to the estrogen effect on the estrogen receptor, progesterone treatment reduces the concentration of its uterine binder within 3 hours.

Studies with nuclear proteins indicate that there exists a component that has binding affinity for progesterone somewhat greater than that observed for the cytoplasmic receptor.

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 studies on the binding characteristics of steroid receptor systems.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Detection of Ovulation in Chronically Instrumented Animals
Contractor: University of Washington
Money Allocated: \$45,600 (FY 72) \$118,308 (FY73)

Objectives: The objective of this research is to devise a technique for sensing the arrival of a cumulus mass in the lower ampulla in chronically instrumented rabbits and primates. The arrival of a cumulus in the ampulla so closely follows the rupture of the ovarian follicle that detection of such an event would serve as detection of ovulation. The investigators propose to develop a cylindrical transducer in the shape of a cuff to be implanted around the oviduct at the ampullary-isthmic junction. Within this cuff on one side would be a piezoelectric crystal which would be stimulated to generate ultrasound and fill the oviductal lumen with ultrasonic radiation. Opposing this crystal would be a receiving crystal, and if a large mass such as the egg and cumulus passes through the ultrasonic radiation field, there would be a Doppler shift in the sound reflected back to the receiving crystal, resulting in a detectable change in the transmitted signal. Other changes that could be measured in the ultrasonic wave patterns produced by a moving object in the oviduct would be changes in the phase shift of reflected and backscattered sound, and plus echo phenomena. Various sources of false negative and positive indications of the moving cumulus would be excluded. Laparotomies or laparoscopies would be done at the time when various signals from the transducer suggest the presence of an egg and cumulus in the oviduct. The ultrasound can be amplified and modulated to produce audible signals so that the presence of a moving object such as an egg and cumulus in the oviduct would be signalled audibly to the investigators. The investigators propose to begin this work with rabbits and extend it to primates. The successful use of this technique would certainly lead to more accurate determination of the time of ovulation in experimental animals and facilitate the various experimental procedures in such animals. The first techniques would involve hard wire connections from the transducer, but ultimately they wish to explore development of a telemetering package for remote observation of unrestrained animals.

Major Findings: Utilizing both ultrasonic and optical transducers the investigators were successful in recording the passage of cumulus masses through the ampullae of exposed rabbit oviducts. The optical transducer appears to have a better signal to noise ratio than the ultrasound ones and will be utilized in the future experiments. In chronically instrumented animals the signals have not been as clear cut as in the acute preparations. A number of technical problems remain to be solved for the chronic preparation.

Significance to Biomedical Research and Program of the Institute: Accurate determination of the time of ovulation is of primary interest to the program of the Contraceptive Development Branch.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Techniques for Study of Oviduct Transport
 and Detection of Ovulation in Primates
Contractor: Case Western Reserve University
Money Allocated: \$123,026 (FY 72) two years

Objectives: It is proposed to investigate the possibility of developing microelectronic sensors and radio-telemetry systems which can be totally implanted in the experimental animal to record several selected parameters believed to be related to ovulation. The proposed telemetry system will measure transovarian impedance, ovarian tubal impedance, ovarian size, ovarian temperature, and body core temperature. Other parameters, such as pressure, tubal motility and the electrical potential, could be telemetered with the same systems. The electrical impedance measurements are unique and have not been previously studied for the ovary, although the method has been successfully used in detecting changes in anatomical structure and histologic changes such as edema and hyperemia. It is believed that the transovarian electrical impedance changes will be easily seen through the ovarian cycle due to follicular growth and atresia. The follicular fluid in the growing follicle is isotonic with serum and therefore a good electrolyte so that the ovarian impedance will be low at the time of ovulation. During ovulation, when the follicle ruptures, there should be a sharp increase in electrical impedance. Following ovulation, when the ruptured follicle forms a corpus luteum, there should be a second increase in impedance. Correlative measurements of gonadotropin levels and basal body temperatures will be the indices of ovulation. In addition, measurement of ovarian temperature as compared to body core temperature will be carried out and it is expected that the differential between these two temperatures will increase during the follicular stage to the point of ovulation, whereupon the body temperature will rise, reducing the differential. For this project it is proposed to use telemetry systems previously developed in this laboratory and to modify them with special attention to the design and modification of transducers for this project.

Major Findings: Instrumentation designed to measure ovarian temperature changes at the time of ovulation has been designed. No biological data are presently available.

Significance to Biomedical Research and Program of the Institute: Accurate determination of the time of ovulation is of primary interest to the program of the Contraceptive Development Branch.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Progesterone-Binding Proteins as a Tool for the Detection of Progesterone Antagonists and Agonists
Contractor: Institute of Medical Research of the Toledo Hospital
Money Allocated: \$74,675 (FY 72), \$81,447 (FY 73)

Objectives: The purpose of this project is to prepare, isolate, purify and characterize a standard cell-free fraction containing receptor proteins from the uteri of rats, rabbits and guinea pigs incubated with ^3H -progesterone and from fresh untreated uteri in order to develop a rapid screening system for the detection of progesterone antagonists and agonists.

Major Findings: Progesterone binding proteins were found to be present in uteri of rats, guinea pigs, and rabbits. Detection required estrogen priming. Binding proteins have sedimentation coefficients between 6.0 and 8.0S, can be isolated from density gradients without major changes in sedimentation behavior. In rabbit, the affinity is $8 \times 10^8 \text{M}^{-1}$ and abundance: 5.7×10^{-12} moles/mg cytosol protein. Binding cannot be accounted for by plasma binding components which sediment at 3.5 to 5.0S.

However, normal cytosol preparations are contaminated with plasma steroid binding components, a fact that has to be taken into consideration when cytosol binding is analyzed.

Comparison of properties of progesterone binding components in rabbit and guinea pig uteri showed approximately same affinity, same sedimentation constant, same abundance in cytosol, that glycerol stabilizes both, that 0.3 M KCl treatment leads to disassociation with loss of binding affinity, that 0.1M P_i buffer leads to disassociation without loss of affinity, that KCl and P_i treated receptor reaggregate (partially) if salts are removed, and in general that in the rabbit is less stable than guinea pig.

Studies in intact rat uteri showed that [^3H] progesterone is metabolized. Two main metabolites were formed: 5 α -pregnane - 3 α - ol - 20 one (30-50% of ^3H in tissue) and 5 α -pregnane - 3, 20 dione (3-5% of ^3H in tissue) none of which bound to progesterone binding protein with high affinity. Rest of ^3H in tissue was progesterone. Higher percent of ^3H in tissue was found in metabolized form before estrogen priming, suggesting that unmodified progesterone is the active substance and is, while bound, protected from metabolism.

Deciduomal tissue in rat takes up more (20%) [^3H] progesterone than control (contralateral) uteri.

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 studies of progesterone binding substances in the uterus.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Prostaglandin: Its Role and Effect in Contraception
Contractor: University of California - Los Angeles
Money Allocated: \$63,767 (FY 72) for two years

Objectives: The objective of this research is to study the role of prostaglandins on calcium uptake and release by the sarcoplasmic reticulum and mitochondria from bovine and human uteri. The thesis is that the action of prostaglandins on uterine smooth muscle is due to the ability of these substances to regulate the intracellular stores of calcium. The primary aim of this research is to help to explain the mechanism of action of prostaglandins on the myometrium. Myometria from pregnant and non-pregnant cows will be fractionated to yield the sarcoplasmic reticulum and the mitochondrial fractions. Calcium transport in these fractions will be examined in the presence of various concentrations of prostaglandins. The proposed studies will be concerned with: (1) the effects of prostaglandins on calcium binding of bovine myometrial sarcoplasmic reticulum (SR) from non-pregnant animals at varying prostaglandin concentrations; (2) the role of progesterone in the sensitivity of uterine SR calcium transport to prostaglandins; (3) the interaction of prostaglandins with the SR membrane; (4) effects of prostaglandins on human myometrium as available; (5) the effects of prostaglandins on mitochondrial calcium binding in pregnant and non-pregnant animals.

Major Findings: The methodology for the study of prostaglandins (PG) on the sarcoplasmic reticulum (SR) has been developed. Sarcoplasmic reticulum from cow uteri has been isolated and the optimum conditions of calcium binding to the SR have been established. While there appeared no change in calcium binding in the presence of 1 ng PG/ml, increased inhibition occurred with increasing PG concentration, amounting to approximately 50% inhibition at 1,000 ng/ml of PGE₂ or PGF₂α.

Significance to Biomedical Research and Program of the Institute: The projected study on the mechanism of action of prostaglandins is directly related to the stated interests of CDB in this group of substances.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Oviductal Fluid in the Rat and Mouse: A Study of the
Chemical and Physical Properties and Mechanism of Formation
Contractor: The Johns Hopkins University
Money Allocated: \$46,014 (FY 73)

Objectives: The objectives of this research are 1) to develop methods for the in vivo collection of oviductal fluid from the rat and mouse; 2) to establish the micro- and ultramicro chemical techniques required for the analysis of the oviductal fluid; and 3) through the use of these techniques, to determine the chemical and physical properties and rate of formation of oviductal fluid throughout the estrous cycle, and to evaluate the influence of a changing hormonal environment on the formation of oviductal fluid in the two species.

Significance to Biomedical Research and Program of the Institute: The practical implications of the results of this approach are clearly related to the development of contraceptive methods based on alterations of oviductal function and thus, to the stated goals of the program.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hypothalamo-Hypophysial Factors in Reproduction
Contractor: Medical College of Ohio at Toledo
Money Allocated: \$82,911 (FY 69), \$70,555 (FY 70), \$56,700 (FY 71)
\$36,000 (FY 72), \$29,574 (FY 73)

Objectives: The principal objective of this research is to develop a reliable and sensitive displacement assay for oxytocin for the purpose of eventually measuring blood levels of this peptide during the menstrual cycle and following coitus. Using this technique, the investigator is assaying oxytocin levels in animals during the estrous cycle and after mating and will eventually apply the method to determining blood levels of this hormone in women. In addition, the structural elements of the oxytocin-binding peptide are being examined and analogues of oxytocin are being synthesized and compared with oxytocin with regards to their ability to bind to the binding peptide.

Major Findings: A radioligand assay for oxytocin utilizing receptors from mammary tissue and uteri was developed. The assay appears to be sensitive to oxytocin and its analogs and the degree of binding appears to be related to the biological activity of the analogs. In the uterine receptor assay prostaglandins fail to displace oxytocin even though they are potent stimulators of uterine activity. This finding suggests the dissimilarity of uterine receptors for oxytocin and prostaglandins.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research directed towards the elucidation of the hypothalamo-hypophysial control of reproductive function.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: New Methods for Fertility Regulation
Contractor: The Upjohn Company
Money Allocated: \$127,684 (FY 69) for two years, \$73,425 (FY 71)
\$88,140 (FY 72 for years)

Objectives: This is a study of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in which the conditions under which $PGF_{2\alpha}$ causes luteolysis and induces the termination of pregnancy will be determined, and the mechanisms by which $PGF_{2\alpha}$ achieves its luteolytic effect will be identified. Pregnancy termination is being studied in rabbits, guinea pigs, and rhesus monkeys by ovarian histology and ovarian and peripheral plasma progesterone levels. The period of pregnancy subject to interruption by $PGF_{2\alpha}$ -induced luteolysis is being established.

The second aspect of the proposal is an attempt to study the processes involved in the luteolytic effects of $PGF_{2\alpha}$. This includes studies of effects on gonadotrophin activity, competitive inhibition of gonadotrophins, direct effects on progesterone synthesis in the ovary by measurement of steroid levels in ovarian venous blood, and of the effects of prostaglandins on steroidogenesis by rat ovaries and primate corpora lutea in vitro. This also includes studies of cholesterol incorporation into progesterone, levels of adenyl cyclase, inhibition of effects of puromycin and cycloheximide, and potentiation of effects by phosphodiesterase inhibitors.

Major Findings:

1. Studies were conducted with hamster uteri and the data for PGF component, although this may be due to the relatively low specific activity of the 3H - $PGF_{2\alpha}$ used in these studies. Significant specific binding of 3H - $PGF_{2\alpha}$ is present throughout the cycle and is maximal on Day 3 (proestrus). The results with monkey tissue indicate the presence of a specific receptor for PGE_1 in the myometrium, but not the endometrium. Evidence for a specific $PGF_{2\alpha}$ receptor in rhesus monkey myometrium or endometrium was not obtained.

2. Tissue levels of $PGF_{2\alpha}$ in rabbit uterine tissue after four hours of superfusion were equal to or greater than tissue levels before superfusion. Measurements of incubated tissue concentrations of $PGF_{2\alpha}$ were made on tissue samples after slicing and, therefore, may not represent physiological tissue levels. Observations on a limited number of monkey tissues indicated that concentrations after eight hours of superfusion were equivalent to pre-incubation levels. Preliminary studies with tissue from rhesus monkeys showed that estradiol- 17β stimulated the synthesis of this prostaglandin by slices of uterine myometrium. Progesterone had no effect on the synthesis of $PGF_{2\alpha}$ by slices of monkey myometrium or endometrium; estradiol- 17β did not alter release of $PGF_{2\alpha}$ from monkey endometrium.

3. In the monkey plasma concentrations of $PGF_{2\alpha}$ are considerably lower than they are in serum. In the human such differences were not observed.

Significance to Biomedical Research and Program of the Institute: The relevance of this study to the contract program is direct, particularly insofar as some of the work is being done in primates.

Proposed Course; This contract will be terminated in FY 74 because the stated objectives will be fulfilled.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Ovulation in the Monkey
Contractor: Oregon Primate Center
Money Allocated: \$44,000 (FY 69) for two years \$47,543 (FY 71)
\$70,612 (FY 72)

Objectives: These studies are directed towards establishing the feedback site of progestins as inhibitors of ovulation in the rhesus monkey using experimental approaches previously used in the rabbit. These approaches are feasible in the rhesus monkey but much base-line information is being obtained. preliminary to critical studies of feedback inhibition of ovulation.

Major Findings: Intrapituitary infusion of LH-RH released a greater quantity of LH from the pituitary than a comparable dosage of LH-RH injected *iv*. On the other hand the route of administration did not change the magnitude of LH release after stalk median eminence extract. Neither the stage of menstrual cycle, ovariectomy, nor three daily injections of 100 μ g of estradiol altered significantly the pattern of LH release after CH-RH.

Collectively, these data suggest that one reason for the relative insensitivity of the monkey to systemic LH-RH is rapid enzymic inactivation in the peripheral circulation before LH-RH can be bound to the LH-secretory pituitary cells. The chemical identity of natural monkey LH-RH remains to be confirmed.

Significance to Biomedical Research and Program of the Institute: Studies of the mechanism of ovulation are clearly relevant to the purposes of the contraceptive development program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Neural Regulation of Ovarian Function
Contractor: Southwest Foundation for Research and Education
Money Allocated: \$29,000 (FY 69), \$31,146 (FY 70), \$31,146 (FY 71),
\$40,271 (FY 72)

Objectives: The objective of the proposed study is to investigate the influence of the anti-inflammatory corticoid triamcinolone acetonide on the menstrual cycle of the baboon. The following end points will be explored: (1) dose-response relationship; (2) the effect on follicular development and on the endometrium; (3) the effect on luteinization when given immediately after ovulation, and (4) effect on the endogenous release of cortisol.

Major Findings: A dose-response study of triamcinolone effect on baboon ovulation has been conducted. Administration of 4 mg. on the first day of menses consistently blocked ovulation and shortened the menstrual cycle. Administration of 2 mg. likewise blocked ovulation in 4 baboons, but only in one was the cycle length changed. Plasma progesterone levels were lowered in all animals. An unexpected finding was made on the influence of the thyrotropin releasing factor (TRF) on ovulation in the baboon. Administration of TRF before the presumed time of ovulation and for several days thereafter blocked ovulation and reduced plasma progesterone levels. Menstrual cycle length was not altered.

Significance to Biomedical Research and Program of the Institute: The study of compounds that can block ovulation is directly related to the published purposes of the Contraceptive Development 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Anti-luteinizing Activity of Follicular Ovum
Contractor: University of Illinois
Money Allocated: \$43,365 (FY 69), \$45,635 (FY 70), \$46,941 (FY 71),
\$28,173 (FY 72)

Objectives: Recent work from this laboratory has shown that removal of the egg and surrounding granulosa cells from a ripe ovarian follicle (rabbit) initiates luteinization and a short spurt in progesterone secretion. Hence, the egg seems to exert an anti-luteinizing activity. A series of well-planned studies to elaborate and determine the significance of this observation are being carried out under the terms of this contract.

Major Findings: The investigation has confirmed the presence of a luteolytic agent in porcine follicular fluid.

Significance to Biomedical Research and Program of the Institute: Reproductive function in the human appears to depend on corpus luteum luteinization and luteolysis which have thus been identified as particularly important topics to the purposes of the contraceptive development program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Isolation of a Bovine Uterine Luteolytic Factor
Contractor: Cornell University
Money Allocated: \$107,232 (FY 69) for three years, \$53,789 (FY 72)

Objectives: The principal investigator has prepared a bovine endometrial extract with the ability to regress corpora lutea of pseudopregnant, hysterectomized hamsters. The active material precipitates with ammonium sulfate and apparently is a large molecular weight protein; the possibility is being investigated that it may be prostaglandin F_{2α} adsorbed to a protein. These studies involve further fractionation and purification of the active material using preparative disc electrophoresis, and study of its effects on corpus luteum progesterone synthesis stimulated by LH, the effects of various modes of administration, and effects on ovarian blood flow and progesterone concentration.

Major Findings: Extracts of bovine luteal endometrium possess luteolytic activity in a lipid soluble fraction which is distinct from prostaglandins. Luteolytic activity is confined to a fatty acid fraction. It may represent a fatty acid precursor of prostaglandins such as arachidonic acid. Injection of arachidonic acid into pseudopregnant hamsters produces luteolysis.

Significance to Biomedical Research and Program of the Institute: Corpus luteum regression, or "luteolysis" has been identified as particularly important to contraceptive development; therefore, this study is directly relevant to the purposes of the program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Endocrine Regulation of the Corpus Luteum
Contractor: Colorado State University
Money Allocated: \$26,706 (FY 69), \$37,220 (FY 70), \$53,618 (FY 71),
\$68,578 (FY 72)

Objectives: The primary objective of this research project is to elucidate the endocrine regulation of the ovine estrous cycle by measuring peripheral serum levels of FSH, LH, prolactin, estradiol, and progesterone. Radio-immunoassay methods are being developed for all these measurements. For the steroids, the methods are validated by comparison with double isotope derivative methods. Methods are also being developed to measure uptake of progesterone-C¹⁴ and LH-I¹²⁵ by uterus and ovaries respectively. Preimplantation embryos will be studied to determine if they have the ability to synthesize LH in vitro, and to metabolize sex steroids in vitro.

Major Findings: Ewes were treated with the synthetic ergot derivative CB-154 in order to ascertain its effect on various endpoints. This drug had no effect on cycle length or the plasma levels of LH, FHS or progesterone. Prolactin levels were decreased to 1% of normal. It was concluded from this study that circulating prolactin could be decreased to very low levels without influencing luteal function.

Studies of ovarian blood flow during the cycle have indicated that the blood flow to the ovary which contained the CL was highest during the luteal phase, while there was little change in blood flow to the ovary which had no luteal tissue. Infusion of PGF₂ α increased rather than decreased ovarian blood flow.

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 describe quantitatively the hormonal components of the estrous cycle in relevant animal models.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Relationships of Endometrial Prostaglandins and Luteal Function
Contractor: West Virginia University
Money Allocated: \$35,337 (FY 69) for two years, \$32,460 (FY 71), \$39,750 (FY 72)

Objectives: The investigators are extracting the endometrium of sheep at various phases of the cycle to determine prostaglandins both qualitatively and quantitatively. The effects of progesterone on the corpus luteum and on prostaglandin levels and the effects of prostaglandin $F_{2\alpha}$ on corpus luteum function in hysterectomized animals are being studied. Corpus luteum function is being evaluated by progesterone levels and length of estrous cycle.

Major Findings: It was previously reported that endometrium from pregnant ewes contained a higher content of $PGF_{2\alpha}$ than the endometrium from non-pregnant animals. Analysis of uterine vein blood in animals pregnant 11-18 days indicate higher levels of PG's than in non-pregnant animals on days 11-16. In sheep insertion of an IUD produces increases in $PGF_{2\alpha}$ in both the endometrium and uterine vein blood. Estrogen treatment on days 9 and 10 of the cycle produces an increase in $PGF_{2\alpha}$ in the venous blood.

Samples have been collected for the comparison of the caruncular and inter-caruncular areas of the sheep endometrium.

A preliminary study of monkey endometria indicates presence of prostaglandins $F_{2\alpha}$ and E_2 .

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 elucidate the factors responsible for the control of corpus luteum function.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Actions of Estrogen and Progesterone upon Uterine Function in Pregnancy
Contractor: Washington University
Money Allocated: \$46,329 (FY 69), \$48,718 (FY 70), \$48,718 (FY 71), \$55,838 (FY 72)

Objectives: This investigation has involved three projects: (1) Removal of corpora lutea in early human pregnancy and observations of plasma progesterone levels, uterine activity and abortion. Progesterone determinations are also made on the enucleated corpora lutea and the placenta. For patients coming to surgery after the first half of the first trimester, pregnancy is terminated by the intra-amniotic injection of hypertonic saline and the effects of this treatment on plasma progesterone and uterine activity are studied. (2) The role of placental estrogen in luteal maintenance is being studied in rabbits and rats with emphasis on questions of timing, dose, duration in treatment and species differences. (3) Electrophysiological studies being conducted on the rabbit uterus and oviduct under a variety of hormonal conditions include: tension measurements in electrically stimulated uterine and oviduct strips; measurements of differential shortening of stimulated muscle strips; and direct measurements of action potentials to establish the extent and velocity of conduction in uterine and oviduct muscles. During FY 73 studies were undertaken to establish the relationship between gestational age and luteectomy-induced complete abortion in human patients and to determine the effect of estrodiol- 17β on serum and corpus luteum progesterone in patients more than 4 weeks pregnant (by Nagele).

Major Findings: Luteectomy at seven weeks of pregnancy (by Nagele) routinely produced an increase of intrauterine pressure and oxytocin response, progress in cervical dilation and abortion due to a rapid decrease in progesterone. In contrast, patients undergoing luteectomy at about 61 days of gestation exhibited only a transient decrease in progesterone which was not followed by abortion. Thus it appears that as long as the corpus luteum serves as the major source of progesterone, it is indispensable for the maintenance of pregnancy. As the luteoplacental shift takes place (about 8 weeks of gestation) pregnancy becomes progressively less dependent upon the corpus luteum as a source of progesterone. Progesterone substitution therapy prevented the consequences of luteectomy. Treatment with estradiol- 17β , which also decreases following luteectomy, did not prevent the sequence of events produced by excision of the corpus luteum.

The investigator has observed that even when luteectomy is performed as early as day 49 of gestation (by Nagele) about half of the abortions are incomplete, the placenta remaining in utero. This may be due to a very early luteoplacental shift (3 weeks after missed menses) and a direct local action of progesterone on the placenta.

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 on the importance of the corpus luteum in early human pregnancy and the timing of the luteal-placental shift.

Proposed Course: Contract objectives have been realized and no further support of this project is anticipated.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Simultaneous Theoretical and Empirical Approach
to the Study of the Rat Estrous Cycle
Contractor: University of Illinois
Money Allocated: \$41,500 (FY 70), \$45,226 (FY 71), \$83,688 (FY &2)

Objectives: The investigator plans a systems analysis approach to the understanding of the mechanisms regulating reproductive cyclicity and acyclicity in the rat. The total approach includes the following stages to be carried out simultaneously: identify from existent experimental data the essential variables and connecting linkages among system components; make a model of the system; simulate the system by computer; perform "experiments" using the model and simulating by computer to compare the model to similar experiments in the real system; use the computer to simulate the results of new experiments; perform these experiments and modify the model if necessary, etc.

Because data on few of the variables needed for constructing an adequate simulation are available from the literature, the investigator will measure the following parameters and variables using radioimmunoassay and competitive protein binding methods for steroids and gonadotrophic hormones: (1) estrogen progesterone, LH, FSH, and prolactin in serum throughout the four-day cycle; (2) distribution volumes and loss rate constants of the above hormones in cyclic rats and following ovariectomy and/or hypophysectomy; (3) setpoints for LH, FSH and prolactin in prepubertal and adult rats; (4) the negative feedback constant for the suppression of LH and FSH secretion by estrogen; (5) ovarian transfer functions, determining the effects of gonadotropins on estrogen and progesterone secretion and on morphology; (6) estrogen, LH, and FSH levels during the onset, steady state and termination of persistent estrus due to continuous light.

Major Findings: The radioimmunoassayable amount of rat LH during the proestrous afternoon surge of LH is less than 100 ng/ml or at most 0.75 ug LH total for a 250 gm rat. Yet an equivalent amount of ovine LH (as measured by RIA) failed to produce ovulation in a hypoxed animal. In fact, it may take greater than five times that amount to cause full ovulation. This suggests that (a) rat LH is not measured as efficiently as the ovine preparation, or (b) that rat LH is more effective in producing ovulation in that species than an exogenous ovine preparation or (c) the temporal sequence of presentation of exogenous hormone is ineffective.

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 on the mechanisms regulating reproductive cyclicity and acyclicity.

Proposed Course: Termination in favor of NICHD Research Grant

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of a Laboratory Screening Procedure for Inhibitors of Progesterone Synthesis
Contractor: Harvard University
Money Allocated: \$45,120 (FY 70), \$45,500 (FY 71), \$83,567 (FY 72)

Objectives: The objective of this contract is to define and evaluate agents which interfere with the enzymatic synthesis of progesterone in the corpus luteum. Specifically, the contract concerns the possibility of finding substances which are specific inhibitors of the mitochondrial system which converts cholesterol to pregnenolone; specifically, substances which act at the cytochrome P-450 site. It is suggested that substances exist which act specifically at this site and are non-toxic; such substances would be potential antifertility agents.

The immediate objective of this contract is to devise a convenient and relatively rapid screening procedure which will enable the prediction and preparation of substances which would be biologically effective for the termination of early pregnancy.

Major Findings:

1. Screening of inhibitors of P-450 cytochrome enzymes has continued. Several potent steroidal and non-steroidal inhibitors were discovered. The compounds of primary interest are the 20-hydroxyl derivatives of pregnenolone. (5-pregnen-3,20 diols). In lower species, the 20 α isomer has very little biological activity, if any, although the 20 β isomer has been reported to have weak progestational activity. In the human, neither is significantly active insofar as progestational activity is concerned. Among the steroid derivatives there are several that not only block the cholesterol side cleavage enzymes but also have the capacity to inhibit the 3- α -ol dehydrogenase and isomerization system.
2. The reliability of the in vitro spectroscopic assay appears to be confirmed by the in vitro biosynthesis assay system. Nevertheless, several compounds that are active in the spectral assay have no effect on biosynthesis. These data must be confirmed.
3. Side-chain cleavage enzymes of the adrenal cortex mitochondria are inhibited by most of the substances which inhibit those enzymes of the corpus luteum but less completely so. The reasons for this are being explored. A preliminary study on the placenta confirms that it too is susceptible to inhibition in a manner similar to the corpus luteum.

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 development of laboratory screening procedures.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Radioimmunoassays for Rhesus Monkey
Gonadotropins
Contractor: Endocrine Laboratories of Madison, Inc.
Money Allocated: \$78,553 (FY 70), \$78,170 (FY 71), \$64,688 (FY 72)

Objectives: This proposal is based on the recognition that rhesus monkey gonadotropic hormones do not cross-react immunologically with their human counterparts or at least not sufficiently to permit their detection in body fluids by radioimmunoassay. In this project, the investigators will collect urine from gonadectomized rhesus monkeys for the isolation of monkey LH and FSH. The urinary gonadotropins will be utilized because of the limited availability of monkey pituitary glands. The gonadotropins thus obtained will be used as antigens to obtain antisera for development of radioimmunoassays. Purification of the hormones will be by procedures previously developed, and by adaptations of methods developed in other laboratories and reported in the literature.

Major Findings: During the course of the past year the contractual effort has been directed primarily toward the stockpiling of crude urinary extracts. Purification of this material is being carried out by NICHD intramural scientists. Chromatographic separation of immunoreactive urinary FSH has been accomplished. Utilization of this material for the development of urinary FSH radioimmunoassay is presently in progress.

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 the Development of new methodologies for the assay of steroid and protein hormones in the blood of primates including man.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Pilot Study for the Large Scale Preparation of
Purified Human Chorionic Gonadotropin
Contractor: Columbia University
Money Allocated: \$47,300 (FY 70), \$85,086 (FY 71), \$194,280 (FY 72)
for two years

Objectives: The objective of this investigation is to undertake a feasibility study to determine the optimal conditions for preparation of large batches of highly purified human chorionic gonadotropin (HCG). Secondary goals of this program are: (1) to obtain data bearing on the chemical and immunological characterization of HCG fractions which appear to be homogeneous in polypeptide content, but possess differing biological activities, and (2) to obtain data on the stability of HCG using both immunological and biological activities as guides.

Major Findings: Distribution of HCG and the β -subunit for radioimmunoassay purposes is now in progress. Further large quantities are being purified and characterized and will be distributed. Requests for mg quantities of these materials are quite high. The subunits have proved to be very useful in studies of gonadotropin 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 the development of procedures for human hormone isolation and purification.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Control of Ovulation and Corpus Luteum Function
Contractor: Medical College of Georgia
Money Allocated: \$43,760 (FY 70), \$25,891 (FY 71), \$41,836 (FY 72)

Objectives: The investigators propose studies to gain insight into the action of estrogens at the hypothalamic and pituitary level. More specific studies will deal with steroid-protein interaction in the rat hypothalamus and pituitary and in the human pituitary. This work will involve the use of both radioactive estradiol and testosterone and the possible identification of the testosterone metabolite which binds to the cytosol fraction. This study will include the use of hormone analogs that bind covalently to the 8S-receptor.

Major Findings: Prior investigations have failed to demonstrate the presence of testosterone receptor in the pituitary gland. It has now been observed, that exhaustive dialysis of ³H-testosterone-incubated cytosol samples, uncovers a small amount of radioactivity that is bound to the cytosol with high affinity. The amount of binding is less than 10% of that of estradiol, and accurs to the same extent in both sexes.

Studies with estrogen binding to pituitary cytosol indicate the presence of two binding systems which makes the interpretation of binding parameters more difficult.

The estradiol binding capacity of the cytosol from female hypothalami is some 20-30 times higher than the capacity of male hypothalami. Differential uptake of estradiol by male and female hypothalami, but not by pituitary, may be the key we have been searching for in attempting to correlate the estrogen feedback mechanism with circulating gonadotropin levels.

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 studies of the action of the pituitary gonadotropins in the reduction of ovulation and the control of corpus luteum formation, function, and regression.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Regulation of the Changes in Corpus Luteum Physiology
Required for the Establishment of Pregnancy in the Rat
Contractor: Case Western Reserve University
Money Allocated: \$53,313 (FY 70), \$53,313 (FY 71), \$45,812 (FY 72)

Objectives: The proposed experiments are designed to shed further light upon the interaction between pituitary LH and prolactin and a placental luteotrophic hormone in the regulation of the rat corpus luteum. Interaction between these agents is assumed to be responsible for differences between the corpus luteum of pseudogregnancy and the corpus luteum of pregnancy, the latter being characterized by (a) increased size, (b) ability to maintain increased peripheral plasma levels of progesterone and (c) ultimate development of independence, i.e., the ability to function in the absence of continued support from the pituitary placenta. The fundamental hypothesis to be tested is that in the non-pregnant state LH has a luteolytic action, and the addition to the system of the placental luteotrophic hormone converts this action to a synergistically luteotrophic action.

Experiments will be carried out in the pregnant rat on three principal problems: (1) increase in size of the corpus luteum of pregnancy, (2) elevation in plasma progesterone levels, and (3) development of corpus luteum independence in the absence of pituitary and placenta. Methods to be used include various surgical manipulations, progesterone determination by competitive binding assays, and prolactin determination by radioimmunoassay.

Major Findings: (1) Metabolic clearance rates for progesterone in rats were 30 liters/day and were not influenced by the reproductive status of the animal, (2) the luteolytic effect of prolactin in pseudopregnant rats has been demonstrated, as has the absence of this effect in pregnant rats, hypophysectomized and hysterectomized rats on day 12. Pilot studies have shown that prolactin does exert a luteolytic effect in pregnant rats after hypophysectomy and hysterectomy on day 9, and (3) autotransplanted pituitary glands can not be used as a source of "pure" prolactin secretion in that they do secrete very low levels of LH and FSH.

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 on corpus luteum physiology.

Proposed Course: Termination in favor of NICHD Grant

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an Automated Immunoradiometric Assay System
Contractor: Veterans Administration Hospital
Palo Alto, California
Money Allocated: \$59,110 (FY 71), \$33,335 (FY 72)

Objectives: The objective of this contract is to establish a simple automated immunoradiometric assay device, capable of measuring low concentrations of a wide variety of antigens. The assay device is made possible by the prior development (by the applicant) of a new type of immunological assay system (the immunoradiometric assay). In this method, purified radioactive antibodies are used as reagents to convert all the unknown antigen into a directly detectable complex. Unreacted antibody is removed by reaction with an insoluble antigen immunoabsorbent. An important but parallel part of the project is the investigation and development of alternative antibody labels.

Major Findings:

(1) The automated immunoradiometric assay apparatus was tested for determination of growth hormone. When large volumes of widely separated samples were analyzed (4ng/ml HCG vs 498 ng/ml HCG) the results indicated good separation. Assay samples of smaller volume or less widely separated have been found to produce greater irregularity and trailing, probably due to diffusion and ineffective washout of the sample.

(2) A new method has been developed for the enzymatic iodination of proteins using a microvolume iodide-specific electrode.

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 on the development of new types of immunological assay systems.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Quantification of Prostaglandins
Contractor: The Uphohn Company
Money Allocated: \$42,913 (FY 71) \$50,241 (FY 72)

Objectives: The objective of this contract is to develop a radioimmunoassay to measure prostaglandins and certain of their biologically active analogs, and subsequently to quantify these compounds in some body fluids and tissues.

Initially, prostaglandin E₂ and F₂ will be conjugated to bovine serum albumin and polylysine, then injected as a suspension with Freund's complete adjuvant into sheep, rabbits and guinea pigs to produce antibodies to the prostaglandin molecules. The affinity and specificity of the antibody will be characterized by comparing the competitive replacement of radiolabeled prostaglandin by several structurally related prostaglandin analogs. Conditions of incubation, methods of separation, and other alterable variables will be examined to optimize the sensitivity and precision of the assay. In addition, a limited number of prostaglandin analogs with biological activity that indicates a potential clinical advantage over natural compounds will be utilized similarly for assay development.

Circulating levels of prostaglandins will be quantitated during parturition in humans and at various stages of the menstrual cycle in monkeys. Absorption of prostaglandin from various formulations will be followed by quantitating prostaglandin in peripheral serum by radioimmunoassay. In vivo biological effects of administering antisera to prostaglandins will be determined.

Major Findings: Radioimmunoassays for PGF₂α and its metabolites have been established. The cross-reactivity of the PGF₂α antibody with the 15-keto and 13-14 dihydro-15 keto metabolites was less than 0.1%. The converse was also true, in that antibodies generated against the metabolites displayed very little affinity for PGF₂α. Relatively higher rates of cross-reactivity were found between the two metabolites, as expected. These results indicate that the keto-hydroxyl configuration at C-15 position is more important than 13-14 double bond, in terms of antibody specificity. These differences were expected, and probably are due to the changes in polarity, as well as molecular configuration of the molecules. The metabolite assays are important because they have much greater biological half-life than does the parent molecule.

Infusion rates of as low as 1 μg/minute significantly increased peripheral 15-keto-13,14 dihydro PGF₂α levels in monkeys, whereas infusion rates of 10 μg/minute or greater were required to elevate PGF₂ levels. Higher infusion rates established the relationship between PGF₂ and the 15-keto-13,14 dihydro metabolite in the rhesus monkey. At these higher infusion rates tested, 10, 40 and 100 μg/minute, metabolite levels were about 10-fold higher than PGF₂α levels.

As previously reported, $\text{PGF}_2\alpha$ was elevated in monkey serum samples, apparently as a result of platelet synthesis during the clotting process. It is of interest that $\text{PGF}_2\alpha$ levels of all plasma samples were below the minimal detectable sensitivity of the assay, 1.0 ng/ml. This may indicate that some in vitro synthesis occurs even in heparinized monkey blood, if not cooled immediately.

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 on radioimmunoassays of prostaglandins.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Model for Studying Inhibition of LH Action on the
Corpus Luteum
Contractor: Mayo Clinic and Mayo Foundation
Money Allocated: \$36,924 (FY 71), \$68,620 (FY 72)

Objectives: A model system has been developed for studying the interaction of human luteinizing hormone (LH) with cells of the corpus luteum using slices of pseudopregnant rat ovaries. The object of this contract is to further validate this model, to define the structural requirements of the LH molecule, to define the requirements of the luteal cell for this interaction, and to seek inhibitors of this interaction. If such inhibitors are found, they may be highly specific abortifacients or contraceptives.

Effort will be made to relate the interaction of LH and the luteal cell to the steroidogenic action of LH. This will be done by assessing the stimulation of estrogen and progesterone synthesis using specific radioimmunoassays. The applicability of this model to human corpus luteum tissue and to the Leydig cells of the male will be investigated.

Major Findings:

1. Autoradiographic studies employing ^{125}I LH have shown that in pseudopregnant rats majority of the silver grains were found over corpora lutea with little or no activity over non-antrum containing follicles or interstitial tissue.
2. Biochemical studies on the gonadotropin binder from the luteinized rat ovaries suggest that the receptor is a lipoprotein with some of the receptor sites covered or shielded by neuraminic acid containing substance, presumably a glycoprotein. Whether RNA is involved in the receptor is speculative.
3. The α and β subunits of LH and HCG do not bind to receptors in ovarian homogenates.
4. Presence of receptors in human luteal tissue has been demonstrated. The data suggest that there is a significant correlation between binding and serum progesterone concentration ($r=0.68$).
5. Treatment of pseudopregnant rats with estrogen increases the number of binding sites while progesterone has no effect.

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 in the relationship between LH and the function of the corpus luteum.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Effect of Prostaglandins on Ovarian Function
Contractor: University of Miami
Money Allocated: \$115,909 (FY 71) for two years

Objectives: The objective of this research is to study the in vivo effect of prostaglandin F_2 on the corpus luteum of the menstrual cycle in women in order to determine what acute effects this compound has on the corpus luteum and to determine if it can be used as a luteolytic agent in humans. The volunteer subjects will be divided into two groups. The first group of normal healthy ovulating non-pregnant females will receive an infusion of prostaglandin F_2 at different times in their secretory phase of the cycle. This will be considered the control group. The parameters for corpus luteum function will be the measurement of plasma progesterone in the peripheral venous blood. The second group will consist of patients already scheduled to undergo abdominal gynecologic surgery for non-endocrine disease. Their surgery will be scheduled at a time a corpus luteum of a certain age can be expected. Prostaglandin F_2 administration will begin at varied times before surgery and progesterone will be measured in peripheral venous blood and in blood obtained from both ovarian veins. In addition, steroidogenesis of incubated luteal tissue will be measured in vitro.

Major Findings: Administration of $PGF_{2\alpha}$ to women during the follicular phase delayed the LH peak and menses by 6-8 days. This study is being continued in rhesus monkeys to ascertain whether repeated injections of $PGF_{2\alpha}$ can suppress the LH peak during the course of several cycles.

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 on the effects of prostaglandins on the corpus luteum.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Prostaglandins in Ovarian Physiology
Contractor: Yale University
Money Allocated: \$25,470 (FY 71), \$15,270 (FY 72), \$78,269 (FY 73)

Objectives: The objective of this research is to study the effects of prostaglandins on ovarian physiology in the rhesus monkey. Specifically, this entails a study of (1) the effects of prostaglandins on steroidogenesis in the developing follicle and corpus luteum; (2) the effects of prostaglandins on development and morphology of the follicle and corpus luteum, and the effects on ovulation; (3) the effects of prostaglandin antibodies and antagonists on ovarian function.

Major Findings: The results of these studies suggest that in the monkey, the administration of DES on the luteal phase of the cycle is followed by a rapid decline in progesterone concentration in the peripheral plasma. In the early luteal phase, there was a "rebound" phenomena in progesterone, while in the late luteal phase this did not occur. In view of the known antagonism between LH and $\text{PGF}_{2\alpha}$ the early luteal phase behavior may represent resistance to $\text{PGF}_{2\alpha}$ because of lingering LH stimulation.

The possibility that the luteolytic action of DES may be related to PGF has been hypothesized due to the concomitant increase in PGF seen at the time of progesterone decline in the majority of animals treated. The present studies however do not establish any definite interrelationship between luteolysis and PGF release.

The results in early pregnancy reveal that DES does not affect the corpus luteum.

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 on the effects of prostaglandins on ovarian physiology.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemistry and Mechanism of Action of Human
Gonadotropins
Contractor: Cornell University Medical College
Money Allocated: \$58,736 (FY 72)

Objectives: The objectives of this project are: (1) to isolate and characterize the gonadotropin binding material from the subcellular fractions of the gonads and (2) to study the interaction of gonadotropins (LH and FSH) with the binding material.

Major Findings: The receptor complex from gonadal tissue has been separated into protein and lipid components. It binds LH and FSH with high affinity. Reconstitution of the receptor has been accomplished. Receptors from bovine corpora lutea have been isolated and purified. Antibodies against the receptor prevent binding of LH and FSH.

Significance to Biomedical Research and Programs of the Institute: Study of gonadotropin receptors may lead in the future to the understanding of the mechanism of action of gonadotropins and formulation of new contraceptive technology. As such it is directly related to the published purpose of CDB.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Molecular Biology of the Human Corpus Luteum
Contractor: New York Medical College
Money Allocated: \$56,436 (FY 72)

Objectives: The objective of this research project is to investigate the normal luteolytic process that the CL of the menstrual cycle undergoes and the factors which control it. The investigator hopes that this research will answer a number of important questions among which are:

1. Does progesterone secretion decrease before or after some other fundamental change in the cellular biology of the corpus luteum (CL).
2. Is there a single molecular event which triggers luteolysis, such as an abrupt decrease in RNA synthesis or an abrupt decrease in enzyme synthesis.
3. If there is such an abrupt event, when in the life history of the CL does it occur.
4. Does the CL of the menstrual cycle become the CL of pregnancy simply by preventing the postulated mechanism.
5. Since human chorionic gonadotrophin (HCG) is probably the messenger for the above transition, what is the difference between the effects of human pituitary luteinizing hormone (HLH) and HCG on the molecular biology of the human CL.
6. What is the relationship between the secretion of progesterone by the human CL and the secretion of other steroids which are known to be synthesized by human luteal tissue, especially estradiol.

Major Findings: An incubation system for human CL slices was developed. The course of steroid release from CL did not exhibit a consistent pattern as a function of the post-ovulatory age. In experiments with added HCG there appears to be a pattern of response as a function of post-ovulatory age. Experiments with prostaglandins have yielded equivocal data.

Significance to Biomedical Research and Program of the Institute: Research on the human corpus luteum is directly related to the stated objectives of the Contraceptive Development Branch.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Prostaglandins in the Secretion of
Gonadotropins
Contractor: Yale University
Money Allocated: \$61,445 (FY 73)

Objectives: The primary objectives of this research project are (1) to examine the possible role(s) of prostaglandins in the mechanisms controlling the pituitary secretion of LH and FSH, and (2) to study the effect of prostaglandin synthetase inhibitors and prostaglandin antagonists on the LH and FSH release induced by synthetic luteinizing hormone-releasing factor (LRF) in vitro.

Major Findings: The results suggest that PGE_1 , PGE_2 , and $PGF_{2\alpha}$, at pharmacological doses, can act directly at the pituitary² to influence LH release. It was found that: (1) the prostaglandins increased LH release, (2) the effect was dose-dependent, and (3) the magnitude of the increase in LH release varied with the different prostaglandins tested. In these studies, the infusion of PGE_2 resulted in the most pronounced stimulation of LH release.

The prostaglandin antagonist, 7-oxa-13-prostynoic acid, as well as the prostaglandin synthetase inhibitor, 5,8,11,14-eicosatetraenoic acid, were found to cause a major suppression in the LH release induced by synthetic LRF. The effect of 7-oxa-13-prostynoic acid was observed to be dose-dependent. The antagonist caused an almost complete (88%0 inhibition of the LH response at a concentration of $6 \times 10^{-5}M$. In contrast, the maximum suppression by 5,8,11,14-eicosatetraenoic acid of the LRF-induced LH release was found to be 50% compared to the control response at both concentrations tested ($6.75 \times 10^{-6}M$ and $6.75 \times 10^{-5}M$).

These results are consistent with the concept that prostaglandins may be involved in the mechanisms controlling the pituitary secretion of gonadotropin.

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 on the effects of prostaglandins on ovarian physiology.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Mammalian Sperm Motility and Ultrastructure
Contractor: Washington University
Money Allocated: \$42,699 (FY 69), \$40,543 (FY 70), \$52,589 (FY 71),
\$70,344 (FY 72)

Objectives: The objectives of this research project are: (1) to study in a variety of species both sperm ultrastructure and sperm motility in an attempt to correlate them, and (2) to study the fine structural difference in spermatozoa before and after capacitation.

Major Findings: In uterine and oviductal spermatozoa the sperm velocity and beat frequency of spermatozoa 3 to 5 hours after insemination is far greater than the velocity and beat rate after 10 to 30 minutes after insemination. Uterine and oviductal sperm 3 to 5 hours after insemination swim extremely rapidly. The swimming rate is related to sperm concentration.

The ultrastructure of rat and hamster spermatozoa appears unchanged at 3 to 5 hours post insemination.

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 on the correlation of sperm function and ultrastructure especially as it is affected by capacitation.

Proposed Course: Termination. Major objectives have been accomplished.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hormonal Control of Sperm Maturation
Contractor: Vanderbilt University
Money Allocated: \$27,527 (FY 69) \$37,083 (FY 70) \$50,394 (FY 71)
\$31,356 (FY 72), \$44,735 (FY 73)

Objectives: This research is directed towards establishing the hormonal factors involved in sperm maturation in the epididymis. The investigator has undertaken a series of endocrine interventions, including ablation and replacement experiments in order to study the effects of these procedures on the fertilizing capacity of epididymal sperm of the male rabbit.

Major Findings: The investigators have determined that a highly specific binding protein for 5α DHT with the characteristics of a receptor exists in the cytosol from the caput epididymidis of sexually mature intact rabbits. The other anatomical regions of the epididymis in these animals have no free binding sites for 5α DHT. Studies in which the ductuli efferentes have been ligated to prevent testicular fluid from entering the epididymis suggest that the binding protein is not of testicular origin. The caput 5α DHT binding molecule is distinct on the basis of sedimentation co-efficient from a plasma 5α DHT binding protein. The epididymal receptor has a K_d of approximately 5.6×10^{-9} M, a sedimentation coefficient of approximately 4.5S is thermolabile, and is protein in nature.

All three epididymal segments contain a cortisol binding component that is distinct from the 5α DHT binding component as determined by sedimentation studies and competition analysis. Unlabeled testosterone is able to compete with [3 H] 5α DHT for binding sites on the caput epididymis receptor molecule, but is approximately 1/5 as effective as unlabeled 5α DHT. Estradiol-17 β , 5α DHT, cortisol, and progesterone are weak or ineffective competitors. The 5α DHT receptor sediments as a single symmetrical peak on linear 5-20% sucroseglycerol gradients with a sedimentation coefficient of approximately 4S. The 5α DHT binding component in the caput epididymis is thermolabile with complete inactivation and/or denaturation occurring upon incubation at 37 $^\circ$ or 65 $^\circ$. Studies using hydrolytic enzymes and the sulfhydryl blocking agent N-ethylmaleimide indicate that the receptor is at least part protein in nature.

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: 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
July 1, 1972 through June 30, 1973
Contraceptive Development Program
Contract and Collaborative Research

Contract Title: Biochemical Studies on Spermatogenesis and Spermatozoa
Contractor: Northwestern University
Money Allocated: \$39,944 (FY 69), \$45,061 (FY 70), \$45,061 (FY 71),
\$37,120 (FY 72), \$5,000 (FY 73)

Objectives: The applicant has published extensively on lactate dehydrogenase isozymes. Relevant to the current proposal are his studies on the LDH-X isozyme found in testis and spermatozoa. The applicant is now studying the role of this isozyme in spermatogenesis and the metabolic economy of epididymal and ejaculated spermatozoa, and determining whether other testis-specific forms of other enzymes exist and what part they may play in the biochemistry and function of the male reproductive system. The applicant asks the question, does LDH-X have any functional role in sperm production, motility or functional capacity? His immediate approach to such studies is to isolate sufficient quantities of the LDH-X isozyme to produce antibodies to this enzyme. Mouse testes are used as the source for the enzyme and antibodies are raised in rabbits. These antibodies to the LDH-X and other subunits, e.g., LDH-1 and LDH-5, will be used to isolate LDH labeled during biosynthesis in the animal using radioactive amino acids. From these data, isozyme synthesis rates or isozyme half-life can be calculated.

Major Findings:

1. Male mice repeatedly challenged with antiserum to LDH-X had lower fertility levels. However, the level of LDH-X activity in the testes of challenged mice was the same as in controls.
2. Male rabbits immunized with mouse LDH-X show lowered fertility. Semen collected from control and immunized rabbits by means of an artificial vagina has been examined for LDH-X content, as well as sperm concentration and sperm motility. In all cases, the quality of sperm motility was depressed from progressive to non-motile within a few days after immunization. Motility was rarely better than vibratory as long as circulating antibody was measurable.
3. Female rabbits injected with mouse LDH-X exhibited lower fertility. The number of fetuses in immunized mated females was lower than in the controls.
4. Treatment of pregnant female mice with antiserum to LDH-X between days 1-4 of pregnancy produced a significant reduction in the percentage of pregnant animals.

Significance to Biomedical Research and Program of the Institute: It is important to establish LDH isozymes turnover rates in the testes since this information is basic to the complete understanding of the normal function of the testes. Studies of this sort are highly relevant to the purposes of the contraceptive development program.

Proposed Course: Termination. Majority of the stated objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male
Contractor: Harvard Medical School
Money Allocated: \$66,656 (FY 69), \$59,944 (FY 70), \$68,151 (FY 71), \$71,702 (FY 72), \$60,000 (FY 73)

Objectives: This research is centered upon the use of modern, high quality ultrastructural techniques to investigate key issues of testicular and spermatozoal morphology function. One project deals with topics in spermatogenesis. The investigators are studying the significance of the chromatoid body and aggregates of spermatocytes within the seminiferous tubules, electron microscope radiographic studies of RNA and protein synthesis in spermatocytes and spermatids and, by the use of mitotic blocking agents, interfering with specific developmental events in spermiogenesis in order to learn more about the function of micro-organelles found in germinal cells.

Major Findings:

1. Study of Sertoli-Sertoli cell junctions by freeze-etch methods revealed 3 types of junctions; typical occluding junctions near the lumen which are permanent; tight junctions which may extend around the entire circumference of a cell near its base, and may periodically dissolve to facilitate passage of maturing germ cells through the epithelium; a few gap junctions. No Sertoli-germ cell junctions were found.
2. Electron microscope studies in many mammals have shown that the amount of interstitial tissue and lymphatics vary widely from species to species. Since these elements are presumably involved in the local actions of androgen, the physiological significance of these interspecies differences should be more fully explored.
3. Similar interspecies differences occur in the boundary tissue of the tubule, and range from the single myoid layer in rodent to the multi-layered boundary tissue in higher mammals; in the latter, the innermost layer is myoid in character and the peripheral layers become increasingly less differentiated toward fibroblasts. The heterogeneity of the boundary tissue is emphasized. The development of the myoid layer during puberty in rodent is mediated by androgen, but it is suggested that androgens do not act directly on boundary tissue, but rather on Sertoli cells which induce a response in the immediately adjacent boundary layer.
4. Studies on interstitial tissue from the seasonal breeding rock hyrax have shown that concomitant with a rise in Leydig cell volume during breeding season there is an increase in plasma testosterone. Ultrastructural changes, particularly in the SER, also accompany seasonal fluctuations.

5. High speed cinematography was used to study in depth the motility patterns in epididymal sperm following administration of α -chlophydrin, a drug thought to decrease sperm motility. No changes in motility patterns were observed.

Significance to Biomedical Research and Program of the Institute: Studies of these topics are deemed highly relevant to the purposes of the contraceptive development program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Formation of Dihydrotestosterone in Male Sex Organs and Transport of Steroid Androgens from the Testis to the Epididymis
Contractor: University of Southern California
Money Allocated: \$36,000 (FY 69), \$45,250 (FY 70), \$53,257 (FY 71)
\$65,188 (FY 72)

Objectives: The contractor is studying epididymal relationships and information of two androgens, testosterone and dihydrotestosterone. He is developing a method for submicrogram quantification of dihydrotestosterone in a biological sample in order to measure the levels of testosterone and dihydrotestosterone in testicular tissues, spermatic venous blood, spermatic lymph, corpus, and cauda epididymis.

Major Findings: The following observations have been made during the reporting period:

- (a) When the dog epididymis is infused via the epididymal artery with a mixture of ^3H -testosterone (T) and arterial blood, ^3H -dihydrotestosterone (DHT) can be found in the venous blood of the infused organ. Selective uptake of ^3H -DHT appears to occur in the epididymal tissue during such experiments pointing to the presence of a DHT binder in this tissue.
- (b) The partition of testicular DHT and T between testicular venous blood and testicular lymph has been determined. Only small amounts of DHT leave the testis via the lymphatic system though selective secretion of DHT into this fluid is highly possible.
- (c) When testes from hypophysectomized, mature rats are implanted with DHT contained in silastic tubing, spermatogenesis will be maintained in experiments lasting 60 days. Dose requirement for DHT in these studies: 9 μg /testis/day.
- (d) Progesterone inhibits testicular formation of ^3H -DHT from ^3H -T. The concentration of progesterone is low in the dog testis.
- (e) The major testicular production site of DHT is outside the cells of Leydig in testes from mature rats.
- (f) Data are accumulating which suggest that HCG inhibits testicular formation of ^3H -T to ^3H -DHT. This process may be stimulated by large doses of FSH.

Significance to Biomedical Research and Program of the Institute: This project is directly relevant to the contraceptive program since epididymal physiology has been identified as a particularly important topic for concentrated attention.

Proposed Course: Termination. The principal investigator is relocating at the University of Trondheim.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Formation of the Subacrosomal Mass (Perforatorium) of the Golden Hamster Spermatozoa
Contractor: University of Houston
Money Allocated: \$14,451 (FY 69), \$15,347 (FY 70), \$15,347 (FY 71), \$20,047 (FY 72), \$20,000 (FY 73)

Objectives: The investigator is studying the origin of the perforatorium of golden hamsters' sperm by following initially the formation of the perforatorium in early spermatids by electron microscopy. The hamster is chosen because of the large size of the hamster perforatorium; however, these studies are being performed, and chemical characterization of the perforatorium attempted.

Major Findings: Immune serum from a rabbit injected with acrosomal ghost preparations inhibits the dispersion of cumulus oophories of hamster eggs seminated in vitro with low concentrations of sperm. This circumstance is consistent with the belief that the acrosome is the primary source of sperm hy luronidase in that antibodies produced to the acrosomal ghost might be expected to prevent the release of acrosomal contents.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research on potential areas of male contraception.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Fine Structure and Function of the Accessory Glands of
the Male Reproductive Tract
Contractor: University of Virginia
Money Allocated: \$12,985 (FY 69), \$12,750 (FY 70), \$18,266 (FY 71),
\$25,851 (FY 72), \$83,685 (FY 73 for 3 years)

Objectives: This study of the fine structure and function of the male accessory organs is being carried out principally in rats, with particular attention being paid to (1) effects of vasectomy, both proximal and distal, to the point of ligation of the vas; and (2) changes which take place in the Wolffian ducts of rat fetuses, before, during, and after onset of androgen secretion (at daily intervals from day 14 until birth). Electron microscopy and electron microscope radioautography are being used to follow the fate of radio-labeled precursor substances in a study of the secretory activity of the accessory glands.

Major Findings: Developmental changes in the prostate and seminal vesicle of the rat occur postnatally in organelles concerned with the formation of secretions. Alterations in the rat epididymis after vasectomy consist mainly of accumulation of masses of membranous material in the light cells of the cauda epididymidis. The fine structure of the rat testis remains normal for at least nine months after vasectomy. Regional specializations in fine structure and function of the vas deferens epithelium occur in normal rats and persist after vasectomy. Cells of the proximal part of the vas deferens are specialized for absorption, while those of the distal portion may produce steroids. Electron microscope radioautography indicates that the protein secretions of the prostate and seminal vesicle are synthesized in the rough endoplasmic reticulum, transported to the Golgi apparatus, packaged into secretory vacuoles, and released into the lumen of the gland. The secretory process is much more rapid in the seminal vesicle than in the prostate. Light cells in the cauda epididymidis of the rat are shown by electron microscope histochemistry to be rich in acid phosphatase.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Electron Cytochemical Studies
of Mammalian Spermiogenesis
Contractor: The University of Texas
Money Allocated: \$35,875 (FY 69), \$58,230 (FY 70), \$64,282 (FY 71),
\$82,127 (FY 72), \$88,533 (FY 73)

Objectives: The research project involves studies of (1) the formation of the manchette in the spermatids during spermiogenesis, by electron microscopy and cytochemistry at the electron microscopic level (ATPase, succinic dehydrogenase, alkaline and acid phosphatases, thiamine phosphatase, aryl sulfatase and β -glucuronidase); (2) the inhibition of microtubule formation in the manchette (colchicine, cold shock, inhibitors of protein and RNA synthesis); (3) the ultrastructure and cytochemistry of seminal vesicles, bulbourethral gland and prostate.

Major Findings: (1) Microtubules of the cells of the seminiferous tubules have been positively identified as the target for reversible Colcemid-induced sterility; (2) Additional evidence has been obtained supporting the role of microtubules of Sertoli cells in maintaining the integrity of the seminiferous epithelium; (3) Experiments with actinomycin D have demonstrated selective uptake of the antibiotic and selective killing of cells at specific stages of spermatogenesis. Also, when actinomycin D is injected intratesticularly, the antibiotic generally remains localized at the site of injection; (4) Preliminary experiments with cytochalasin B indicate that cytoplasmic microfibrils in the Sertoli cells play an important role in spermatid-Sertoli cell junctions; (5) New observations on the ultrastructural localization of marker enzymes have been reported; and (6) Studies of the effects of castration on the fine structure of the epididymis of the rat and the localization of epididymal phosphatases have been completed.

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

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Role of the Epididymis in Sperm Maturation
Contractor: Pennsylvania State University
Money Allocated: \$30,706 (FY 69), \$58,660 (FY 70), \$65,359 (FY 71)
\$89,736 (FY 72), \$177,465 (FY 73 for 2 years)

Objectives: The specific objectives are: (1) to determine actual sperm production rate in bulls; (2) to compare ultrastructure and biochemical characteristics of spermatozoa recovered by cannulation of the rete testis with those of cells recovered by cannulation of the vas deferens; (3) to characterize biochemically the testicular effluent and, by comparing this with seminal plasma and accessory gland fluids, to establish the nature of the epididymal secretion; and (4) to determine if there is a substance in the testicular effluent which might exert a local control on spermatogenesis or epididymal function.

Major Findings: Dihydrotestosterone was not detected in rete testis fluid, but its concentration in cauda epididymal plasma was uniquely high (14 ng/ml) and about twice that of testosterone. Metabolic responses of cauda epididymal sperm to added androgens were generally similar to those of ejaculated sperm when incubated in the presence of glucose. However, their responses were very different from those reported earlier for testicular sperm. In rabbits, both inositol and total lipid concentrations were greater in tissue from the caput than from the cauda epididymidis. Tissue slices from all regions of the epididymis incorporated radioactivity from inositol-U-14C into lipid, but incorporation was greatest for the cauda epididymidis. The concentration of phospholipid in testicular sperm was twice that in cauda epididymal or ejaculated sperm. Choline plasmalogen was the major phospholipid in all cell types and constituted about 40% of the total pool in sperm which have traversed the epididymis. The phospholipid-bound fatty acids also differed between testicular and cauda epididymal sperm. Rete testis fluid and cauda epididymal plasma contain androgen-binding proteins, but further studies are needed to determine if these are of high affinity. Bovine cauda epididymal and ejaculated sperm are of similar fertility (88% cleaved ova) while testicular sperm apparently are infertile (0%).

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 studies directed towards the development of new male contraceptives.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control Mechanisms of Sperm Motility
Contractor: State University of New York
Money Allocated: #30,836 (FY 70), \$83,170 (FY 71), \$34,523 (FY 72)

Objectives: The applicant proposes that sperm motility is the result of a control system consisting of a free-running oscillator, an amplifier, a power supply, and a feedback loop. The investigator proposes a number of novel experiments designed to investigate the nature of this control system and its components. Specifically, he proposes: (1) To measure the electrical activity of the oscillator in the centriole by inserting glass microelectrodes into the head of sperm immobilized first by low temperature, and to search for electrical potentials of the same frequency as the tail wave. (2) To destroy by a laser beam, areas of the centriole. A glass microelectrode will then be inserted into the head of the immobilized sperm to monitor the electrical activity, and to attempt electrically to reactivate the mechanism for motility. (3) To inhibit the action of the oscillator and its coupling, by specific chemicals such as xylocaine and procaine. (4) To investigate the feedback mechanism by inhibiting respiration, and then measuring the change of frequency of the tail wave.

Major Findings: The elastic rigidity (stiffness) of impaled bull sperm flagella has been determined by a manipulatory technique which permitted direct analytical treatment of the experimental system. It was found that ATP acts as a plasticizing agent, while ADP does not. The stiffness measured for flagella in medium without ATP was 15 times greater than the value measured with 10mM ATP present. The rigor-like stiffness measured with no ATP present is reversible by ATP and seems to be correlated with a transition in the state of the contractile system. These findings are analogous to the state of rigor, relaxation, and activity in muscle.

The oscillatory properties of bull sperm flagella have been investigated at raised viscosity, under KCN inhibition and thiourea. The result show that the contractile system of bull sperm flagella is self oscillatory, and is not controlled by an external oscillator. At high viscosity the moments developed by the contractile system are increased compared to at normal viscosity. This indicates a strong mechanochemical feedback of the external medium onto the contractile system.

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 studies in which electrophysiological techniques are applied to elucidating complex reproductive processes such as sperm motility.

Proposed Course: Termination. Major objectives have been achieved. Work is continuing with the support of a NICHD grant.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Immunological Investigation of Spermatogenesis, Sperm Transport, and Fertilization in the Mammal

Contractor: University of Miami

Money Allocated: \$92,300 (FY 70), \$58,275 (FY 71), \$78,025 (FY 72)
\$60,000 (FY 73)

Objectives; The objective of this research project is to study the effects of antibodies on spermatozoa in the female tract of mammals, using bivalent and univalent fragments of guinea pig and goat anti-rabbit semen antibody.

The investigators will examine the fertilizing capacity and transport of antibody-pretreated spermatozoa following intrauterine artificial insemination, in an attempt to establish whether antibodies can inhibit spermatozoan passage from the uterus through the tubes to the site of fertilization. In addition, intratubal and/or in vitro insemination experiments with univalent and bivalent antibody-treated spermatozoa will be performed in an attempt to determine if antibody treatment inhibits capacitation and whether antibody can inhibit the fertilizing capacity of capacitated sperm. Anti-sperm antibody inhibition of sperm head enzymes thought to be responsible for ovum penetration will be tested by preparing extracts of these enzymes and examining their activity on appropriate substrates in the presence of univalent and bivalent antibodies.

Major Findings: The purification of sperm hyaluronidase has been continued. A 300 to 400 fold increase in specific activity has been achieved. The preparation obtained exhibits two protein-staining bands following acrylamide gel electrophoresis. The final resolution of the two bands is in progress.

The investigator has demonstrated that purified sperm hyaluronidase can produce antibodies that are both species and tissue specific. This is an important finding for the possible preparation of an antifertility vaccine.

Injection of female rabbits with the purified enzyme preparation produced anti-hyaluronidase antibodies in 5 out of 6 animals. The sera from control rabbits were devoid of anti-hyaluronidase activity.

Univalent anti-semen antibodies block the sperm-leucocyte attachment that is normally observed with spermatozoa recovered from uteri. Likewise, isoimmunized females fail to respond to the leucotactic factor.

Sera from infertile women were tested for the presence of anti-hyaluronidase antibodies. Although majority of the sera produced sperm agglutination or immobilization only a smaller proportion were active against sperm hyaluronidase. Whether the presence of anti-hyaluronidase antibodies in sera from infertile women is related to their infertility is conjectural at this point.

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 studies which utilize immunological techniques to study reproductive processes.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Enzymes of the Sperm Acrosomes, Occurrence of the Inhibitors of These Enzymes in Seminal Plasma and the Role of the Enzymes and Inhibitors in Capacitation of Sperm and in the Penetration and Fertilization of Ova

Contractor: University of Georgia

Money Allocated: \$29,494 (FY 70), \$45,835 (FY 71), \$44,462 (FY 72), \$69,986 (FY 73)

Objectives: The objectives of this research project are: (1) to isolate and purify the five acrosomal enzymes from mammalian sperm; (2) to demonstrate their role in fertilization; (3) to elucidate the role played by enzyme inhibitors in seminal plasma (including decapacitation factor, DF); (4) to attempt to correlate DF activity with the inhibition of a new sperm neuraminidase; and (5) to study uterine enzymes and their possible role in capacitation.

Major Findings: The following acrosomal enzymes were obtained in pure state: acrosin (acrosomal proteinase), hyaluronidase, aryl sulfatase and sperm neuraminidase. Substrate and inhibitor specificity studies on acrosin showed that it is a unique serine endopeptidase inhibited by arginine. Inhibition of acrosin by synthetic inhibitors such as tosyl lysine chloromethyl ketone led to the use of synthetic enzyme inhibitors as antifertility agents. Methods were developed for the purification of hyaluronidase. The pure hyaluronidase obtained has greater specific activity than the best known hyaluronidase reported in the literature. Pure hyaluronidase has an absolute requirement of cations for its activity. Pure aryl sulfatase has two isoenzyme activities, A and B. Its inhibition by certain ions and not by others and its substrate affinity showed that acrosomal aryl sulfatase is of unusual type.

Pure aryl sulfatase is inhibited by crude decapacitation factor. The physiological role of pure acrosin causing the dissolution of the zona pellucida and sperm neuraminidase altering the macromolecular composition of the zona was demonstrated. A new method for sequential removal of acrosomal enzymes was developed. Seminal plasma acrosin inhibitor was purified and its ability to inhibit fertilization demonstrated. Inhibition of fertilization by specific sialoproteins was demonstrated.

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 studies of the role of sperm acrosomal enzymes and their inhibitors in capacitation and fertilization.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Neurochemical Control of Sperm Motility
Contractor: Medical College of Ohio
Money Allocated: \$45,558 (FY 70), \$45,558 (FY 71), \$48,330 (FY 72)
\$50,992 (FY 73)

Objectives: The investigator will apply biochemical, electron microscopic, and electrophysiological procedures to the study of control of flagellar movement as related to sperm motion. More specifically, the applicant will conduct a systematic analysis of the role of acetylcholinesterase in the regulation of the propulsive motion of spermatozoa. The study will be conducted in three phases:

1. Sperm cell suspensions will be subjected to microcolorimetric analysis of acetylcholinesterase activity, rating of motility, timing of swimming duration, electron microscopic cytochemical procedures, and microelectrode determinations of electrical potentials. The effects of specific acetylcholinesterase inhibitors, such as eserine, on sperm propulsion will also be studied.
2. In the second part, the investigator will examine the developmental and maturational aspects of flagellar growth in relation to the development of sperm motility.
3. The third part will be directed toward the environmental interactions, both physical and chemical, between the sperm cells and the medium.

Major Findings: The studies dealing with the origins and redistribution of sperm AChase during mouse spermiogenesis have been completed. As maturation progresses localization becomes more distinct within the midpiece and the flagellum. This may reflect the association of the enzyme with the propulsive mechanism.

Changes in Ca^{++} concentration produced changes in sperm membrane potential as well as changes in the amplitude of flagellar wave.

Addition of eserine to the sperm suspending medium not only caused a drop in sperm cell potential from --10mv to --3mv, but also altered the swimming characteristics from a progressive type to a circular pattern.

DMSO increases the permeability of the sperm to ACh. In the presence of DMSO (10mM/L) ACh (1-10mM) altered the potential by increments until polarity reversal brought it up to +6mv, clearly mimicking the Ca^{++} phenomenon.

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 on sperm motility.

Proposed Course: Termination. The stated objectives of the contract will be fulfilled after this contract year.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Electron Spin Resonance and Radioisotope Labelling
Studies of Mammalian Reproductive Cell Membranes in
the Evaluation of New Methods of Contraception
Contractor: University of Hawaii School of Medicine
Money Allocated: \$56,099 (FY 70), \$66,606 (FY 71), \$68,387 (FY 72)

Objectives: In this research project, electron spin resonance (ESR) spin-labelling, will be applied to the study of sperm membrane interactions and sperm transport in vivo. The method relies on the ease of labelling sperm cells with a lipid-seeking spin-label which causes no loss of sperm motility or function. The spin-label is capable, through analysis, of reflecting structural changes in the membrane of the sperm. These changes will be measured during sperm capacitation and interaction of sperm with genital fluids and chemical contraceptives. The same kind of label, but tagged with C^{14} , will be used to measure in vivo transport of sperm in the uterine tract of experimental animals especially primates.

Following preliminary work aimed at establishing the feasibility of using such a spin-label for detecting alterations in sperm membrane characteristics, such studies would be extended to the in vivo case. Miniaturized electron spin resonance detectors would be developed for implantation directly in the tract so that alterations could be monitored directly. The investigators also propose to use a radioisotope label for studying sperm transport within the female tract. In this case the sperm would be labelled with a fatty acid carrying not a paramagnetic nitroxide, but a heavy concentration of C^{14} ; the investigators have designed a small Geiger-Mueller tube (2mm diameter, 5mm in length) which could be implanted in the uterine tract to detect in vivo transport of the labelled sperm.

Major Findings:

1) Spin-labelling of epididymal spermatozoa with sulphhydryl reagents indicates that the differences observed between caput and cauda sperm can not be accounted for by the differences in total number of S-S crosslinkages. Large differences exist in partitioning of the label between highly immobilized sites and free or partially immobilized sites within the sperm membrane. There is a dramatic difference between caput and cauda, the former showing a much more rapid labelling with a higher percentage of free to bound labels. These results would seem to indicate a much more flexible structure for the caput sperm than the cauda sperm which is contrary to what one might expect if disulfide crosslinking parallels rigidity since the latter is greater in caput than cauda. This flexibility difference would also account for the differences observed between caput and cauda sperm when reacted with detergents. Caput sperm are strongly affected by detergents but not cauda, this it would appear that the cauda membrane is less porous or contains blocking groups that prevent the detergent molecules from interacting with the structural proteins within the membrane.

2) Capacitation increases sperm motility and raises the sperm cyclic AMP levels. This elevation in cyclic AMP is sufficient to account for the motility changes.

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 studies which make use of state-of-the-art technology to study reproductive processes such as sperm capacitation and transport.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Testis and Sperm Specific Antigens of Man and Certain Other Primates

Contracter: University of Michigan

Money Allocated: \$21,140 (FY 71), \$21,768 (FY 72)

Objectives: Immunization with testis and sperm antigens is a possible means of human contraception. However, the use of the materials will depend upon knowledge of the characterization and purification of these antigens. This proposal is intended to yield information on the chemical and immunologic nature of immunogens specific for testis and sperm, the possible influence of the epididymis on the antigenic molecules, and the effects of antibodies against these antigens on sperm viability and function. The use of immunosera from infertility patients will permit detection of those testes and sperm antigens that are immunogenic in man and their possible relationship to infertility. These immunogens would be purified and characterized. The effects of antibodies induced by the different antigens upon sperm will be determined by laboratory tests involving agglutination, immobilization and cervical mucus penetration. Possible changes in the chemistry of sperm antigens in the epididymis will be associated with the maturation of sperm cells. After characterization and purification of the immunogens future plans call for isoimmunization of male and female monkeys to determine the effects on the fertility process.

Major Findings: Six specific antigens have been identified in acetic acid-Triton X-100 extract of human testis. Five of the antigens induce precipitating antibodies and one the sperm immobilizing antibody. At least 4 to 5 testis-specific antigens have been identified for rhesus monkey and baboon. One of the most significant findings to date is the high degree of cross-reactivity among the testis antigens of man, rhesus monkey and baboon as determined by immobilization test, immunoprecipitation tests and immunofluorescence. Human sperm and rhesus sperm were interchangeable in the immobilization tests with the various antisera. If this cross-reactivity is consistent for the antigens associated with inducing infertility the possibility is open to study specific human antigens in the rhesus monkey in regards to immunization and antifertility effects.

Significance to Biomedical Research and Program of the Institute: Immunologically induced infertility could be a useful contraceptive method and its study is highly relevant to the contraceptive development program especially since the work will be done on primate material.

Proposed Course: Termination. The study is not providing unequivocal data.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contraceptive Development Studies for Males: Oral
Steroid Hormone Administration
Contractor: University of Washington School of Medicine
Money Allocated: \$118,056 (FY 71) for two years

Objectives: This contract proposal is designed to evaluate the combined effects of two androgenic steroids on spermatogenesis when administered to normal adult human males. The ultimate goal is to develop an effective oral contraceptive technique for males.

At the present time there is no compound available for effectively inhibiting human spermatogenesis which is non-toxic or absent from adverse reactions which would render the preparation either unacceptable to the patient or to his physician.

Major Findings: In an initial clinical trial, Danazol[®] (an isoxazole derivative of ethynyl testosterone having anabolic activity) was administered to normal adult male volunteers alone or in combination with testosterone propionate or testosterone enanthate to determine the effects on sperm output. The drugs were administered for four months. Danazol[®], orally (p.o.) (600 mg/day), produced a modest reduction in sperm counts, but Danazol[®] (p.o.) plus testosterone propionate, 10 mg intramuscularly three times weekly, reduced sperm counts to below 2 million/ml in 3 of 4 men. Danazol[®] (600 mg/day, p.o.) plus testosterone enanthate (200 mg intramuscularly once monthly) dropped sperm counts to less than 1 million/ml in 3 of 3 men within eight weeks. The loss of libido and potentia observed with Danazol[®] alone was prevented when the testosterone esters were added to Danazol[®] as a combined regimen. A much more extensive clinical trial employing Danazol[®] together with the orally active androgen, methyl testosterone, is currently in progress.

Significance to Biomedical Research and Program of the Institute: Development of contraceptive techniques for the male is highly relevant to the contraceptive development program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunologic Localization of Prostaglandins in the
Reproductive Tract of Rabbit
Contractor: Yale University School of Medicine
Money Allocated: \$39,850 (FY 72)

Objectives: The objective of this research is to study the localization of prostaglandins in the uterus, oviduct and on sperm during the capacitation process. Antibodies to prostaglandins will be coupled with peroxidase incubated with the respective tissues and subsequently reacted with diaminobenzidine. The last reaction produces electron dense material which can be subsequently studied with EM. The localization studies may provide an answer for the site of action or synthesis of prostaglandins at the cellular level.

Major Findings: Methods have been developed for conjugating prostaglandin A to gamma globulin and subsequently to horseradish-peroxidase. Initial attempts to utilize this complex for the intracellular localization of prostaglandins within the rat seminal vesicle were only partially successful.

Significance to Biomedical Research and Program of the Institute: The projected study on the localization of prostaglandins is directly related to the stated interests of the Contraceptive Development Branch in this group of substances.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Changes in the Sertoli Cells in Rats
Under Various Hormonal Conditions
Contractor: University of Texas Medical School, Houston
Money Allocated: \$126,804 (FY 72) for three years

Objectives: The objective and scope of the present study is to conduct a detailed investigation of the ultrastructure of mature and developing Sertoli cells of rats under various experimental conditions. Specific emphasis will be placed on the possible hormonal interaction which may be operative in Sertoli cells. In vivo and in vitro studies will be made of testes from developing and adult animals deprived on gonadotropic hormones, replaced with gonadotropins or androgens or treated with a cytotoxic agent, furadroxyl.

Major Findings: Electron micrographs of testes that were perfused respectively at 4, 10, 20, 31, and 40 days after hypophysectomy are presently being evaluated. One rat for each of the preceding listed days was perfused and fixed for electron microscopic examination. Qualitative estimates indicate that by 20 days after hypophysectomy, the number of profiles of Sertoli cells increase, whereas the seminiferous tubule decreases in diameter. The entire testis atrophies and there is considerable loss of cells in the seminiferous epithelium. Transverse sections of the epithelium show large numbers of contiguous Sertoli cells. There is an apparent increase in the extent of junctional complexes formed between these closely packed Sertoli cells of the 20, 31, and 40 day hypophysectomized rats. The nature of the junction complexes are currently being investigated using horseradish peroxidase as a tracer.

Mitochondrial reorientation in some spermatocyte cytoplasm occurs as early as the fourth day after hypophysectomy. In controls the mitochondria are randomly scattered in the cytoplasm. However, in hypophysectomized rats the mitochondria of the spermatocytes align around the cell periphery in an almost evenly spaced manner. Adjacent border regions of spermatocytes have mitochondria of one cell practically paired in a 1:1 relationship with those mitochondria of the other cell.

Numerous lysosomal bodies are apparent throughout the cells of the seminiferous epithelium and the appearance of large lipid-like inclusions in the Sertoli cytoplasm occurs at approximately 20 days following hypophysectomy.

Significance to Biomedical Research and Program of the Institute: Understanding of the regulatory function of the Sertoli cells may suggest approaches to interference with seminiferous tubule function. The study is clearly relevant to the goals of the program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hormonal Control of the Epididymis
Contractor: Bureau of Biological Research, Rutgers University
Money Allocated: \$62,539 (FY 72) for two years

Objectives: The primary objective of this research is to determine the biochemical composition of rat epididymis under a variety of hormonal conditions. Among the biochemical endpoints that will be evaluated are: (1) total protein, (2) nucleic acids, (3) alanine and aspartic transaminases, (4) glycogen, (5) glucose-6-phosphate and 6-phosphogluconate dehydrogenases, (6) free amino acids, (7) fatty acids, (8) lactic dehydrogenase and alkaline phosphatase.

The influence of the following hormones and hormone antagonists will be evaluated: (1) various androgens, (2) estradiol, (3) cortisol and (4) cyproterone acetate, A-nor progesterone and MER-25.

The influence of castration or the deletion of testicular fluid (efferentiectomy) on epididymal composition will be assessed.

The biochemical assessment will be carried out on the cauda and the caput of young adult male rats.

Major Findings: Initial biochemical analysis of caput and cauda epididymis revealed no difference in protein or nucleic acid concentration between the two segments, nor were there differences in transaminase activity. However, aspartic transaminase activity was significantly greater than alanine transaminase. Also, glycogen concentration was greater in the cauda than in the caput, whereas glucose-6-phosphate dehydrogenase activity was just the reverse.

Compositional changes following castration include a decrease in endoplasmic reticulum, glycogen and succinic dehydrogenase activity. Protein concentration increased and RNA concentration declined in the caput, but not in the cauda segment.

Testis fluid as well as testosterone from the testis contribute to the maturation and maintenance of the epididymis. Unilateral ligation of the vasa efferentia was followed by a decrease in epididymal weight and a reduction in 5α -dihydrotestosterone and finally loss of sperm.

Unilateral efferentiectomy studies showed that alanine and aspartic transaminase activity was greater in epididymal tissues from the operated side.

Treatment of castrated rats with testosterone, dihydrotestosterone and dihydroepiandrosterone showed only slight regional differences in response, the cauda being somewhat more sensitive.

Significance to Biomedical Research and Program of the Institute: The study of epididymal function is one of the stated goals of the contraceptive development program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Sperm Fertilizing Capacity
Contractor: The Worcester Foundation for Experimental Biology
Money Allocated: \$45,000 (FY 73)

Objectives: The investigator will study the particulate nature of the sedimentable decapacitation factor (DF) and attempt to characterize the biochemical makeup of fraction I and II vesicles from epididymal and seminal plasma. The interaction of the vesicles with sperm membrane components will also be investigated. Specific objectives of this contract are:

- a) to determine the DF activity of fraction I and II vesicles
- b) to investigate the biochemical makeup of these vesicles
- c) to determine the interaction between the vesicles and spermatozoa
- d) to elucidate the mechanism of decapacitation by these vesicles and attempt to exploit this for male contraception purposes.

Significance to Biomedical Research and Program of the Institute: The study of capacitation and decapacitation is directly related to the published purpose of the Contraceptive Development Branch to support research of possible fertilization inhibitors.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Analysis of Anti-Sperm Antibodies in Monkey Sera
Contractor: University of Colorado Medical Center
Money Allocated: \$7,150 (FY 73)

Objectives: Analyze sera from monkeys previously immunized with monkey spermatozoa for anti-sperm antibodies. This study will complete the analysis previously initiated under another contract.

Significance to Biomedical Research and Program of the Institute: The immunological studies undertaken in this project are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Termination. This study will complete the objectives of the initial contract.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study on Human Acrosomal Proteinase
Contractor: Wayne State University
Money Allocated: \$71,785 (FY 73 for 3 years)

Objectives: The objective of this contract is to investigate several aspects of biochemistry and immunology of the purified human acrosomal proteinase. The primary interest is to ascertain whether antisera against this enzyme can block fertilization. Secondarily the relationship of enzyme activity to the process of semen liquifaction and to the presence of naturally occurring enzyme inhibitors will be investigated.

Significance to Biomedical Research and Program of the Institute: Sperm acrosomal proteinase facilitates ovum penetration by the spermatozoon. The work to be carried out on this project is directly related to the published purpose of the Contraceptive Development Branch to support research dealing with fertilization inhibition.

Proposed Course: This 3 year contract should fulfill the stated objectives.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Dynamics of Spermatogenesis: Quantitative Morphological
and Physiological Characterization of Isolated Meiotic Cells
Contractor: The Johns Hopkins University
Money Allocated: \$78,481 (FY 73) for 2 years

Objectives: This is a proposal to isolate, separate, and characterize meiotic germ cells in rat testes using the lg sedimentation system. Uptake and retention of radioactive steroids (particularly ^3HT) by various types of meiotic cells which have been isolated by gradient analysis will be studied. Further, efforts will be made to maintain isolated meiotic cells in tissue culture and to determine if differentiation of these cells occurs.

Significance to Biomedical Research and Program of the Institute: The experimental approach to studying spermatogenesis presented has considerable implications for the development of fertility control methods in the male, which is directly responsive to the stated goals of the program.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Macromolecular Synthesis in Spermatogenic Cells Studied
by Cell Separation Methods
Contractor: University of Texas
Money Allocated: \$25,296 (FY 73)

Objectives: The investigator proposes to develop further and improve the existing methods for separation of various populations of mouse testicular cells. Solid data in pure testis cell populations are needed, and once these have been obtained, selective modification of tissue function and consequently, sperm production or fertility should be feasible.

Significance to Biomedical Research and Program of the Institute: An ultimate goal of acquiring this basic knowledge would be the possible inhibition of sperm development in the testis by drugs or immunological means, which is relevant to the purposes of CDB activities.

Proposed Course: Once purity and homogeneity of cells has been accomplished, the investigator plans to undertake two specific aspects of testis biochemistry. Therefore, this will 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Androgen Receptors in the Testis
Contractor: The University of Texas Medical Center
Money Allocated: \$111,744 (FY 73) for 2 years

Objectives: The investigator proposes the following studies:

- 1) Demonstration of the presence of androgen receptors in the testis.
- 2) Localization of the receptors in one or both of the major compartments of testicular tissue, i.e., seminiferous tubules and interstitial tissue.
- 3) Characterization of the androgen receptors and study of the relationship between soluble and nuclear receptors.

Significance to Biomedical Research and Program of the Institute: An understanding of the cellular sites of binding and the nature of the interaction between androgens and specific receptor molecules will allow for a more rational assessment of androgens and anti-androgens as male contraceptive agents and is related to the stated goals of the program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Metabolism of Sperm Nuclear Proteins During Spermiogenesis
Contractor: Harvard Medical School
Money Allocated: \$42,847 (FY 73)

Objectives: The nuclear protamine, rich in cysteine residues, undergoes oxidation of free sulfhydryl groups to form intermolecular disulfide bridges thus becoming a complex keratinoid network stabilizing the genome. The initial phase of disulfide bond formation is proposed to occur during nuclear condensation of the spermatid and is followed by a more gradual transition during maturation of the spermatozoa in the epididymus.

The objective of this proposal is to verify, by amino acid analysis, that mouse and rabbit sperm nuclear protamine are rich in cysteine residues. With this basic information an intensive biochemical study will be undertaken on the formation of protamine disulfide bonds which is suggested to occur during epididymal maturation of spermatozoa and a function of the epididymal environment.

A further study is planned to test the validity of the hypothesis that initial condensation of the spermatid chromatin is induced by formation of disulfide bonds at the carboxy-terminal and amino-terminal cysteine residues of the protamine molecule. Thus, progressive formation of additional disulfide bonds induced during epididymal maturation of the sperm occurs primarily between internal cysteine residues.

Significance to Biomedical Research and Program of the Institute: A study of protamines and their chemical modification during spermatogenesis and epididymal maturation of spermatozoa could provide basic information required to elucidate mechanism of action of potential antispermatogenic agents and is related to the stated goals of the program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemical Requirements for Fertilization and
Development of Rabbit and Human Ova
Contractor: University of Georgia
Money Allocated: \$34,122 (FY 69), \$45,351 (FY 70), \$46,519 (FY 71)
\$42,999 (FY 72)

Objectives: This research project involves the study of at least three enzymes present in sperm, the corona-removing enzyme (CRE) and two trypsin-like acrosomal enzymes which, the applicant hypothesizes, are involved in the penetration and removal of the corona radiata, the zona pellucida and the vitelline membrane. The applicant further postulates that "decapacitation factor" (DF) is a low molecular weight material which inhibits the CRE and thus blocks fertilization or capacitation. Both rabbit and human materials are used to study these points. Included as a related objective is the development of an effective medium and the proper physical conditions for in vitro fertilization and cleavage of human ova obtained from ovarian tissue removed at local hospitals. .

Major Findings:

- (1) Studies with bovine decapacitation factor (DF) indicate that it is labile to trypsin and pronase but the DF activity is not destroyed by chymotrypsin digestion. Rabbit DF activity is destroyed by both trypsin and chymotrypsin.
- (2) Corona penetrating enzyme was demonstrated to be proteolytic enzyme from the acrosome and it is different from the previously isolated acrosomal proteinase.
- (3) Treatment of capacitated rabbit spermatozoa with sialic acid containing mucins blocks both in vitro and in vivo fertilization. This suggests that sperm neuraminidase participates in the capacitation process by removing sialic acid from sperm membranes.
- (4) In vitro fertilization of squirrel monkey ova has been achieved.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to elucidate the mechanisms involved in sperm capacitation and in the critical process of fertilization.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Ovulation and Capacitation in Non-Human Primate
Contractor: Michigan State University
Money Allocated: \$24,351 (FY 69), \$18,100 (FY 70), \$26,314 (FY 71),
\$42,648 (FY 72)

Objectives: The objectives of this research project are: 1) to develop a reproducible technique for induction of ovulation in primates; 2) to determine the fertilizable life of primate ova; 3) to carry out further studies on the need for sperm capacitation in the primate.

Major Findings: In vitro fertilization of squirrel monkey ova has been achieved. This was confirmed by the presence of sperm vestments and two pronuclei.

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 studies on the necessity for capacitation in primates.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morpho-Physiologic Studies on Mammalian Gametes Prior to and During Fertilization In Vivo and In Vitro
Contractor: Harbor General Hospital
Money Allocated: \$23,783 (FY 69), \$42,416 (FY 70), \$45,978 (FY 71), \$39,714 (FY 72), \$52,519 (FY 73).

Objectives: The main objective of the proposed research is to study in vitro fertilization of primate ova for the purpose of (1) defining the experimental conditions suitable for gamete interaction, and (2) investigating the morphologic changes associated with fertilization and early embryonal development. Two species of primates are to be used: the free-ranging rhesus macaque and the squirrel monkey (*Saimiri sciureus*).

Major Findings: Significant progress has been made in the following areas:

1. Fine Morphology of follicular oocytes

High resolution light microscopy and electron microscopy studies performed on wedges of ovarian tissue obtained from adult macaques have elucidated for the first time the morphologic changes which are associated with the process of oocyte maturation in ovarian follicles of free-ranging macaques. The study revealed that oocyte maturation is accompanied by salient mitochondrial changes which are obviously the expression of profound metabolic changes and may indicate ongoing steroidogenic activity in the oocytes of large antral follicles. Structural and functional differences were observed in ovaries of free-ranging and laboratory rhesus.

2. Fine Morphology of Oocytes maturing in vitro

Oocytes of free-ranging rhesus monkeys are capable of proceeding to maturation in vitro in percentages which are comparable to those of cultured oocytes of other species including humans. Their maturative process is associated with structural changes identical to those noted in human oocytes maturing in vitro.

3. Fine morphology of ejaculated spermatozoa and male reproductive organs.

Semen of free-ranging rhesus was analyzed for volume, sperm number and motility. A light microscopic evaluation of sperm morphology was performed and a portion of the semen was also prepared for electron microscopy. The study demonstrated the occurrence of seasonal changes in the semen of free-ranging macaques, as well as another radical difference between free-ranging rhesus and laboratory rhesus: in the latter, semen production and composition remain constant throughout the year.

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 as it pertains to the mechanisms involved in the critical process of fertilization.

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
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Sperm Capacitations as Target for Contraception
Contract: Vanderbilt University
Money Allocated: \$44,278 (FY 70), \$44,278 (FY 71), \$63,392 (FY 72)

Objectives: The objective of this research project is to perform animal studies aimed at clarifying the role of leukocytes in the capacitation process and improving the methodology for the study of capacitation. Alkylating agents or extracorporeal irradiation of blood will be used to produce systemic leukopenia in an attempt to eliminate leukocytes from the endometrium and uterine lumen. A second objective of the project is the development of a reliable system for in vitro fertilization of human ova.

Major Findings:

Animal Studies: Inhibition of leukocytic response to presence of sperm in female rabbit genital tract indicated that polymorphonuclear leukocytes are not a factor of prime significance in sperm capacitation mechanism. Alkylating agent (nitrogen mustard) used to abolish leukocytic response, however, inhibited capacitation significantly, presumably by interfering with epithelial lining function. Acute early inhibition (within 12-24 hours) was followed by temporary recovery, followed in turn by slower depression. Inhibition was maximum in uterus, less in oviduct, and even less when both sites were acting sequentially on sperm.

Human Studies: A technique for direct oocyte recovery from any exposed ovary was developed. From 139 oocytes so recovered, 37% were atretic. Of 76 presumably non-atretic oocytes only 23 (30%) completed extrusion of second polar body in culture. Among these, 6 (26%) became fertilized in vitro. In an experiment using oocytes from hormonally primed patients, the addition of FSH, LH and HCG to fertilization medium resulted in significant (P .01) increase in sperm penetrating ability as compared to controls. Such effect is attributed to hormonal stimulation of follicular cells present in fertilization environment. Experimental evidence was thus gained that capacitation is a prerequisite for human fertilization as it is in other animals.

The contractor has extended his studies on the in vitro maturation and fertilization of human oocytes. The average recovery rate (about 3 oocytes per patient) was independent of age (range 20-45 years) or gestational status. Oocytes were classified into three categories: degenerate or atretic, healthy but non-ovulatory and preovulatory. Oocytes in the later two categories have been studied in several culture media and the degree of maturation and/or fertilization was studied by light and electron microscopy. The serial electron photo micrographs required to demonstrate the degree of maturation or fertilization have not been completed 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 on new methods of

studying complex reproductive functions such as sperm capacitation and in vitro fertilization.

Proposed Course: This contract has been terminated as original objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Effect of Progesterone and Estradiol on RNA Synthesis in the Rabbit Oviduct and its Relationship to Early Embryonic Development

Contractor: University of Colorado Medical Center

Money Allocated: \$26,197 (FY 70), \$26,197 (FY 71), \$32,344 (FY 72)

Objectives: The objective of this investigation is to study RNA synthesis as a function of hormonal environment in both the embryo and the oviduct during the early stages of environment in the rabbit. Embryos, produced following superovulation, will be transferred to host females whose hormonal status will be altered by endocrine ablation, and estradiol and progesterone administration. The effects of these hormones on implantation, uridine incorporation into RNA, and the type of RNA synthesized will be determined in both the embryo and the oviduct.

Major Findings: Significant progress has been made in the following areas:
RNA synthesis: A wide variety of RNA species are synthesized in preimplantation embryos. Nucleic acid hybridization analyses indicate that at least the equivalent of 60,000-90,000 unique base sequences 1000 bases in length are transcribed in 6-12 day embryos. Ribosomal RNA is synthesized as early as the 16 cell stage and a burst of ribosome production occurs in the early blastocyst. If maintained in vitro embryos which have gone through a burst of ribosome production may become blocked or arrested, and ribosomes are not released to the cytoplasm. Ribosome release can be initiated in these embryos by the addition of a millipore-trapped component from uterine secretion.

Protein Synthesis: Approximately 100 electrophoretically separable proteins, 75-80 of which are labeled with methionine S³⁵, appear in the 84-hour blastocysts. The pattern of labeled proteins changes significantly during cleavage and blastocyst formation. Little if any ribosomal protein is synthesized until 60 hours of development.

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 studies of the hormonal control of intra-cellular processes.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Transfer of Maternal Macromolecules to Mammalian Eggs
Contractor: University of California Medical School
Money Allocated: \$29,000 (FY 70), \$39,480 (FY 71), \$53,075 (FY 72)

Objectives: The principal investigator will examine in detail the macromolecular transfer process in the preimplantation mammalian embryo, working primarily with oocytes and embryos of the mouse and rabbit. There are four general areas of investigation: (1) the composition and origin of mouse and rabbit blastocyst fluid. The blastocyst fluid constituents will be analyzed and the evacuated blastocysts placed in various media. By this means, it is hoped to learn more of the factors controlling the passage of macromolecules into blastocysts. Blastocysts will also be cultured in various antisera to see if they are detrimental to development. If such sera are found, females will be immunized passively or actively to see if development stops in vivo. (2) The composition and origin of rabbit oviductal fluid. Studies will be made of the macromolecular components of rabbit oviduct secretions and these will be compared with the constituents of blood of normal female rabbits. Antisera or analogs will be tested for adverse effects on embryonic development. (3) Electrophoretic studies will be conducted of the enzymes and antigens of the preimplantation mouse embryo. Attempts will be made to identify components which might be blocked to arrest development. (4) Attempts will be made to destroy selectively specific tertiary follicles in the mouse ovary by antifollicle sera.

Major Findings: (1) Twelve hour embryos showed a low level of spontaneous uptake of protein from the medium as indicated by grain count; this baseline level could not be decreased by DNP or theophylline but could be stimulated to near maximal levels by addition of insulin or cAMP to the medium. With the advent of cleavage, spontaneous uptake rose to a high at 48 hours; this high level could not be stimulated further but it could be inhibited. Seventy-two hour embryos had a moderate protein uptake but this spontaneous activity was not affected by treatment with stimulators or inhibitors. (2) It is concluded that: (a) protein uptake by mouse embryos occurs in culture; (b) uptake during cleavage is an active energy-utilizing process; (c) membrane transport is a factor in protein uptake; (d) metabolic characteristics of transfer change with embryo age.

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 on preimplantation mammalian embryos.

Proposed Course: Termination in favor of grant support.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Endocrine Control of Human Oocyte Maturation
Contractor: Peter Bent Brigham Hospital
Money Allocated: \$23,213 (FY 71) \$40,552 (FY 72)

Objectives: The objective of this contract is to explore a new approach to contraception involving the control of maturation of the human oocyte. Mammalian oocytes are in a resting stage of meiosis until shortly before ovulation. At this time meiosis resumes. The investigators wish to find out what hormones stimulate the resumption of meiosis in humans. They propose to administer these hormones before ovulation in order to induce a premature maturation of the oocyte. They anticipate that if this is done, when ovulation does occur the over-mature oocyte will be incapable of being fertilized.

Major Findings:

1. Changes in human oocyte morphology related to maturation appear to be correlated with follicular growth. The nuclear morphology of ova from small follicles 2-3 mm in diameter appears to be granular, while that of ova from 4-6 mm follicles is fibrous. Chromosomes of ova from 7-8 mm follicles are at diplotene. First metaphase chromosomes are observed in ova of follicles 9-10mm. Ova from 11-13 mm follicles contain the first polar body, which is perhaps the final stage of ovum development within the follicle before ovulation.

2. The liquor folliculi within which the ovum is undergoing maturation is rich in steroidal hormones. It is possible to demonstrate changing concentrations of steroids, progesterone, estrone and estradiol-17B in follicular fluid associated with the growth of the follicles. This is particularly striking during the late follicular phase of the menstrual cycle. Progesterone concentration is maximal when the follicles are about 7-9 mm in diameter. Estrogen concentration begins to rise in some follicles of 9 mm and gradually increases until they are 12-13mm in diameter. It appears that the antrum of the Graafian follicle is a reservoir of concentrated steroid hormones.

3. Ovum degeneration occurs frequently in follicles of "normal" ovary and also in pathology, such as, dermoid cyst and polycystic disease. Ova without normal chromosomal complement are prevalent in the follicles during late luteal phase and from older women approaching menopause. These ova usually have different structures within their nuclei. It is likely that these ova will not mature and represent initial stages of ovum atresia.

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 on new approaches to contraception involving the control of human oocyte maturation.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Fertilization of Mouse Ova In Vitro by Epididymal Sperm
Contractor: The Jackson Laboratory
Bar Harbor, Maine
Money Allocated: \$57,700 (FY 72) for two years

Objectives: The investigators have been highly successful in obtaining in vitro fertilization (95%) of mouse ova by epididymal sperm. In this contract proposal, it is proposed to define the chemical, physical, temporal, and genetic factors that have contributed to this success.

To the objective of obtaining fertilization in the shortest interval and with the minimum number of sperm, the investigators will study the effect of varying systematically, singly or in combination, ingredients of the medium and other factors of his in vitro system; among the important parameters to be studied are the necessity of subjecting epididymal sperm to capacitation, the effect of egg denudation, acrosome reaction, and patterns of attachment of the sperm head to the zona pellucida.

Major Findings:

1. The in vitro fertilization method employing epididymal spermatozoa has been standardized.
2. Preincubation of epididymal spermatozoa in Whitten's defined medium resulted in earlier ovum penetration than when unincubated spermatozoa were used. This indicates that "capacitation" in a simple medium can take place.
3. Glucose is required for "capacitation". Fructose can not be substituted for glucose.
4. Epididymal sperm produce CO₂ from glucose at twice the rate than from fructose. There is no evidence for a pentose shunt.
5. Washing of epididymal spermatozoa does not lower their fertilization ability.

Significance to Biomedical Research and Programs of the Institute: The work to be carried out on this project is directly related to the published purpose of the Contraceptive Development Branch to support research aimed at clarifying factors responsible for successful fertilization.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hormonal Requirement for Implantation in the Rhesus
Monkey (Macaca mulatta)
Contractor: University of Wisconsin
Money Allocated: \$78,335 (FY 73) for 2 years

Objectives: It is proposed to use the egg transfer technique to determine the requirement of ovarian hormones for survival of the blastocysts and implantation in the rhesus monkey. Blastocysts will be obtained from animals that have ovulated and mated naturally or have been treated with gonadotropins. The recipients will be ovariectomized two weeks before use and given daily injections of progesterone prior to egg transfer. If implantation does not occur, recipients will be given estrogen in addition to progesterone.

Significance to Biomedical Research and Program of the Institute: Knowledge concerning the hormonal requirements for implantation in the monkey is related to the stated goals of the program.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Steroid Hormone Receptors in Human Cervix
Contractor: The University of Texas Medical School at Houston
Money Allocated: \$70,706 (FY 73) for 2 years

Objectives: The investigator proposes to establish the existence of specific cytoplasmic estradiol and progesterone receptors in human cervical tissue. An attempt will be made to identify these binding proteins in 100,000 g supernate fractions from tissue homogenates and the individual receptors will be identified with regard to size, steroid specificity and binding parameters. The investigator will develop assays using dextran-coated charcoal to measure the number of binding sites per mg of protein and DNA. Once these assays are established, the number of cytoplasmic receptor sites for estradiol and progesterone in human endocervical and exocervical tissue at various stages of the menstrual cycle will be studied. Measurements of the plasma and uterine content of progesterone and estradiol at the time uteri are removed from receptor assay will be made. This should allow accurate calculation of binding parameters and total number of sites. Finally, the transfer of both estrogen and progesterone receptors to the cervical nuclei will be examined.

Significance to Biomedical Research and Program of the Institute: The characterization of hormone receptor complexes in human cervix is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new female contraceptives.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: New Methods of Fertility Regulation: Specific Binding of Luteinizing Hormone to Cells of the Corpus Luteum and Inhibition of this Process.
Contractor: University of Texas - Houston, Texas
Money Allocated: \$119,340 (FY 69) for three years; \$84,187 (FY 72) \$19,754 (FY 73)

Objectives: The objectives of this contract are twofold: (1) to prepare well characterized ovine luteinizing hormone subunits for distribution to other investigators via the Center for Population Research; (2) to develop a radioligand assay for testing compounds structurally related to luteinizing hormone (LH) for biological effects, e.g., inhibition of LH function.

Major Findings: (1) The isolation of LH from 200 pounds of ovine pituitary is approaching completion and approximately 2 grams of the purified LH has been processed to the subunit stage. Initial samples have been sent to sub-contractors for evaluation. A large pooled batch is being prepared for reference characterization of the material to be delivered to the Center for Population Research; (2) Using radioligand assays, and correlating results by bioassay (ovarian ascorbic acid depletion assay) testing has been completed on LH-amino group derivatives. The three types of amino group derivatives involve maleyl derivatives (converting the residues from positive to negative charges), acid amidination (a derivative that retains the positive charge) and carbamylation (which converts the residue to a neutral amino acid derivative). It is concluded that the lysine amino groups are not significantly involved in the binding of the alpha and beta subunits to each other, but are involved in the biological activity at the receptor site.

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 elucidate the mechanism of action of the pituitary hormones.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Development of Inhibitors to Estrogen Biosynthesis
Contractor: Worcester Foundation for Experimental Biology, Inc.
Money Allocated: \$43,634 (FY 70), \$43,634 (FY 71), \$74,745 (FY 72)

Objectives: The investigator seeks to develop inhibitors of estrogen biosynthesis, in particular specific inhibitors to the aromatase enzyme system which converts androstenedione to estrogens. Greater emphasis is to be placed on in vivo testing.

Major Findings: A systematic evaluation of the ability of steroids containing various functional groups to inhibit estrogen biosynthesis is being carried out. Androstenedione-4-¹⁴C is incubated with human placental microsomes in the presence and absence of a potential inhibitor and the exact amount of estrogen produced in each case is determined with the aid of tritiated estrogens as recovery markers.

The 19-chloro group makes androsta-4-ene-3, 17-dione inert as an estrogen precursor but does not interfere significantly with binding to the aromatizing enzyme. A variety of modifications to the 17 β -hydroxyl group decreased inhibition; the best groupings for C-17 remain the ketone, the 17 β -alcohol and its short chain esters.

The more promising inhibitors were tested as antifertility agents in rats. Four of the compounds were 90-100% effective in blocking fetal development at the 50 mg/kg level while one (4-acetoxy testosterone-17-acetate) was 100% effective at the 12 mg/kg level and one (4-hydroxytestosterone-17-formate) was 100% effective at the 2 mg/kg level. The first three effective ones, as a rule, partially delayed mating as an estrogen synthetase inhibitor might do while the last one completely blocked mating. This compound was subsequently found to have 50% of the androgenic activity of testosterone.

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 studies of the biosynthetic pathways for ovarian steroids with special attention being paid to possible points of inhibition for the purpose of fertility control.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Antifertility Testing of Prostanoid Acid Derivatives
Contractor: Ayerst Laboratories
Money Allocated: \$180,640 (FY 71); for two years

Objectives: The purpose of this project is to synthesize a series of 11-deoxy prostaglandin analogs and to test their antifertility effect in animals.

Major Findings: This contract involved the handling of large quantities of chemical intermediates in multistep syntheses in order to obtain a sufficient quantity of chemically pure material for biological evaluation. A total of 23 11-deoxy prostaglandin analogs and related compounds were synthesized and screened in the interruption of pregnancy test in hamsters. None of the compounds tested approached the activity of the natural prostaglandins in this assay. The best compound was found to have only 1/20th of the antifertility activity of PGF_{2α} in hamsters when administered subcutaneously. Some of the compounds were totally effective in the hamster (S.C.) but only at high doses (20 to 100 times that of PGF_{2α}) at which concomitant toxic and gastrointestinal side effects were frequently observed. None of the compounds were significantly active orally in the hamster and none were significantly active subcutaneously in the rat. The results indicate that elimination of oxygen in the 11-position of PGF_{1α} PGE₁ and their 13, 14-dihydro derivatives leads to considerable loss of 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 and evaluation of prostaglandin-like compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Antifertility Compounds
Contractor: Stanford Research Institute
Money Allocated: \$108,908 (FY 71), \$82,798 (FY 72), \$83,647 (FY 73)

Objectives: The purpose of this project is to synthesize a series of side-chain silicon-containing steroidal compounds and test their antifertility effect in animals.

Major Findings: The introduction of silicon into certain steroids markedly affects their biological response and causes a favorable separation of antifertility activity from estrogenic activity. One compound, 17 α -triethylsilylethynylestradiol, has shown a 4-6 fold increase in antifertility potency (postcoitally) in rats when compared with ethynyl estradiol but only shows 40%-75% of the estrogenic activity in rats. The same compound also shows a sufficiently favorable separation of activities in rabbits as to warrant preliminary studies in monkeys and this work is currently in progress. Several other trialkylsilyl analogs of ethynyl estradiol also show good separation of antifertility and estrogenic activity in rats. Further biological evaluation is in progress. Extension of this work to chemical modifications of testosterone has yielded compounds showing little or no androgenic 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 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Prostaglandin Analogs for Biological Studies
Contractor: University of Chicago
Money Allocated: \$46,675 (FY 71), \$20,943 (FY 72), \$48,502 (FY 73)

Objectives: The purpose of this project is to prepare prostaglandin-like compounds in chemically pure form and in sufficient quantities for evaluation as antifertility agents in animals.

Major Findings: A large number of prostaglandin analogs have been synthesized. The most interesting substances discovered are the acetylenic prostaglandin analogs, 13-dehydro-PGF_{2α} (I), ent-13-dehydro-15-epi-PGF_{2α} (II), and 13-dehydro-PGF_{3α} (III). All three compounds possess antifertility activity in the hamster, two of them (compounds I and III) from 2 to 5 times greater than that of PGF_{2α}, depending on the route of administration, subcutaneous or peroral. They probably act by a mechanism involving luteolysis since both I and II showed complete suppression of progesterone secretion in the ewe when infused into the ovarian artery at 25 μg/hr. All three substances failed to serve as substrates of placental prostaglandin 15-dehydrogenase and were in fact inhibitors of that enzyme. The most remarkable finding was that II possesses 1/500th of the activity of PGF_{2α} in that assay. Compound II also showed about 1/5th to 1/6th of the antifertility activity of PGF_{2α} in the hamster. It is concluded from the above that the receptors mediating the smooth muscle response on the one hand and the luteolytic effect on the other possess different structural specificity. This opens the possibility of discovering even greater selectivity among prostaglandin analogs.

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 prostaglandin 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesizing and Testing of Antifertility Compounds
Contractor: Collaborative Research, Inc.
Money Allocated: \$137,316 (FY 71) for two years

Objectives: It is the purpose of this project to synthesize a series of ergoline derivatives and to test their antifertility effect in mice and rats.

Major Findings: Seven ergoline derivatives were synthesized and tested for antifertility activity in rats. All of the compounds tested were significantly less active than the standard, D-6-methyl-8-cyanomethyl ergoline, in a pregnancy termination assay in Sprague-Dawley rats. The results indicate that further work on these ergoline derivatives is not warranted at this time.

Significance to Biomedical Research and Program of the Institute: The work undertaken to synthesize these polyheterocyclic compounds and test them for antifertility activity is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize Nitrogen-Containing Analogs of Prostaglandins
Contractor: University of New Hampshire
Money Allocated: \$27,418 (FY 72)

Objectives: The purpose of this project is to synthesize azaprostaglandin analogs and to test these compounds for antifertility activity.

Major Findings: During the contract period, six compounds, n-octanoylprolyl-5-aminovaleric acid, n-octanoylprolylglycylglycine, n-octanoyl-5-oxoprolyl-5-aminovaleric acid, n-octanoyl-5-oxoprolylglycylglycine, n-hexanoylglycyl-L-prolyl-5-aminovaleric acid methyl ester and n-hexanoylglycyl-L-prolyl-5-aminovaleric acid have been prepared and submitted for evaluation as anti-fertility agents in hamsters and rats. The syntheses of the 3-oxo- Δ^4 -proline and 5-oxo proline series are in progress and will be completed shortly.

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 prostaglandin 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Novel Compounds of Potential Anti-Fertility Activity
Contractor: University of California
Money Allocated: \$23,994 (FY 72)

Objectives: The purpose of this project is to synthesize cis- and trans-4,6-cyclopropane analogs of certain contraceptive steroids which, due to the unique geometrical conformations imposed by the introduction of the cyclopropane ring, could competitively inhibit progesterone at the receptor site and result in an antiprogestational compound. These compounds will be tested for antifertility activity.

Major Findings: The syntheses of four 4,6-cyclopropane steroid analogs have been completed during the past year: In the cis-series, 5 β -H, 17 β -acetoxy-4-6-androstane-3-one (1), 5 β -H, 4,6-cyclopropanoprogesterone-3,20-dione (2) and 5 β -H, 4-6-cyclopropano-19-norandrostane-3,17-dione (3) have been prepared. In the trans series, 5 α -H, 4-6-cyclopropanoprogesterone-3,20-dione (4) has been prepared. Evaluation of these compounds for antifertility activity and/or other hormonal activity is in progress.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Prostaglandin Analogs
Contractor: Research Triangle Institute
Money Allocated: \$51,089 (FY 72)

Objectives: The purpose of this project is to synthesize 10-oxa-prostaglandin analogs lacking the 11-oxygen function and to test these compounds for antifertility activity.

Major Findings: The total synthesis of the diastereoisomers of methyl 10-oxa-11-deoxy-15-ketoprostaglandin-E₁, was achieved by reaction of the corresponding aldehyde and dimethyl 2-oxoheptylphosonate. Its basis structure was firmly established by spectral analysis (ir, nmr and mass spec.). This substance is the immediate precursor of the methyl ester of 10-oxa-11-deoxy-15 (S)-15-methyl-prostaglandin-E₁, the synthesis of which is one of the objectives of this contract and which will be completed shortly.

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 prostaglandin-like compounds and prostaglandin antagonists 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis of Potential New Agents for the Control of Fertility
Contractor: University of Rhode Island
Money Allocated: \$26,660 (FY 72)

Objectives: The purpose of this project is to prepare 9(10→19) abeosteroids (substances related to 19-nor steroids but having an extra methylene group between ring A and C) and to test their antifertility and hormonal effects in rats.

Major Findings: It was found that the original synthetic scheme failed to produce the 9(10→19) abeosteroid. Although Dr. Abushanab has tried a variety of methods to prepare an important intermediate in his synthetic scheme, none of them have been successful. An alternate more promising scheme for the synthesis of 9(10→19) abeosteroid starting from 3-methoxy-9, 10-seco-1,3,5(10)-androstatriene-9,17-dione which was, in turn, obtained by fermentation of testosterone with species of pseudomonas has been devised.

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 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis and Testing of Silicon Substituted Steroids
as Potential Antifertility Compounds

Contractor: Research Triangle Institute

Money Allocated: \$30,312 (FY 72)

Objectives: The purpose of this project is to synthesize silicon ring-substituted steroids related to estradiol and to test these steroids for antifertility and estrogenic activity.

Major Findings: The total synthesis of DL-6-sila-6,6-dibenzyl-3-methoxy-17 β -hydroxyestra-1,3,5(10), 8-tetraene (an important intermediate in the synthesis of the final product) has been successfully accomplished by an application of the Torogov approach starting from silatetralone. The preparation of the final products, the silicon substituted steroids, 6,6-difluoro-6-silaestradiol 3-methyl ether and related 6,6-dialkyl derivatives, 6-silaestradiol and 6,6-difluoro-6-silaestradiol is in progress and will be completed shortly.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize and Furnish Steroidal Compounds with Anti-fertility Activity
Contractor: Laval University
Money Allocated: \$110,772 (FY 72) - for two years

Objectives: The purpose of this project is to synthesize 11-oxa, and 11-aza analogs of certain contraceptive drugs for biological evaluation of their effects on the reproductive system. Preliminary biological evaluation indicates that 11-oxaprogesterone has enhanced ovulation inhibiting activity and reduced progestational activity on subcutaneous administration when compared with progesterone.

Major Findings: 11-oxa-5 α -pregnane-3,20-dione has been successfully synthesized from methyl 3,9,20-trioxo-5 α -pregnan-12-oate. This compound is the immediate precursor of 11-oxaprogesterone and of most of the other 11-oxa steroids, the desired final products of this investigation. Two compounds, 11-oxa-5 α -pregnane-3,20-dione and 11-oxa-5 α -pregn-1-ene-3,20-dione, have been submitted for evaluation as competitive inhibitors in progesterone binding assays.

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 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize and Furnish Peptide Inhibitors of Luteinizing Hormone-Releasing Factor/Follicle Stimulating Hormone-Releasing Factor
Contractor: University of Wisconsin
Money Allocated: \$35,560 (FY 72)

Objectives: The purpose of this project is to synthesize competitive inhibitors of LH-RH/FSH-RH (LRF) by replacing certain hydrophobic amino acid residues in LRF by unusual amino acids to increase the hydrophobic properties of the amino acid normally present in that position while providing a minimal steric hindrance between the releasing hormone at that position and its receptor. Such competitive inhibitors are potential antifertility agents.

Major Findings: Eight of the nine target amino acids were prepared in racemic form; of these, three have been resolved using cleavage of the N α -chloroacetyl derivatives by carboxypeptidase A as the method for resolution. These resolved amino acids will be substituted for tryptophane in LRF.

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 LRF 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contract Program for Obtaining, Synthesizing, and
Testing Antifertility Compounds
Contractor: Hazleton Laboratories
Money Allocated: \$44,800 (FY 72)

Objectives: The purpose of this project is to study and confirm the anti-fertility activity of an antibiotic mixture in dogs and several species of small laboratory animals and to identify the active agent.

Major Findings: An antibiotic mixture fed to a large beagle breeding colony prevented pregnancy in 100% of the mated females. Conception rates rapidly returned to normal upon discontinuation of the medicated feed. The active antifertility agent has been shown not to be the antibiotic itself, but rather a mixture of quaternary ammonium salts which are currently being tested in rats and rabbits.

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 chemical agents 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize Novel Derivatives of Progesterone and Testosterone
Contractor: University of Pittsburgh
Money Allocated: \$66,779 (FY 72) \$26,851 (FY 73)

Objectives: The purpose of this project is to prepare unique chemical modifications of certain contraceptive steroids which, due to the introduction of unsaturation and the 10α (retrograde) configuration, will produce changes in the geometry of the steroidal conformation and thus might lead to selective changes in steroidal receptor attachments and therefore to interesting physiological consequences. These steroids will be tested for antifertility activity and other hormonal effects.

Major Findings: Four compounds, two in the 10β (normal) unsaturated testosterone and androstenedione series, and two in the 10α (retrograde) series were synthesized and found to be devoid of significant androgenic activity. Six other variously unsaturated steroids (normal and retrograde) in the progesterone and ethisterone series have also been synthesized and biological testing is in progress.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Production of New Type of Contraceptives Based on Derivatives of Luteinizing Hormone-Releasing Hormone (LH-RH)

Contractor: Tulane University

Money Allocated: \$51,716 (FY 72) \$71,000 (FY 73)

Objectives: The purpose of this project is to prepare and evaluate biologically decapeptide analogs of LH-RH which involve modifications of various amino acid residues in order to produce an inhibitor of LH-RH (LRF).

Major Findings: Twenty LRF analogs were synthesized and tested. It is concluded that the active site in LRF is in the N-terminal tripeptide part of the molecule and that the remainder of the molecule is involved in transport and binding. The investigator presents data to show, for the first time, the synthesis of an LRF antagonist which is active in vivo. Future work will establish the potency of this LRF inhibitor.

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 LRF 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Structure-Activity Studies of Small Peptides which Release LH and FSH.
Contractor: Tulane University
Money Allocated: \$59,171 (FY 72), \$110,570 (FY 73)

Objectives: The purpose of this project is to biologically evaluate new synthetic polypeptides, which may affect the reproductive system, based upon structural modifications of (1) the decapeptide, LH-RH/FSH-RH (LRF) and (2) a synthetic tetrapeptide which was found to release LH, but not FSH.

Major Findings: Twenty-eight peptide analogs of LRF were synthesized and evaluated biologically. One compound, a tripeptide, p-Glu-His-Trp was found to be effective as a partial antagonist of LRF in the release of LH, but not FSH, at rather high doses in vitro and in vivo. Much structure-function information was also gained. Thus, analogs involving [Arg]⁸LH-RH point to the significance of the guanidino group for activity and its participation in an ionic mechanism for release and potency related to a basic moiety at this position. Other derivatives suggest the importance of the proximity of the protonated Arg relative to the receptor. In addition, the importance of the terminal amide function for hormonal potency was established.

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 LRF 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Gonadotropin Releasing Hormone (Gn-RH) to Establish Antifertility and Other Endocrine Activity
Contractor: Abbott Laboratories
Money Allocated: \$125,300 (FY 72) \$7,750 (FY 73)

Objectives: The purpose of the project is to (1) develop methods for the large-scale synthesis of the gonadotropin releasing hormone, (2) develop and evaluate chemical and biological tests to establish the integrity of the product and the reproducibility of different batches, (3) subject the synthetic hormone to physiological, pharmacological and toxicological testing in laboratory animals, (4) make at least ten grams of the synthetic hormone available to the CDB, (5) prepare a brochure of information on the hormone for use by clinical investigators, and (6) apply for an IND.

Major Findings: All of the goals stated in the objectives have been achieved. Ten grams of Gn-RH (LRF) have been made available to the CDB for distribution to qualified investigators. The biological work necessary for an IND has been completed. The IND for LRF was submitted in November 1972 and, therefore, has been in force since about January 1, 1973. This IND will enable Abbott to conduct clinical trials with LRF to establish its safety and efficacy in inducing ovulation in infertile women and its safety and efficacy as a contraceptive agent.

Significance to Biomedical Research and Program of the Institute: The work undertaken to synthesize Gn-RH and conduct extensive biological studies in laboratory animals is an integral part of the contraceptive development program.

Proposed Course: Termination. All of the contract objectives have been achieved.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Study of the Factors that Control Diffusion Rates in Controlled-Release Contraceptive Systems.
Contractor: Southern Research Institute
Money Allocated: \$84,101 (FY 72, 15 months)

Objectives: This proposal aims to quantify individual factors affecting diffusion rate of steroidal contraceptive drugs through polymeric materials (membranes). Diffusion rate is the combined effect of the solubility of the drug in the polymer, the diffusivity of the drug in the polymer and the partition coefficient between the drug in the polymer and the solution into which it is diffusing.

The study will show the difference in the transport rates between the various drugs and polymers, the influence of the above physical constants on the rate and also indicate whether dissolution or diffusion of the drug in the polymer is rate limiting. Further, an attempt will be made to establish the relationship between the drug structure and the structure of the polymer in the diffusion process.

Major Findings: In order to determine quantitative values for the factors that affect diffusion of contraceptive steroids through polymeric materials, further refinements of the experimental techniques have been made. Equilibrium solubilities at 37°C were measured for four selected steroids (norprogesterone, progesterone, androstenedione, and estrone) in crosslinked polydimethylsiloxane (PDMS), polytrifluoropropylmethylsiloxane fluid (FS1265, Dow Corning), and water. Partition coefficients of each of the four steroids between PDMS and the fluids were determined, and diffusion coefficients for each steroid through PDMS were also measured.

Significance to Biomedical Research and Program of the Institute: The study will provide much needed information on the type of polymer to be used with a given steroid to either inhibit or enhance the rate of drug release and is therefore directly relevant to the objectives of the CDB.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Novel Steroidal Gestagens
Contractor: University of Illinois at Chicago Circle
Money Allocated: \$30,033 (FY 73)

Objectives: The purpose of this project is to synthesize ring B bicyclo [2.2.0] hexene derivatives of progesterone and ring A/B cyclopene derivatives of progesterone, which may have the ability to compete with progesterone for the receptor protein and thereby act as antiprogestational agents. These compounds will be tested for antifertility 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: 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.

July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis of Novel Steroids with Potential
Antifertility Activity
Contractor: Temple University
Money Allocated: \$26,990 (FY 73)

Objectives: The purpose of this project is to synthesize novel spiro steroids, ring A bicycles [3.2.0] heptanes, and cyclopropyl steroids. These compounds will be tested for antifertility 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: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptive and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Ring A Bi-and Tricyclo Steroids with Potential Antifertility Activity

Contractor: Stanford Research Institute

Money Allocated: \$55,543 (FY 73)

Objectives: The purpose of this project is to synthesize steroids containing bridged bi-and tricyclo systems in ring-A, which will result in alteration of the binding of the steroid to the receptor protein. These compounds will be tested for 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 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Novel Steroids for Fertility and Population Control

Contractor: University of South Carolina

Money Allocated: \$71,963 (FY 73) for two years

Objectives: The purpose of this project is to synthesize pentacyclic analogs of known effective contraceptive steroids which, due, to the unique conformational changes imposed by the introduction of bridged rings in either ring A or ring D, could competitively inhibit progesterone at the receptor site and result in an antiprogestational compound. These compounds will be tested for 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 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Steroidal Compounds Possessing a Bridged Bicyclic A-ring with Potential Antifertility Activity
Contractor: Research Triangle Institute
Money Allocated: \$ 26,860 (FY 73)

Objectives: The purpose of this project is to synthesize bicyclo [2.2.0] hexene derivatives of steroids possessing the zero bridge between carbon atoms 1 and 4 and test these compounds for antifertility 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: 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of 12 α -Methyl Prostaglandins Related to
PGF_{2 α}
Contractor: University of Pittsburgh
Money Allocated: \$72,736 (FY 73) for two years

Objectives: The purpose of this project is to synthesize 12 α -methyl PGF_{2 α} , and 12 α -methyl-15 β -methyl-PGF_{2 α} and to test these compounds for antifertility activity. The extra methyl group may serve to block one of the pathways of elimination of the 11 α -hydroxy group in the prostaglandins. This novel structural modifications would be important to study insofar as structure-activity relationships of the prostaglandins are concerned.

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 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Potential Inhibitors of
Luteinizing Hormone Releasing Factor

Contractor: Indiana University Foundation

Money Allocated: \$98,462 (FY 73) for two years

Objectives: The purpose of this project is to synthesize potential inhibitors of luteinizing hormone releasing hormone (LRF) in which unnatural and unusual amino acids are to be substituted for certain natural amino acids in LRF.

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 potential inhibitors of LRF 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Non-Steroidal Nitroheterocycles with Potential Antifertility Activity
Contractor: Rensselaer Polytechnic Institute
Money Allocated: \$32,458 (FY 73)

Objectives: The purpose of this project is to synthesize several series of five-membered heterocycles containing electron withdrawing substituents for evaluation as possible antifertility agents.

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 non-steroidal heterocycles for male 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Novel Steroids as Potential Antifertility Agents
Contractor: The John C. Sheehan Institute for Research, Inc.
Money Allocated: \$27,072 (FY 73)

Objectives: The purpose of this project is to synthesize a new class of steroidal compounds with a cyclopentane ring fused at C₄ and C₆ as potential antifertility agents.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Irreversible Inhibitors of
Uterine Estradiol Binding Factors
Contractor: Research Triangle Institute
Research Triangle Park, North Carolina
Money Allocated: \$64,490 (FY 73)

Objectives: The objective of this project is to synthesize and test a variety of derivatives of estradiol which would have the ability to bind to estrogen receptor sites in the target tissue. These derivatives would contain bridging groups terminating in an arylsulfonyl fluoride. This reactive group could possibly form a covalent linkage to the receptor and thus, would elicit either an estrogenic or antiestrogenic condition in the target tissue. Such compounds may be useful as contraceptive agents.

Significance to Biomedical Research and Programs of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to develop 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study of Compounds for Inhibition of Spermatogenesis
Contractor: The Childrens Hospital of Philadelphia
Philadelphia, Pennsylvania
Money Allocated: \$23,843 (FY 73)

Objectives: Certain steroidal compounds have been found, in vitro, to selectively block testicular conversion of prenenolone to testosterone, but not adrenal conversion to corticosterone. It is the objective of this contract to study these compounds in vivo for testicular pregnenolone inhibition and subsequent blockage of spermatogenesis.

Significance to Biomedical Research and Programs of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch of developing new contraceptive agents for use by the male population.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Device and Technique for Blocking Fallopian Tubes
Contractor: Franklin Institute Research Laboratories
Colloids and Polymers Laboratory
Money Allocated: \$78,866 (FY 71), \$124,620 (FY 72), 15 months

Objectives: The purpose of this project is to develop a new device and application technique for accomplishing the blocking of fallopian tubes with medical grade silicone rubber for reversible sterilization. The device is to be evaluated in rabbits for installation, compatibility, antifertility efficacy and reversibility and in monkeys for safety.

Major Findings: Continuation of rabbit studies shows that the devices will remain in place for at least 50 days with no adverse tissue reaction. The silastic plugs appear to offer an effective barrier to the ascent of spermatozoa and the descent of ova and yet they can generally be removed from the oviducts in toto. While the investigators have obtained preliminary evidence for the reversibility of this system in rabbits (pregnancies following device removal), this work must be extended and confirmed.

Chronic studies in rabbits and monkeys are in progress to ascertain the safety of this procedure. The extrusion of the uncured elastomer into the oviducts of monkeys via the uterine ostium has proven to be extremely difficult because of the anatomical configuration of the intramural portion of the fallopian tubes. Efficacy studies therefore, will probably be deferred to a carefully controlled series of human clinical trials should the animal safety studies justify continuation of this project.

Significance to Biomedical Research and Program of the Institute: The development of a device for accomplishing the blocking of fallopian tubes for female sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Grafted Hydrogels in Contraceptive Application
Contractor: Franklin Institute Research Laboratories
Money Allocated: \$108,356 (FY 71), \$16,704 (FY 72) \$7,297 (FY 73)

Objectives: The purpose of this project is to prepare hydrogel-coated intra-uterine devices and hydrogel-coated intra-uterine devices impregnated with copper and to study these devices in rabbits to determine their tolerance and effectiveness as antifertility devices. Conventional IUD's have already shown promise and it is anticipated that there will be greater tolerance and less medical problems with a hydrogel-coated IUD than with the conventional IUD. A series of studies will be performed with complete, scaled devices on the rabbit initially and then based on these animal results, in the human.

Major Findings: Rod-shaped IUD's were constructed by covalently grafting a 0.5mm thick coating of water-swallowable poly (2-hydroxyethylmethacrylate) hydrogel onto Ba SO₄ - pigmented polyethylene rods and copper wound polyethylene rods. Uncoated polyethylene IUD's and hydrogel-coated polyethylene IUD's with and without copper were inserted into the uterine horns of rabbits. Retention of both hydrogel-coated devices over a two-month period was about 50% while all of the uncoated devices were expelled. No pregnancies were observed in animals bearing the copper-containing hydrogel-grafted IUD while about 40% of the rabbits bearing the hydrogel-coated device alone bore young. No evidence for either copper or hydrogel fragmentation was found. The hydrogel coating did not appear to alter the rate of in vivo copper release compound with reported release rates from copper "7" and "T" type IUD's.

Significance to Biomedical Research and Program of the Institute: The development of hydrogel-coated IUD's for female sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This contract was terminated in FY-73, the original objectives in animals having been accomplished.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an Intra-uterine Sterilization System
for the Female
Contractor: IIT Research Institute
Medical Engineering Center
Money Allocated: \$247,977 (FY 71), \$519,465 (FY 72)

Objectives: The purpose of this project is to develop a prototype system for intra-uterally sterilizing the female by occlusion of the utero-tubal junction. The occlusive devices would be introduced through the cervical os, and be guided into the tubal location by a specially designed steerable hysteroscope. This would simplify the procedure for the physician or specially trained paramedical personnel, in a non-operative procedure. The design and development effort will be supported with biological and animal experiments and will lead eventually to clinical use in humans.

Major Findings: The contractor has fabricated several prototype steerable hysteroscopes which have been successfully employed in visualizing the uterine ostia of the oviducts in extirpated human uteri as well as in anesthetized baboons under actual operating room conditions. A sliding cervical seal is an integral part of the instrument and permits the expansion or flushing of the uterine cavity without leakage through inlet and outlet ports located in the hysteroscope tip. Pulsing the uterus by alternate expansion and deflation aids in the rapid location of the tubal ostia. The instrument will facilitate the placement of tubal occlusive devices in the intramural portion of the oviduct and will provide a convenient means of performing sterilization by electro-cautery. Improved fiberoptic bundle resolution, depth of field and increased range tip steerability are currently in the design phase and will be incorporated into the next generation of hysteroscopes. An improved dual viewing eyepiece has been designed and is now being fabricated.

Several prototype occlusive devices have been designed and are being fabricated of various materials including medical grade silicone rubber impregnated with ceramic particles to encourage device retention and tissue ingrowth. Tubal gauging for the fabrication of devices of appropriate size has been obtained on extirpated human uteri. The investigators have also found that soft-tipped devices can negotiate the tortuous path of the utero tubal junction more readily than rigid devices and can be implanted to a greater depth than rigid probes. Mechanical aids for device retention within the oviduct are being evaluated.

Implantation of prototype devices in baboons under actual operating room conditions will commence shortly and evaluation of the steerable hysteroscope in patients scheduled for hysterectomy will be undertaken within the next few months.

Significance to Biomedical Research and Program of the Institute: The development of a transcervical approach to female sterilization eliminating the ~~need~~ necessity for abdominal surgery is highly relevant to the mission of the CDB.

Proposed Course: This is expected to be a continuing contractual efforts leading to the development of a simple nonsurgical means of female sterilization. Clinical studies are already planned and will be initiated within the next few months.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Research and Development of Contraceptive Devices and Materials. Development of New Sterilization Techniques.
Contractor: Abcor, Inc., Cambridge, Massachusetts
Money Allocated: \$345,000 (FY 71) for two years

Objectives: The purpose of this project is to develop (1) a reversible intravasal plug with a hollow center into which a removable pin can be inserted, and (2) an absorbable intravasal stent that would facilitate reanastomosis of the cut end of the vas following vasectomy. A variety of materials will be evaluated for the devices and the effectiveness of these new sterilization techniques will be studied in the guinea pig.

Major Findings: The selection of materials and the fabrication of the Reversible Intravasal Plug have been completed. Preliminary results in guinea pigs have shown satisfactory adhesion and ingrowth of the vas luminal epithelium into the outer fiber flock of the vasal plug. Most of the animals had complete azoospermia after implantation of the plugs.

Various techniques have been developed for the preparation of stents. These have been tested in animals, and preliminary results show that they are well tolerated. Evidence of recanalization of the vas lumen was obtained by the presence of sperm in the distal segment of the vas after implantation of the stent. This was also confirmed by sperm counts.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the utility of these contraceptive devices as the development of devices for male sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Design and Development of Implantable Contraceptive
Devices for Use in the Male

Contractor: IIT Research Institute

Money Allocated: \$150,000 (FY 71) \$194,476 (FY72)

Objectives: The purpose of this project is to conduct a feasibility study on the design of devices suitable for intravasal implantation for the purpose of reversibly occluding the vas. Biological studies will be conducted in animals to determine the reversibility and tissue compatibility of the devices.

Major Findings: After numerous experiments with mechanical valve devices which have been poorly tolerated in the vas deferens, these investigators have evolved an all soft device, which is compliant and appears capable of conducting sperm for extended periods. New approaches to tissue ingrowth materials have also evolved, which have a soft flexible quality compatible with the new devices.

The "soft" occlusive devices have been implanted in 15 dogs. Fourteen of these dogs have produced motile sperm following implantation. In two experiments, the soft device was employed to anastomose the vas deferens of dogs which had been vasectomized approximately 3 months earlier. Viable sperm transport was rapidly restored in one of two dogs, providing further encouragement for the "soft" design approach in device development.

Significance to Biomedical Research and Program of the Institute: The design and development of implantable devices for male sterilization is directly relevant to the purposes of the CDB activities.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Use of Reversible Vasectomy Device in the Guinea Pig
Contractor: New York Medical College
Money Allocated: \$70,000 (FY 71), \$59,933 (FY 72)

Objectives: The purpose of this project is to ascertain the usefulness of a reversible intravasal valve as a contraceptive device in the male guinea pig. Because of the confidential nature of this aspect of the CDB program, disclosure of the nature of the device cannot be made until release of information will be allowed.

Major Findings: Of the original 10 animals implanted with the Bionyx control (reversible vasectomy device) in the closed (or "off") position, 6 "leaked" small numbers of sperm. These were found to be operative failures--incorrect appositioning and immobilization of the device. An additional 13 animals so implanted have remained aspermic.

Six of the original 10 animals implanted with the device in the open (or "on") position remain in the study and continue to put through sperm in their once-a-week collection. Ten additional animals have been implanted, but only 5 have thus far passed sperm.

Tissue ingrowth has presented no problems; adverse tissue reactions or corrosion of the valve have not been observed.

Results are encouraging, but what needs to be evaluated is the fertilizability of sperm passing through such devices.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the usefulness of this contraceptive device, as the development of devices for male sterilization is directly relevant to the purposes of the CDB activities.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Resersible Sterilization by an Intravasal Spermicidal Device

Contractor: Southwest Foundation for Research and Education

Money Allocated: \$41,678 (FY 72)

Objectives: The objective of this research is to develop an intravasal device which is spermicidal in its effect. This device is intended to produce reversible sterilization of the male without blocking sperm passage through the male tract. Such an approach may eliminate the potential immunological side effects of those devices which produce sterilization by temporary or permanent blockage of the vas deferens.

Major Findings: Methods for collection and evaluation of guinea pig semen have been worked out. Procedures have been established for anesthesia and surgical placement of the devices. The diameter and design of the devices have been established. A method of reducing the incidence and severity of sperm granuloma has been developed using a tissue adhesive to close the incision on the vas deferens. Catheter devices have been successfully installed in thirteen animals. Five animals have 1.5 cm devices, five have 1.0 cm devices and three have 0.5 cm devices installed bilaterally in their vasa deferens. Although there is a slight trend toward reduction of % motility following installation of the devices, there is insufficient data available at this time to draw conclusions. It does appear rather certain however, that the marked reduction of sperm quality which was observed in the pilot study in the baboon is not observed in this study.

Significance to Biomedical Research and Program of the Institute: Development of new techniques and devices for production of reversible sterilization is one of the stated goals of the program.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development and Evaluation of a Reversible Vasectomy
Prosthesis
Contractor: University of Missouri
Money Allocated: \$38,290 (FY 72)

Objectives: This research is directed toward the development of a reversible vasectomy prosthesis, as well as parallel studies of the effects of the prosthesis and of temporary vas occlusion on gametogenesis.

Particular attention will be paid to the effect of the prosthesis on the tissues of the vas deferens over a prolonged period of time. Tissues will be evaluated by histopathologic studies.

The effects of temporary occlusion of the vas on gametogenesis will be evaluated following semen collection and evaluations. The testes will later be studied histologically and gametogenesis further evaluated.

In order to accomplish these studies a vasectomy prosthesis of silastic has been designed. This prosthesis is inserted into the lumen of the vas and the musculature of the vas sutured to a cuff on the prosthesis. Occlusion is obtained by folding the tube on itself and ligating it. Sperm passage is obtained by removing the ligature and allowing the tube to become patent.

The initial studies will focus on development of the tube design, surgical implantation of the tube, effects on the vas deferens for short periods, and the effects of occlusion for 5-6 month periods on gametogenesis.

Later studies will evaluate the effects of the tube on the vas deferens and gametogenesis for longer periods of time.

Major Findings: Measurements of the bull vas deferens and supporting anatomical structures indicate the bull is very similar to man in vas size and character, thus providing an excellent experimental model in vasectomy studies.

Bilateral vas prosthesis have been implanted into 12 bulls. Semen has been recovered at various times in four of six bulls implanted with the prosthesis in the "open" position, indicating the vas can indeed be kept patent for a period of time. There appears to be no external signs of pathology in these animals, although microscopic studies have not as yet been completed. Morphological evaluation of spermatozoa is presently being completed.

Significance to Biomedical Research and Program of the Institute: Study of reversible vasectomy devices and of their effects on reproductive function is the goal of the program aimed toward the development of new contraceptive technology.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Proposal for the Study of a Long Acting Injectable
Progesterone Contraceptive
Contractor: Brookdale Hospital Center
Money Allocated: \$118,614 (FY 71); for two years

Objectives: The purpose of this project is to prepare and evaluate two progesterone contraceptive drug release systems to determine the possibility of designing a progesterone preparation which would release relatively constant amounts of the agent sufficient for contraceptive purposes for at least three months. The two systems involve steroid-lipid pellets and collagen implants. Steroid lipid pellets will be prepared by processing eutectic mixtures of progesterone with various lipids, primarily sterols, in an apparatus permitting preparation of pellets of any desired diameter. Collagen implants will be made by binding progesterone to collagen solution and inducing varying degrees of crosslinking. In vitro and in vivo studies in hamsters and rabbits will be undertaken to determine the rate of dissolution of these different matrices and the rate at which progesterone will be liberated.

Major Findings: The contractor has studied the in vivo release rates of a eutectic mixture of progesterone and cholesterol in rats, hamsters and rabbits. Results in rats and hamsters were disappointing, in part due to the difficulty of obtaining sufficient blood samples from these species over long periods for adequate hormone determinations.

Weekly blood and urine samples and bi-or tri weekly fecal samples were monitored for progesterone levels following implantation of ^{14}C - ^4C -progesterone/cholesterol pellets in the hind leg of rabbits. No problems were encountered with the implantation site (walling off from the surrounding tissue, inflammatory reaction, etc.) except where aseptic procedures were not followed. Constant plasma levels of progesterone could be maintained for about 3 months after which the concentration rapidly declined. About 90% of the administered material could be accounted for. It appears that sufficient plasma levels of progesterone could be maintained by this method in women to provide contraceptive protection.

Significance to Biomedical Research and Program of the Institute: The development of progesterone drug release systems for female contraceptive measures is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: Since the original objectives of this contract program have been realized, no additional funding is anticipated.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Feasibility Study of Intrauterine Microcapsules as a Prolonged Drug Release Form
Contractor: Battelle Memorial Institute
Money Allocated: \$61,200 (FY 71), \$86,240 (FY 72)

Objectives: The purpose of this project is to investigate the feasibility of employing a microencapsulated drug dose form as an intrauterine antifertility measure. Feasibility will center largely on maintaining microcapsules within the uterus. The drug delivery system would involve the microencapsulation of progestagens, spermatocidal agents, or cervical mucosal thickeners. After microencapsulation of the designed drug agent, these microcapsules would be inserted in the uterus for a controlled and prolonged release of the drug.

Major Findings: The contractor has concentrated on determining the distribution of various sizes of tissue microcapsules and microspheres within the reproductive tract of rabbits following intrauterine placement. Microspheres $15 \pm 5\mu$ or $50 \pm 10\mu$ in diameter were found in both the uterus and fallopian tubes one week following placement in the uterus while microspheres $115 \pm 10\mu$ in diameter were confined to uterus. In contrast, microcapsules of all three sizes were found equally distributed between uterus and oviducts. Ascension of the 115μ microcapsules through the uterotubal junction is probably due to their flexible membrane, a feature not shared by the rigid microspheres. Mating did not influence the migration of $15 \pm 5\mu$ diameter microspheres.

Of special significance was the observation that the $15 \pm 5\mu$ diameter microspheres migrated into all segments of the uterus and oviducts following insertion into the vagina and then mating.

These studies demonstrate the need for distribution studies prior to the development of a drug release system utilizing small particles introduced into the reproductive tract.

Work is in progress to determine the rate of loss of microspheres and microcapsules from the vaginal orifice and the degree of escape from the oviducts into the peritoneal cavity.

Significance to Biomedical Research and Program of the Institute: It would be important to conduct a feasibility study of intrauterine microcapsules and microspheres as a prolonged drug release form as the development of intrauterine antifertility measures is directly relevant to the purposes of the CDB activities.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biological Testing Facility for the Evaluation of New
Antifertility Drugs and Devices
Contractor: Mason Research Institute
Money Allocated: \$844,057 (FY 72) 30 months

Objectives: The objective of this contract program is the establishment of a testing facility capable of rapidly exploiting potential leads in the control of fertility. Using a broad spectrum of antifertility and endocrine screens and assays, the biological profiles of a number of standard drugs will be established against which experimental compounds will be evaluated. Sources of compounds for testing include synthetic programs supported by the CDB and numerous private, public and governmental laboratories throughout the world. The staff of the CDB will review all compounds submitted for evaluation to determine the manner in which each drug will be studied.

The testing of compounds in a single laboratory using standard protocols will permit a more meaningful comparison of experimental drugs and their potential for clinical utility.

Major Findings: Baseline studies, including endocrine profiles and control data, have been completed on 16 biological standards. This information will provide the means for a direct comparison of the activities of new compounds. Fifty-four experimental drugs have been submitted for screening, assay and/or mode of action studies. Several promising leads have been identified and one such drug is now being evaluated in subhuman primates.

Several new test procedures are being developed jointly by the Contractor and CDB staff. These include mode of action studies on postovulatory contraceptive agents and in vitro tests for drugs affecting uterine and oviductal motility.

Procedures have also been developed to safeguard proprietary interests in both compounds and devices.

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 stated goals.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Implantable Bio-Absorbable Capsules as Slow-Release
Contraceptive Drug Delivery Systems
Contractor: Abcor Inc.
Money Allocated: \$254,856 (FY 73 for two years)

Objectives: The contractor proposed to formulate a biodegradable sustained release drug delivery system consisting of microcapsules containing progesterone. The system will be evaluated for its ability to alter the characteristics of cervical mucus and thus produce its contraceptive effect. The microcapsules will be injected directly into cervical tissue and it is hoped that such a delivery system will not interfere with menstrual cyclicity and avoid other systemic side effects. It can also be utilized for the delivery of other contraceptive drugs where sustained long term delivery of the drug is desired.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development and Testing of an Implantable Contraceptive
Delivery System
Contractor: Dynatech R/D Company
Money Allocated: \$245,972 (FY 73 for 2 years)

Objectives: It is proposed to develop and test new implantable drug delivery, systems based on synthetic polymers which slowly release the incorporated drug. The implantable material will consist of tissue compatible hydrolyzable polymers material such as polyglycolic acid, polylactic acid, as copolymers in which the synthetic progestin d-norgestrel will be dispersed. As the polymers undergoes slow hydrolysis, the physically entrapped drug will be released. At the end of a planned period of time both the drug and polymer material are absorbed by the surrounding tissues.

Significance to Biomedical Research and Program of the Institute: The development of improved 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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Study of Biodegradable Polymers for the Sustained
Delivery of Contraceptive Drugs
Contractor: Research Triangle Institute
Money Allocated: \$299,994 (FY 73 for two years)

Objectives: It is proposed to develop a biodegradable polymer drug delivery system for male and female fertility regulation. The release of the appropriate drug will be dependent on the rate of polymer by hydrolysis and will be independent of diffusion rate.

The specific polymers which will be evaluated are the aliphatic polyesters. They will be selected on basis of tissue compatibility, biodegradability, synthetic availability, and structured variability. The performance of the delivery system will be evaluated by a number of different tests.

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
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Clinical Evaluation of a Fluid-Filled Intrauterine Device
Contractor: Tecna Corporation and University of California
Money Allocated: \$51,980 (FY 73)

Objectives: The major objective of this contract is to clinically evaluate a new fluid-filled intrauterine device (IUD). Preliminary data indicate that the device is well tolerated and provides high contraceptive efficacy. The IUD will be inserted into 100 women for an anticipated 1050 women months. This represents a minimal sample upon which statistically valid conclusions can be made.

Significance to Biomedical Research and Program of the Institute: Development of new contraceptive devices is directly related to the published purpose of the Contraceptive Development Branch.

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

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Intrauterine Release of Estriol for Contraception
Contractor: Michael Reese Hospital
Money Allocated: \$133,000 (FY 73)

Objectives: The objective of this contract is to investigate the local contraceptive action of the hormone estriol. The estriol will be delivered to the uterine cavity by means of an intrauterine device which will be composed of polymeric membrane which can control drug release. The devices will be evaluated initially in rabbits and subsequently in baboons. If local action of the released estriol can be demonstrated a clinical trial will be initiated. The release of small quantities of estriol directly into the uterine cavity should circumvent undesirable systemic effects.

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 is expected to be a continuing contractual effort leading to the development of improved IUD's.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Androgen Polydimethyl-siloxane Implants: Metabolic Fate of Testosterone and Contraceptive Efficacy.

Contractor: The Johns Hopkins University

Money Allocated: \$64,000 (FY 73)

Objectives: The investigator proposes to study the effect of testosterone released by PDS implants in different amounts on spermatogenesis, testosterone production rates, plasma testosterone and ICSH concentrations, and accessory sex organ size and morphology in monkeys. The objective is to compare the efficacy of PDS testosterone implants to create azoospermia without elevating plasma testosterone and causing accessory sex organ hypertrophy.

Significance to Biomedical Research and Program of the Institute: These experiments should provide the rationale for subsequent experiments testing the efficacy of steroid-filled PDS implants in the human male. Development of new techniques for the control of fertility in male is one of the stated goals of the program.

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

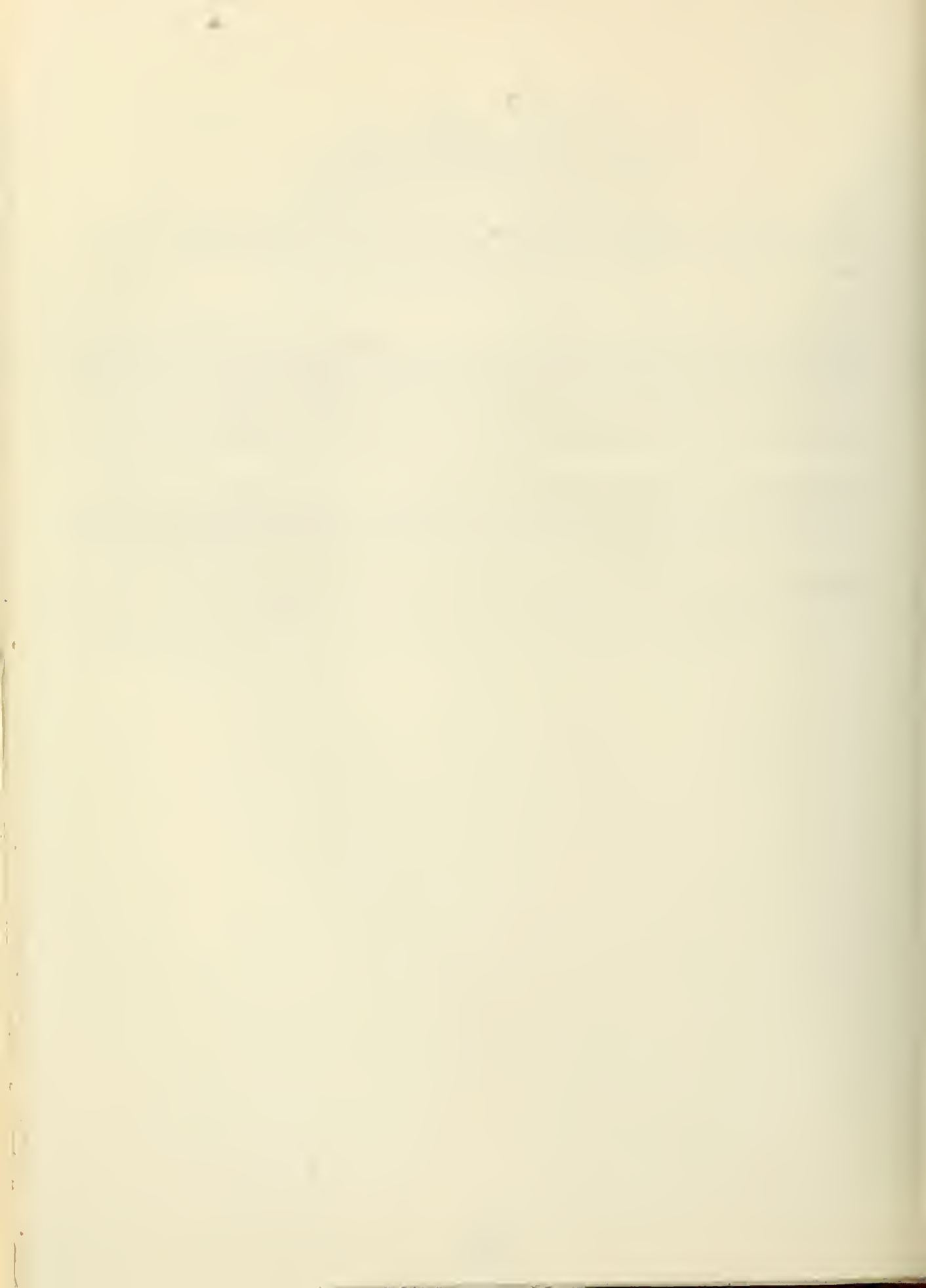
NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Systems Approach to Vaginal Delivery of Contraceptive
Drugs - Methodology and Mechanism for Absorption
Contractor: University of Michigan
Money Allocated: \$126,361 (FY 73 for 2 years)

Objectives: The proposed studies are aimed at developing suitable methodology in an appropriate animal system and obtaining firm, baseline data on vaginal absorption of model compounds and contraceptive steroids. The data generated will be used in delineating the general barrier properties of the vaginal mucosa and in developing quantitative, integrated models describing both the release of drugs from vaginal devices and subsequent drug absorption.

Significance to Biomedical Research and Program of the Institute: The study of drug absorption from the vagina can further our knowledge on the characteristics of drug absorption from this local site. The work is directly related to the published purpose 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
July 1, 1972 through June 30, 1973

Population and Reproduction Grants Branch
Center for Population Research

The Population and Reproduction Grants Branch within the Center for Population Research has the responsibility of administering basic research and training grants in the biological, clinical, biomedical and social (behavioral) areas relating to reproduction and population phenomena and aimed at gaining a fuller understanding of normal and abnormal reproductive and early developmental processes in animals and human beings. It is concerned with all aspects of population fertility and infertility, particularly studies likely to yield new information and methods for the development of safer, more acceptable contraceptives, as well as with the training of young investigators to accelerate and broaden the research effort.

As of March, 1973, grant funding of \$22,197,000 supports 347 research grants (\$18,845,000), 28 training grants (\$2,539,000), 16 fellowships (\$140,000), and 30 research career development awards (\$672,000). This funds research and training in the areas of reproductive biology and contraceptive development, animal behavior and ecology, and the behavioral-social aspects of population research. No new training awards may be anticipated after January 29, 1973, so the current funding after this date represents merely continuation of previous commitments, with no new trainees being added to the programs.

The mission of the Branch has been broadened with the development of the Population Research Center Award Program, which currently is funding seven such Centers. Eight fully functional Centers are anticipated within another year. The program was initiated on July 1, 1970, to provide core support and new program development for non-profit organizations conducting research and training relevant to population problems. This program represents a commitment by the National Institutes of Health to the long-term support of institutions with demonstrated ability to conduct research and research training in areas that seem likely to hasten progress toward the solution of problems in reproductive biology as well as those related to human population growth, structure, and distribution. Population Research Centers are designed to facilitate multidisciplinary communication and effort in the population field, give visibility to the research area, and provide administrative support and a physical environment conducive to scientific productivity. Ongoing Centers have been established at: (1) The Population Council, New York City, Biomedical Division, Dr. Sheldon Segal, Director; (2) University of Texas, Population Research Center, Dr. Harley Browning, Director; (3) Vanderbilt University, Department of Obstetrics and Gynecology, Dr. M.-C. Orgebin-Crist, Director; (4) University of Wisconsin, Center for Demography and Ecology, Dr. Halliman Winsborough, Director; (5) Harvard University Medical School, Center for Population Research, Dr. Roy Greep, Director, and (6) University of Chicago, Ben May Laboratory, Dr. Elwood V. Jensen, Director. The latest addition to this group is Johns Hopkins University, Department of Population Dynamics, Dr. Wiley H. Mosley, Director.

Total funding for the Centers Program is \$10,176,053. The Centers at the Universities of Texas and Wisconsin are in the behavioral-social field, the ones at Johns Hopkins University and the Population Council involve both fields, while the other three relate to the biomedic field.

The second annual meeting of the Directors of Population Research Centers and Program Projects was held in July. The meeting was held at Vanderbilt University, the first institution to receive a Population Centers award, and included the Directors of the seven Population Centers awards as well as the Directors of the eight Program Projects. The purpose of these annual meetings is to facilitate the exchange of information and to foster better communications about significant new developments among the institutions holding these large awards. One result of this meeting was the decision to prepare and publish annual progress reports from all the Centers and Program Projects. The first such publication appeared in January. The Directors meeting was followed by a useful and interesting Conference on Receptors for Reproductive Hormones. This scientific meeting which dealt with the role of receptors in the mechanism of action of both sex steroid and gonadotropic hormones was organized by Dr. Bert O'Malley, the Director of the Center at Vanderbilt at the time, and will be published within the next few months by Plenum Press of New York City. Dr. O'Malley has since moved to the Baylor College of Medicine, Texas. Dr. Marie-Claire Orgebin-Crist is the new Director of the Center at Vanderbilt.

The first annual report of accomplishments in population research at the population research centers and program projects consists of summaries of the objectives of each program, the disciplines and staffs represented, the core facilities being supported, and brief descriptions of the activities and research achievement in reproductive biology and social science research supported in 1972. Although constrained in compliance with the request for brevity, the descriptions of the research programs and progress attest to the high quality, depth, breadth of the programs as well as to the competence and productivity of the staffs and high relevancy to the guidelines and goals of the Center for Population Research.

In contrast to Population Research Center Grants, Program Project Grants provide research support for the investigators as well as core facilities. This type of research support involves the concerted attack of scientists in different, but related disciplines on a common well-defined goal or problem area. Scientists are encouraged to pool their resources, expertise and interests for the examination of more than one facet or phase of a problem through a multidiscipline approach. During this year the Program funded nine program projects; four of these were new awards. Current funding encompasses all aspects of reproductive research and training, both clinical and non-clinical, biomedical and behavioral.

Program Projects are located at Columbia University, Department of Obstetrics and Gynecology, Dr. Raymond Vande Wiele, Director; University of Hawaii, Department of Anatomy, Dr. Vincent DeFeo, Director; University of Michigan, Department of Pathology, Dr. A. Rees Midgley, Director; Northwestern University, Department of Physiology, Dr. Oscar Hechter, Director; Ohio State University, Department of Animal Reproduction Teaching and Research Center, Dr. Noland VanDemark, Director; Oregon Regional Primate Research Center, Department of

Reproduction, Dr. William Montagna, Director; University of Pennsylvania, Department of Obstetrics and Gynecology, Dr. Luigi Mastroianni, Director; and University of Texas at Austin, Department of Zoology, Dr. Antone Jacobson, Director. These program projects involve a total funding of \$8,585,798, and are all in the biomedical field.

As of April, 1973, the Branch had the following numbers of approved but unfunded applications (01 year awards):

1) Center applications (3)	\$617,231
2) Program Project applications (4)	\$631,875
3) Project Research grants (170)	\$5,699,570

In biomedical research the scope of the current funding at \$16,166,000 is indicated by the range of the following topics:

- A. Morphology and associated function of all tissues involved in reproduction. Development of structure and function of the hypothalamic-hypophysial-gonadal axis. Role of the sex steroids.
- B. Mitosis and meiosis in gamete formation. RNA and protein synthesis.
- C. Gamete maturation, liberation, transport and fertilization.
- D. Biochemical studies of semen, and tubal fluids.
- E. Pre-implantation differentiation; implantation. Immunologic aspects of reproduction. Biology of the blastocyst.
- F. Synthesis and physiological function of all hormones involved in reproduction, prostaglandins. Effects of steroid hormones on their target tissues. Receptor or binding proteins.
- G. Localization and identification of steroid and protein binding sites, membrane biology, subcellular actions, molecular structure.
- H. Pheromone and endocrine control of sexual behavior, circadian rhythms, environmental influences (light).
- I. Gonadotropin releasing-factors and their control of reproductive processes. Feedback mechanisms.
- J. The influence of environmental variables (light) and population density on reproduction. Translation of signals (signals) into reproductive events. Pineal gland.

Behavioral-Social Science Population Research Grants Program

During fiscal 1973, the behavioral-social science program continued to grow, as exemplified by the following increases in the number of grant applications: FY 1970 - 25; FY 1971 - 125; FY 1972 - 151; FY 1973 - 228.

Members from a variety of disciplines submitted research grant proposals. As in FY 1972, members of 21 disciplines submitted proposals, and about the same disciplines were involved. The disciplines were represented as follows: Economics - 29%; Psychology - 19%; Sociology - 15%; Anthropology - 9%; Demography - 5%; Political Science - 5%; Geography - 3%; Public Health - 3%; Statistics - 3%; History - 1%; Obstetrics and Gynecology - 1%; Anatomy - 1%; Medicine - 1%; and Education - 1%. Members of the following disciplines submitted one proposal each: Social Work, Communications, Home Economics, Physics, Genetics, Operations Research, and Psychiatry. There was only a small change in the pattern of the disciplines submitting proposals, with some increase in Economics (16% to 29%) and a drop in Psychology (24% to 19%). It is still imperative that the behavioral sciences of Anthropology, Political Science, and Geography be stimulated and encouraged to become more involved in population research. At the same time, if vigorous programming efforts are not continued in Psychology, Sociology, Demography, and Economics, the number of proposals in these disciplines may drop.

Proposals receiving priorities during Fiscal 1973 which warrant funding were mainly in the area of fertility. In this proposed research, analyses were to be undertaken of a number of factors which may affect fertility, including social, psychological, cultural, economic, ethnic, educational, and other factors. Also included among the studies of fertility are several concerned with problems of, and attitudes toward, abortion, while one investigator is studying the psychological effects of vasectomy. Family planning intentions and behaviors are also being investigated. A few studies are concerned with problems of the family, careers, and sexuality.

A few proposals are investigating migration, with special attention to rural migrants in urban areas and the migration of black people to the South.

Population growth and distribution is an area which is receiving some attention in the research grant proposals, and the relationship between population density and human behavior is being investigated.

A workshop on Research on Behavioral Aspects of Surgical Contraception was held in Bethesda, Maryland, on June 18-19, 1973.

During this year, several staff changes have occurred in the Branch. Dr. Everett Wilson, who joined the Center in June 1971 as Branch Chief, resigned to return to his former position at Sam Houston State University in Huntsville, Texas. In his place, Dr. William A. Sadler has been appointed Branch Chief. Dr. Sadler, former Dean, Department Head, and reproductive physiologist at Texas Southern University, joined the staff last summer as science administrator. Dr. Allyn J. Waterman retired last August, and was appointed Health Scientist Consultant to the Center. Dr. William Spillane, a social scientist with training and experience in public health, joined the staff in September, 1972, as social science administrator. Two scientists, enrolled in the Grants Associate Program, National Institutes of Health, spent three to six weeks in the Branch during this year: Dinesh Sharma, Ph.D., D.Sc., and Luigi Giacometti, Ph.D. They participated in the daily work of the Branch, and completed a program assignment designed to assist them in developing a knowledge of the programs and goals of the Center for Population Research and its relation to the NICHD.

The Population and Reproduction Grants Branch has administered as one aspect of its program the development of conferences, symposia and workshops to assess the present state of knowledge of selected areas of basic reproductive biology deemed particularly pertinent to future contraceptive development, as well as to identify areas where imaginative approaches may accelerate research development and progress. A further purpose is to identify and encourage investigators who may not now be actively working in the field so that interdisciplinary exchange may be effected and rapid progress may be made in solving the complex problems of basic human reproduction. The latest symposium was held on the corpus luteum in conjunction with the fifth annual meeting of the Society for the Study of Reproduction at Michigan State University, June, 1972. Organized and directed by Dr. Gilbert S. Greenwald, University of Kansas Medical Center, President of the Society, it discussed such topics as: the cytology of the corpus luteum (Enders), biochemistry (Savard), function in several mammalian laboratory species (Hillard), function in large domestic animals (Hansel, Concannon and Kukaszewska), regulation in the primate (Knobil), and progesterone: implications for fertility control (Warren). The proceedings appeared in Volume 8, March 1973, of the Biology of Reproduction, the Society's journal. Two workshops were held at this meeting; Neuroendocrine Techniques and Electron Microscope Techniques Relevant to Reproduction.

This symposium continues a series supported by the Population and Reproduction Grants Branch. Previous topics in the series have included: male reproduction, gametes and fertilization, environment and reproduction, and immunoreproduction. The proceedings in each instance have been published in the journal of the Society. The purpose, as in all other symposia, was to review the corpus luteum as it relates to reproductive processes in the female, present the subject to reproductive physiologists with varied backgrounds and thus to touch many areas, to inform them of the present status of the field, to provide new research data and to probe the potential for future research, and to examine the role of the corpus luteum in the infertile human. Presentation of the potential for research in reproductive control may invite new investigators and new concepts, as well as interested students in the field.

The book, Biology of the Cervix, edited by Kamran S. Moghissi, M.D. and Richard J. Blandau, M.D., Ph.D., and published by the University of Chicago Press, has recently come off the press. It reports on the proceedings of a conference organized and directed by Drs. Moghissi and Blandau, which was held in June 1971 at the University of Washington's Lake Wilderness Conference, Seattle. This conference brought together more than 50 participants from Australia, England, India, Sweden as well as the United States, who reviewed and discussed a wide range of current studies concerning the structure, function and regulation of the Cervix and its role in reproduction in an effort to advance knowledge about this important pathway, encourage active participation of scientists from all possible relevant disciplines, and assess and facilitate development of new approaches to the methodologies of regulation of human fertility. It is a companion volume to symposia held in other years; important topics that have been discussed previously include: fetal homeostasis, comparative reproductive failure, the mammalian oviduct, biology of the blastocyst, and oogenesis.

Because of its location at the entrance to the uterus, this important segment of the reproductive tract subserves strategic roles in sperm transport and at the time of birth. Abnormalities of the cervix and cervical secretion may cause infertility in animals and human beings. Its secretion, the cervical mucus, influences the passage of sperm from vagina to uterus, and this same secretion in its several states is being used to interpret in the rhythm method of family planning the period of the menstrual cycle when the fertilizable egg may be present in the human uterus. Through possible interference with normal biophysical and biochemical properties of cervical mucus it may be possible to create a biologic barrier to sperm penetration at this site without significant alteration of reproductive and metabolic processes. This latter possibility has led to a resurgence of interest in reassessing the role of the cervix and its secretion in reproduction and contraception. The task of the conference was to update relevant information regarding the role and control of the cervix in reproduction, to assess current investigative procedures and to outline those areas requiring future investigative effort.

A conference on "The Biology and Pathology of Aging Gametes" was supported in June, 1973. Organized and directed by Dr. Richard J. Blandau, University of Washington, School of Medicine, this conference brought together at the University's Lake Wilderness Conference Facility, Seattle, thirty-five scientists of whom twenty-one presented papers, from France, England, Canada, Netherlands, Poland, Japan and the United States to discuss this important basic topic. Dr. Thaddeus Mann, University of Cambridge, England, lent his counsel to its formulation, and was one of the participants. Contributed papers and relevant discussions will be published in the near future.

Aging of the gametes may lead to partial deterioration or loss of developmental vitality so normal development may be interrupted. Wide spread use of the rhythm method in family planning could significantly increase the possibility of more aged ova being fertilized. It is recognized that conditions of over-ripeness may be one of the causes of developmental irregularity or failure. The time of ovulation cannot yet be determined with accuracy. Abnormal development may express itself in death and spontaneous abortion or resorption of embryo or fetus or more importantly, in the birth of a child with gross abnormalities or possibly with more subtle deficiencies even including such parameters as a lowered intelligence.

The ever increasing number of vasectomies as a means of family planning has led to the formation of more "human sperm banks" for the possible future use in the production of progeny. There are insufficient data in man as to the length of time sperm retain not only their fertilizing ability, but, actually their ability to produce normal progeny after prolonged periods of cryogenic preservation.

In this conference, the participants explored and evaluated the gamete aging process at the cellular and molecular levels with the purpose of analyzing major problems in each area and open the way for new direction of research. Among the topics of the contributed papers were: biochemical and genetic changes in aging gametes and reproductive organs, preservation of gametes, teratologic factors as cause of reproductive failure, chromosome behavior, and effects on ovulation and implantation.

Research Excerpts

Beyond the need for goal-oriented research associated with population problems arising with the significant expansion of the world population, there is an undisputed need for continued support of fundamental investigations which will furnish basic information concerning reproduction. Studies at the biochemical, genetic, physical, physiological, behavioral, and early developmental levels in both the normal and the pathologic, the in vivo and the in vitro, the vertebrate and the invertebrate, are continuing to help towards an understanding and appreciation of human development. Support for over 400 grants has enabled basic biomedical-behavioral scientists throughout the nation to identify their research interests in population research and basic reproductive biology with the mission of the Center. The discovery of new, safer, more acceptable methods of regulating family size will require the cooperative efforts of biomedical and social scientists from many different disciplines including: biology, chemistry, physics, bioengineering, sociology and psychology. The problems associated with human infertility continue to be of concern; failure to have children can be as disturbing to healthy family life as too many children and/or too closely spaced children. The field of male fertility has been quite neglected, probably for various reasons, including the difficulty involved in research in this problem area.

Since the last annual report there have been numerous developments in the basic reproduction field among our Grantees. Areas showing particular progress and promise basic to contraceptive development include gamete development and maturation, gene activation and other aspects of the mechanism of action of steroid hormones, ovulation, gamete transport, fertilization in vitro, implantation, corpus luteum initiation and maintenance, useful cell culture lines, and many others. The prostaglandins and their analogues are being rigorously researched, but there is still question as to their ultimate potential as contraceptives to be administered in the regulation of fertility other than as abortifacients. The side effects pose a hurdle, and the appropriate dosages and best avenues of administration are not yet clear, let alone which of them offer the best possibilities for continued investigative effort in the area. There is emerging evidence that a prostaglandin may be indirectly involved in the chain of control of the corpus luteum, e.g., PGF 2a may have such a luteolytic effect in some mammals, but as yet this has not been convincingly demonstrated for the human when physiological amounts of the substance are used. A new avenue being studied is intravaginal administration.

Areas of the reproductive systems considered currently to offer the maximum potential for contraceptive intervention have been identified as including: the oviduct, ovum and blastocyst, corpus luteum, ovary and ovulation, spermatozoa and fertilization, epididymis, vas deferens, and receptor mechanisms operative for steroid hormones.

A survey by title of the currently supported research grants in the biomedic field has shown investigations being conducted within the broad program guidelines from germ cell development to implantation in placental mammals, including the human. This survey does not take into consideration the multitudinous researches being conducted within the large Program Project and Center Grants. But reference to such grants listed above in the biomedic field will reveal

that these pretty well cover many of the same topics as listed below, with some emphasis of course, on specific areas.

Male

The complicated processes of spermatogenesis and spermiogenesis continue to be investigated in depth by studies on nuclear proteins (17 grantees): hormone regulation, biochemical processes involved and intercellular relationships and physiology. Six grantees are looking at the immuno-biology of the testis, and 22 others are seeking answers to questions relating to its metabolism, regulation, steroidogenic activity, contribution to the seminal plasma, culture in vitro, biochemistry and sperm production. Interest is being shown in the immunologic reaction and endocrine changes following vasectomy (6 grantees), male contraception (1), and the biochemistry and origin and role of the seminal plasma (11). Many questions are being asked about spermatozoa (36 grantees): freezing for artificial insemination, mechanism of motility, maturation (capacitation) within the male or female ducts, enzyme content, acrosome, nucleotides, chromatin, and metabolism. The action, roles, and production of androgens are interesting 6 grantees; male contraceptive (5); also the histophysiology, role, biochemistry, and receptors of steroids in the epididymis and vas deferens (15).

Female

Reproduction in the female has received by far the most attention from grantees this past year: physiology and control of the estrous and menstrual cycles (11 grantees); biology, structure-function relationships, radioimmunoassay of the gonadotropins (8); the action of steroid and peptide hormones (49)(control, biosynthesis, effects, secretion, localization, mechanism of action and receptors, inhibitors of release, metabolism); oxytocin (3); specific organ hormone receptors or binding proteins (11). The ubiquitous prostaglandins are occupying the attention specifically of 14 grantees (as contraceptives, effects on reproductive ducts, uterine luteolytic factor synthesis, analogues), but these are also included as minor components of the research programs of many more grantees.

The hypothalamic-hypophysial-gonadal axis is interesting many investigators (28). Problems are being attacked that include the neuro-endocrine control centers, releasing factors and their synthesis, feed-back mechanisms, environmental factors influencing hypothalamic centers, role of the pineal gland (3), pheromones and hormone influences on sex behavior (13), the mechanism of biological rhythms (5).

There is no part of the female tract that has escaped attention: ovum development-including human (9); maturation and protein synthesis in the oocyte (5); oogenesis-DNA, RNA, protein synthesis (11); ovarian function (5); ovulation-process, detection, genetic mechanisms (11); follicular physiology and fluid (8). Also being investigated are: biology of the oviduct--its role, source and changing constitution of the luminal contents; transport of gametes, blocking agents, smooth muscle physiology, RNA and protein synthesis, hormone receptors, nerve and vascular supplies (15); biology of the uterus--decidualization, hormone receptors and binding sites, control of, luteolytic

factor, uterine proteins, changing nature of the luminal fluid (27); biology of the cervix (3); biology of the corpus luteum--control of, luteinization, metabolism, binding of proteins, luteotrophic and luteolytic factors (23).

The process of fertilization has long been of interest to reproductive biologists. Twenty-five grantees are specifically emphasizing this phenomenon by continuing to study the biochemistry of the process, membrane changes, molecular aspects, and in vitro fertilization. Another stage in early development that also has been of interest because of the theoretical possibility of relating it to fertility regulation is the blastocyst (16). This stage spends 3-4 days in the uterus (longer in some mammals) before implantation is initiated. Investigators have been interested in its metabolism, protein synthesis, reaction to hormones and other drugs, fluid in its blastocoel, its trophoblastic component, relation to the uterine fluids, and implantation (14).

Other grantees are studying: the origin of germ cells (2), reproductive senescence (2), methods of sterilization (3), in vitro methodologies for pure cell lines, fertilization, and subsequent development (31), reproduction and obesity (1), development of reproductive function, nucleocytoplasmic interactions, hormone synthesis (17), immunologic aspects of reproduction (5), and the action and composition of intrauterine devices (7).

Examples of some of the current research. Corpus luteum.

Regression of the corpus luteum of pregnancy occurs during the fourth week of gestation in the rhesus monkey. The specific factor(s) responsible have not been identified, although this has been associated with a marked decrease in monkey **chorionic gonadotropin (MCG) which occurs about this time.** The CL can be removed as early as 21 days postmating without altering the course of pregnancy in the rhesus monkey; it has been assumed that the placenta in this species assumes steroidogenic function of the regressed CL at least from 21 to 40 days of pregnancy. Yet the placenta, during the early stages of pregnancy, produces only small quantities of progesterone (Treloar, Wolf, and R.K. Meyer).

The critical endocrine role of the rat placenta in supplanting the hypophysis and maintaining corpus luteum function has been affirmed (Linkie and Niswender). The luteotropic activity was also demonstrated following administration of placental tissue to the hypophysectomized pregnant rat; it is dose related and the active factor can be lyophilized repeatedly without loss of activity. It is also characterized as a heat-labile protein of approximately 25,000-50,000 molecular weight.

Culture of Cells in vitro.

The culture of cells in vitro, whether somatic or reproductive, provides the investigator with a tool that can be utilized in basic studies of hormones at

cellular and subcellular levels. Granulosa cells from ovarian follicles are being grown in vitro, and those from large ones provide an ideal model system for studying the mechanisms underlying the luteinization of these cells (Channing). Prostaglandins are not luteolytic when added alone to monkey granulosa cells, but can inhibit the luteinizing action of administered luteinizing hormone on such cells. Prostaglandins do stimulate progesterin secretion and morphological luteinization of both monkey and human granulosa cell cultures. The effectiveness is PgE₂ or PgE₂ > 77 PgA > PgF_{2a}. That luteinization truly occurs has been confirmed at the fine structural level and the course of differentiation in culture has been correlated with progesterin secretion rates. In a study (Channing) to determine whether or not prostaglandins are an intermediate in luteinizing hormone (LH) and human chorionic gonadotropin (hCG) action upon monkey granulosa cells, it was found that prostaglandin may be an intermediate in the action of LH or hCG in luteinization, but the inhibitors employed may provoke necrotic effects in the cultured cells. LH and FSH and prostaglandins stimulate luteinization in pig granulosa cultures via increased cyclic -3',5' -AMP production, and the latter prevents the decrease in progesterin secretion which occurs at about 12 days in cells cultured in LH-containing media for 20 days.

Receptors

Progesterone receptor activity has been reported to be present in specific tissues of a variety of animals. A specific progesterone-binding component has been found in the uteri of both mouse and rat (O'Malley and colleagues) and has the properties expected of a hormone receptor. This conclusion has been based on the findings of tissue and hormone-binding specificity, by the similarities between it and other previously described steroid hormone receptors, and by the effect of the estrous cycle and estrogen treatment on tissue levels of these binding components. The receptor binding is estrogen dependent. The functional nature of the receptor and the primary focus of the regulatory aspect of estrogen are areas of future study.

Oviduct

Pacemaker action is not localized within the rabbit oviduct, conduction is slow and limited, and the intraluminal pressure cycles are of irregular and complex shape with slow rate of rise and often with low amplitude. Apparently, in the isthmus, as in the uterus, the conduction of the electric activity between distant regions is promoted by estrogen and disturbed or suspended by estrogen deficiency, or progesterone treatment in combination with estrogen. Variations in the generation and propagation of the electric activity suggest that homonal control of the activity pattern could be one important mechanism in the regulation of the egg transport (Brandin and Talo).

The mouse oviduct is an organ whose genomic regulation by hormones is different from that in the uterus. DNA content was not altered by varied experimental treatments. Most, if not all, of the effects of progesterone on oviductal function, either directly or in concert with estradiol, are mediated by mechanisms independent of those involved in the genomic regulation of RNA and protein synthesis (Bronson and Hamilton). The hamster is unique in the extensive portion of gestation devoted to the preimplantation period, and use of this fact has been made in an analysis of a biologically active factor from oviductal contents that causes a decrease in ovarian function in pro gravid females (Kent). It appears to be a polypeptide with a molecular weight below 700. The assumption is made that this factor influences the oviduct, which, in turn, affects ovarian function.

Preimplantation Embryo

The early discovery that 8-cell mouse embryos can develop into blastocysts in a simple chemically defined medium (Whitten, 1956), and the demonstration that blastocysts produced in this way can also develop into normal mice when transferred to uterine foster mothers (McLaren and Biggers, 1958) opened up a new area of investigation. The metabolic capabilities of the mammalian preimplantation embryo change with development; some biochemical characteristics are determined by events during oogenesis, whereas others are a consequence of the activity of the new genome when the paternal genes become active. This early embryo is dependent on special environments which vary from one part to another of the upper genital tract; the ampullary region of the oviduct provides the first of these special environments (Biggers).

It is known that progesterone imbalance near the time of fertilization can interfere with fertilization. Gamete transport and capacitation may be affected, leading to asynchrony between developing embryo and the uterine environment or to defective gametes even to ones with chromosomal abnormalities with impairment of developmental potential (Chang). The question is being asked whether progesterone has a direct effect upon the preimplantation embryo. In a recent study it has been shown that progesterone at sub-contraceptive levels, which block neither ovulation nor fertilization in the rabbit, may adversely affect embryo development in vitro, in treated mothers, and following transfer to control recipients (Allen and Foote).

During early pregnancy in the rat the maximal uterine glycogen stores, achieved at estrus, decline in a consistent manner along with the enzymes of glycogen metabolism. An increase in glycogen turnover occurs, however, as indexed by the activity levels of synthetase and phosphorylase at a time period of early pregnancy consistent with embryo implantation. These changes in uterine glycogen metabolism suggest that glycogen may play an important role in the nutritional welfare of the preimplanting embryo and may also be a limiting factor to the energy metabolism associated with nidation (Demers, Yoshinaga and Greep).

The zona pellucida of mammalian embryos does not exclude molecules on the basis of size within the normal range of individual protein molecules (Hastings, Enders and Schlafke).

IUD

One physical method of fertility regulation involves the intrauterine device (IUD). Many reports on the efficacy, acceptability, and safety of this device are available; however, bleeding, pain, and an occasional pregnancy continue to be major side effects. Expulsions may go unnoticed. There are several types of the IUD in current use. The introduction of the Tatum "T" device and the copper T (TCu 200) has opened a new concept in the field of intrauterine conception, with the hope of overcoming the above mentioned adverse effects.

A foreign body in the uterus provokes an inflammatory reaction, as found in laboratory animals, and the entering neutrophils die and degenerate thus releasing their contents into the uterine luminal fluid. Extracts of neutrophils are toxic for early embryos growing in culture. The inflammatory reaction also occurs in human uteri with IUD's, conspicuously in the endometrium directly in contact with the IUD. Neutrophils tend to adhere to the IUD surface along with other cells including macrophages. The adjacent endothelium is characterized by a leukocytic infiltration into the region. Thus, an IUD causes a chronic, low-grade inflammatory reaction, focused on this foreign body. It has further been shown that the contraceptive effectiveness of an IUD is well correlated with its surface area in contact with the endometrium. This is consistent with the view that the effectiveness of an IUD may be the result of the induction of a foreign body response; the greater the area of endometrial involvement the more effective is the IUD.

Uterine secretion during the progestational phase is thought to involve an apocrine process, whereby the apical portion of luminal and glandular epithelial cell bulges into the lumen and bursts to release cellular contents. This, together with degenerating neutrophils, forms a fluid environment toxic for preimplantation embryos. The magnitude of a foreign body response depends upon the type of foreign material implanted, some being more toxic than others. It could therefore be expected that the effectiveness of an IUD might depend upon the material employed. Nearly all IUD's currently used are of polyethylene or some similarly inert plastic which cause only minor foreign body reactions. Perhaps this is not good.

Vasectomy

Vasectomy, a wide spread birth control method, is followed in time by changes throughout the entire male duct system. In the rhesus monkey the ductuli efferentes become greatly enlarged, with thickened basal lamina in the site of antigen-antibody complexing and reduced number of ciliated epithelial cells. Spermatozoa become agglutinated in the lumen and subsequently ingested by macrophages. There occurs an autoimmune response to spermatozoa, which may aid in sperm disposal. Little or no change in testicular tissue is associated with long-term vasectomy, and spermatogenesis is not affected. The level of circulating spermagglutinins greatly increases, as well as the antibody. The autoimmune changes both in the epididymis and the serum after long-term vasectomy

may result in a reduction of successful pregnancy after surgical re-anastomosis of the vas deferens (Alexander).

The antigens in human semen are of interest due to their role in immunologic infertility and as possible factors in future methods of population control. Their identification is now possible. Nine antigens in human seminal plasma have recently been identified. Four of these albumin, transferrin, aB1 globulin, and immunoglobulin G originate from blood plasma. Lactoferrin aB1 globulin and aB2 globulin arise in the seminal vesicles and phosphatase and another B2 globulin come from the prostate gland. Lactoferrin is also produced within the epididymis and possibly the prostate; it is found as a sperm-coating antigen present on the surface of both ejaculated and spermatocele spermatozoa (Quinlivan and Sullivan).

Mammalian seminal plasma in which the spermatozoa are transported through the male ducts of epididymis and vas deferens consists of contributions from the entire reproductive system: testes epididymis, accessory glands and vas deferens. Quantitative determination of the chemical components under normal and pathological conditions has thrown light on their source, secretory pattern and roles. The gain in the method of choice of vasectomy as a male contraceptive has led to interest in the after effects of this method. One of these is the possible immunologic or biochemical changes in the semen. With reference to the enzymes found in seminal plasma, aspartate aminotransferase (SGOT) activity is reported to be correlated with sperm density, and its amount in the plasma suggests a decreased synthesis by the accessory organs (prostate gland) in the infertile man. Most of the activity of this enzyme occurs in the first portion of the ejaculate. Leucine aminopeptidases appear to have their source in the spermatozoa. They show decreased enzyme activity with oligo- and azoospermia and in individuals with chromosomal abnormalities. Other enzymes (isocitric dehydrogenase, alanine aminotransferase), fructose, and protein content apparently do not vary following vasectomy and seem to be correlated with sperm numbers.

Oral Contraceptives

A number of publications in recent years have reported on clinical aspects of the administration of quinestrol=quingestanol (QQ) as a once-a-month contraceptive pill. The method is based on the knowledge that orally administered quinestrol is a long-acting estrogen. After absorption, it is stored unchanged in body fat from which it is subsequently released to exert its action, presumably, by conversion to ethinyl estradiol. But in one recent study a relatively high incidence of pregnancies resulted (Taymor and colleagues, Harvard Medical School) in a small number of tested patients. A rise in serum estrogens and LH level occurred, but there was no evidence of ovulation by progesterone patterns. It is postulated that long-acting exogenous estrogen inhibits FSH release and prevents cyclic changes in FSH secretion necessary for full follicular development. But FSH is not completely inhibited and after 21 to 27 days endogenous estrogen is secreted, and LH activity may even peak at an ovulatory level. Failure to ovulate is probably due to failure of LH to achieve a high enough level. It would appear that there is probably a narrow margin of safety in terms of hypothalamic-pituitary suppression. A brief delay in taking the medication might result in a more significant LH

surge, and, if this occurred in the presence of a mature follicle, ovulation could occur. Variations in dosage and frequency of administration are being studied.

Among the progestogens, norethindrone may alter or lower triglycerides, chlormadinone is without effect and Delalutin lowers blood levels as does ethynodiol diacetate. Data from cases using the combination-type oral contraceptive suggest the triglyceride changes are chiefly associated with the estrogen content. The mechanism of action of the estrogens on this aspect of lipid metabolism is not known, but an increase in the liver's production of triglycerides has been reported.

Thromboembolism has been linked with oral contraceptives, particularly with those with high estrogen content. This has stimulated the search for lower dose oral contraceptive combination. Interest in microdose progestin contraception therapy continues unabated, especially as estrogen may not be involved. One example is 37.5 µg. d-norgestrel (dl-13B-ethyl-17B-hydroxygon-4-en-3-one). The minimum contraceptive daily dose of dl-norgestrel has been suggested at 75 µg. Current thoughts on the mechanism of action of microdose norgestrel have ranged from subtle changes in the endocrine balance to biochemical changes in the cervical mucus. Ovulation occurs in at least a considerable proportion of women or in some cycles. There is a typical corpus luteum in the majority, and absence of LH peak during the menstrual cycle in some cases. It seems, in general, that norgestrel in microdose induces depression of the corpus luteum which in turn alters endometrial functions. Elevated fasting plasma triglyceride levels beginning several weeks after therapy was begun indicating altered lipid metabolism, which disappear about 6 months after discontinuation of the drug. Mestranol, ethinyl estradiol, and stilbestrol are all capable of elevating blood triglycerides.

Development

A knowledge of the patterns of differentiation of structure and particularly function of an organ or organ system, along with the establishment of its interrelations with other organs, hormonal and nervous, can furnish valuable clues to the investigator of adult reproductive phenomena. Function, for example, both within and between organs (hypothalamic-hypophysial-gonadal axis) does not just emerge de novo all at once, but as a series of steps expressing the parental genome and involving cellular interactions. For instance it takes the association of Rathke's pouch and neural infundibulum anlage to even bring the hypophysis into existence. While endocrinologists have paid little attention to this area of investigative endeavor in the past because of engrossment with the adult animal, some are turning their attention to development in the expectation that useful ideas may emerge.

Progesterone imbalance near the time of ovulation in the rabbit can disturb normal fertilization by interfering with normal egg and sperm transport. It has been reported that capacitation is slower in the presence of progesterone. When the normal time of fertilization is delayed, gametes age; fertilization may be prevented or inherently defective zygotes may result, in which case chromosomal abnormalities can occur. These subsequently may produce developmentally impaired young (Blandau). It has been reported that a higher incidence of chromosomal defects were found in aborted fetuses from women who

conceived within 6 months after discontinuing use of the pill, suggesting that imbalance of this hormone may have been responsible for chromosomal defects in the fertilized egg.

Progesterone seems to hasten the transport of the cleavage embryo to the uterus, with the result that the embryo arrives in the uterus before the latter is prepared for it. Thus, the embryo may survive initially, but may not remain viable to implant under the hostile conditions of asynchrony between the developing embryo and the uterine environment. Finally, progesterone may have a direct effect on the embryo. Under conditions where progesterone is present at subcontraceptive levels, which block neither ovulation nor fertilization, there is a tendency to suppress development of morulae into blastocysts and a reduction in viability. But, if implantation is successful, typical embryonic development follows.

By 90 days after birth the epithelial cells lining the oviduct appear similar to those of the adult rat; the secretory granules, and release of these into the tubal lumen, may be observed. This has potential as a model system (McCarron and Anderson).

When neonatal rodents receive steroid treatment, this makes them more sensitive as adults. Resulting development of peripheral tissues can be altered via two pathways: directly by the injected steroid itself, and indirectly via altered brain, pituitary, and hence, gonadal action, as shown by tests with hormones in the adult animal. RNA synthesis and rate of growth in the adult were significantly hastened in the neonatally primed rat. Relatively pure biochemical priming was seen in the seminal vesicles of the primed mouse, but such primed glands accumulated testosterone-1, 2- H^3 at the same rate as the control, (Bronson, Whitsett and Hamilton). Thus, neonatal androgenization may result in seminal vesicles similar to the control except that they have the capacity to more rapidly synthesize RNA and structural protein when challenged in adulthood by testosterone.

It is known that the fetal testis produces testosterone as early as the 14th day with a peak at 18 days, which is probably responsible for Wolffian duct stabilization and hypothalamic differentiation of the male fetus. If failure occurs in this system, disorders of sexual differentiation may appear. There is a sudden and rapid decline in testicular testosterone after birth in the rat. At eighteen days the male duct system undergoes retrogression in the absence of androgens.

The testis is an organ responsible for further sexual differentiation of the male fetus, and steroid androgens play an important role in this process. Androgenic steroids seem responsible for stabilization of the male duct system and development. The regression of the female duct system also appears to be controlled by the fetal testis, but a different mechanism is involved since such regression cannot be induced by any known androgen. Steroid androgens are also necessary for development of a "male" type hypothalamus. Enzymes are present in the fetal testis that are capable of converting progesterone to testosterone in the rat; also ones for converting pregnenolone to testosterone have been found in human, rat, and mouse fetal testes; acetate can be converted to testosterone (human, rat).

In mammals the germ line is established early in embryogenesis (man, mouse, rat, rabbit, cow), and all gametes are derived from this line of primordial germ cells. In the mouse embryo, the most extensively studied among mammalian embryos, primordial germ cells have been identified at around 8 days of development by virtue of their high alkaline phosphatase activity. They appear first at the base of the allantois and subsequently are reported to migrate to the gonadal ridges. The origin of the first of these cells is not known, nor is much known about the mechanisms of their migration.

Oocytes

Mammalian oocytes, including those of the human female, enter meiosis during fetal life and reach the diplotene stage of prophase before or shortly after birth. They then enter a long period of inactivity which typically ends when certain individual oocytes, for some unknown reason, resume meiosis shortly after ovulation in each menstrual cycle or prior to atresia. But any oocytes removed from their follicles resume and often complete meiosis in relatively large numbers and rather synchronously. This usually occurs irrespective of the stage of follicular development prior to culture and at a rate comparable to that in graafian follicles stimulated with gonadotropins. Morphological characteristics are similar too (Zamboni and Thompson, Harbor General Hospital and D. Smith, Harvard Medical School).

Testosterone production can be stimulated by cyclic AMP (adenosine 3',5'-monophosphate) the latter is the intracellular mediator of the actions of the sex hormones. Both FSH and ICSH increase the amount of cyclic AMP (in dog testis). Testicular tubular adenyl cyclase is low at 25 days of age in the rat and increases to a maximum at 60 days of age (rat), a period associated with the arrival of sexual maturation.

Photoperiods

Photoperiods may continue to play an important role in timing the endocrine events of the reproductive system in pseudopregnancy, even in the absence of cyclicity. Exposure to constant light alters ovarian progesterin secretory patterns by lowering the progesterone/20 -OH-P ratio at an earlier time (Piacaselo and colleagues). Young female rats raised in instant light exhibit earlier vaginal opening. This also stimulates FSH secretion from transplanted pituitaries in hypophysectomized rats. Adult females in reduced dietary intake may stop cycling, but can be induced into proestrus or estrus by exposure to continuous light. The presence of functional corpora lutea (pregnancy or pseudopregnancy) can delay the onset of constant light induced persistent estrus; constant illumination disturbs the pattern of progesterin secretion.

Pheromones

Mice produce two primer pheromones (Whitten); one in the male urine induces earlier puberty, shortened estrous cycles, increased clutch of eggs, and pregnancy block in receptive females. These responses involve gonadotropin synthesis and release, but the response depends on the content within which the pheromone acts. Pregnancy block may require some identifying substance

that first arouses the pregnant female. The second pheromone is produced by females and delays puberty or lengthens or even abolishes estrous cycles in other females. Both pheromones appear to be volatile chemicals and act through the olfactory receptors. The production of pheromones and the response to them appears to be genetically determined. Some mice can continue to cycle in the absence of olfactory bulbs. Whereas others become anestrus. While genetically determined, it is not known where the genes have their primary action. Pregnancies in mice may become blocked when recently mated females are removed from their stud males and placed with alien males. It seems that that is due to gonadotropin release at an ovulating level induced by the pheromone and that reduced prolactin secretion is secondary. Administration of physiological doses of gonadotropin also block pregnancy confirming the above hypothesis (Hoppe and Whitten).

Reproduction in Aged Animals

Reproduction failure in aged females is well documented. Recently it has been reported that ovarian venous blood progesterone levels are lower in old pregnant rabbits than in young pregnant ones (Maurer and Foote). There is no difference in the parameters of ovarian function between aged and young does until after day 12 of pregnancy. It seems that the decrease in fecundity in aged animals is not caused by lack of progestin secretion, but rather the absence of viable fetuses may cause luteal regression, and thus failure is directly associated with uterine inability to initiate and maintain pregnancy (Spilman, Larson, Concannon, and Foote).



NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Following rapid expansion during FY 72, new activities of the program to evaluate the medical effects of fertility regulating agents presently in use declined sharply during FY 73 in order to permit consolidation of the large number of new projects initiated and in response to an unchanged budget. One review panel met in December 1972 to consider a series of revised proposals to study the clinical effects of vasectomy. Studies of the relationship between outcome of pregnancy and contraceptive use were discontinued or not implemented because of administrative difficulties with the contractors and funding requirements of higher priority. Symposia on Contraceptive Agents and a Workshop on Oral Contraceptive Drugs were held as scheduled during the Fifth International Congress of Pharmacology, July, 1972, and a Symposium on Oral Contraceptives and High Blood Pressure was held at the University of Florida in March, 1973, as planned. A workshop including collaborators in the studies of nutritional biochemistry as related to contraceptive use was held in November 1972 and a similar workshop for investigators studying epidemiological relationships between genital system cancer and use of hormonal agents was held in March, 1973. Staff of the Branch was reduced by one clerical member.

Since a large proportion of the program including studies of comparative pharmacology, dietary nutrients and the medical effects of vasectomy was initiated during late FY 72, these projects were only in early stages of operation during FY 73 and have yielded little new information. A number of ongoing studies are undergoing appropriate redirections while others are concluding.

A total of 17,000 women has been recruited into the Contraceptive Drug Study at the Kaiser Foundation Hospital, Walnut Creek, Calif., and further expansion of the cohort has been discontinued. Emphasis has been placed on longitudinal followup utilizing the Automated Multitest Laboratory (AML), surveillance of hospital records and an annual questionnaire. Cross-sectional analyses of the screening data have been virtually completed and, as a result, a modified version of the AML will be applied in the future to selected sub-populations in the cohort. Particular attention will be paid to monitoring for cardiovascular events, especially hypertension, as a model for developing and evaluating the system. Reports of progress are now regularly published in the literature and a three-volume monograph which will include both published work and supplementary tables is now in preparation.

Studies of the relationship between cancer of the breast and cervix and oral contraceptive use have been proceeding actively. (White, Paffenbarger) Epidemiological case-control studies of the relationship of breast cancer and oral steroid use have been undergoing continuing analysis and completion of patient recruitment will be accomplished early in FY 74. On the basis of preliminary information, no relationship between the occurrence of malignant disease and

drug use is apparent. Longitudinal studies of the effect of oral and mechanical contraceptives in patients with diagnosed abnormalities of cervical cytology had revealed no differences during the nearly three years of initial observations. (Stern) However, in patients followed for longer periods of time a slight increase in abnormal cytology appears in the oral contraceptive users. Although it was planned that the study would conclude during FY 73, the data are now undergoing further analysis in order to determine whether the studies should be continued to evaluate this finding in more detail. The multi-clinic longitudinal study of correlations between abnormal cervical cytology and contraceptive use has recruited more than half of the desired cohort of 30,000 women and recruitment should be completed by early FY 74. (LoSciuto) During the past year particular attention has been paid to standardizing criteria for interpretation of cytological abnormalities. Workshops for the collaborating investigators were held during July and December 1972 and another is planned for June, 1973. Analysis of the prevalence rate of dysplasia as related to contraceptive use and other demographic factors is now in preparation. Preparation of Volume I of the "Handbook of Steroid Contraceptives", a photo album with descriptive information about oral contraceptive products which will be of use to both study interviewers and family planning clinics, will be completed during FY 73.

Analysis of data from the Collaborative Study of Stroke in Women of Reproductive Age is now proceeding and an initial publication will be available during late FY 73. An eight-fold increased risk of thrombotic stroke was demonstrated in oral contraceptive users and the role of other contributing risk factors is under analysis. (Heyman) In addition, recruitment of the cohort into a multi-hospital study of other forms of thromboembolism will be completed during FY 73 and the final analysis should be available during FY 74. (Stolley) The studies of new procedures to predict impending thrombotic events was subjected to controlled evaluation during FY 73 and analysis should be available early in FY 74. (Fletcher) Investigation of the effects of contraceptive steroids on the blood vessel wall, blood flow and coagulation function in monkeys have been completed and are now under analysis. (Loeb) Further exploration of the effects of oral contraceptives on blood pressure have revealed differences in the potency of hypertensive effects produced by various progestational agents. (Crane)

Other studies of the effects of contraceptive steroids on drug metabolism by the liver have suggested that long-term use of these agents causes inhibition of the metabolism of other drugs; these findings are under evaluation. (Bressler) The Central Repository for Tissues derived from required animal testing protocols for contraceptive steroids has been established at the Armed Forces Institute of Pathology and is receiving accessions from both industry and FDA-sponsored projects. (Hansen) Workshops for the program participants were held in October, 1972 and April, 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Five-Year Prospective Study of Abnormal Cervical Cytology
Contractor: Temple University, Institute for Survey Research
Money Allocated: \$1,230,000 (FY 1973 Funds)

Objectives: This is a five-year prospective study of the possible relationship between changes in cervical cytology and the use of various methods of contraception. Thirty Thousand women will be recruited from 10 geographic location and Papanicolaou smears, contraceptive history and sociological and demographic information will be collected. After the first year of recruitment, patients will be followed with annual Pap smears and current medical history. Papanicolaou smears will be interpreted both by a central as well as the local cytological laboratories. The principal objective of the study is to determine whether there is any relationship between the use of steroid contraceptive drugs and changes in cervical dysplasia; in addition, any cases of breast cancer which are detected during the course of the study will also be evaluated. Other background information with regard to changes in contraceptive use and the general characteristics of the contraceptive-using population will also be analyzed.

Significance to Biomedical Research and Program of the Institute: It is clear that the steroid drugs used in oral contraceptive preparations have a stimulatory effect on the tissues of the uterine cervix as well as the breast. Whether these changes could result in a development of cancerous lesions remains to be determined. Since the etiology of genital system cancer is likely to be multifactorial, it is important that comprehensive and detailed studies of all factors including contraceptive use be collected simultaneously on the population at risk. The project is relevant to the mission of the Center for Population Research and its goal of assessing the medical effects and possible risks of contraceptive use and their correlation with other environmental factors.

Proposed Course: Initial recruitment of the patient population began in ten locations during May 1972. Although proceeding at a somewhat slower than expected pace, more than half of the anticipated cohort of 30,000 women has now been recruited into the study and patients initially recruited are now returning for followup visits. During the past year, particular attention has been paid to standardizing criteria for interpretation of cytological abnormalities. Workshops for the collaborating investigators were held during July and December 1972 and another is planned for June 1973. Analysis of the prevalence rate of dysplasia as related to contraceptive use and demographic factors is now in preparation. Preparation of Volume I of "The Handbook of Steroid Contraceptives", a photo album with descriptive information about oral contraceptive products which will be of use to both study interviewers and family planning clinics, will be completed shortly.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Contraceptive Drug Study
Contractor: Kaiser Foundation Research Institute, Walnut Creek, Calif.
Money Allocated: \$812,000 (FY 1973 Funds)

Objectives: The objective of this program is to evaluate a variety of biochemical and medical parameters as related to contraceptive use in women who are members of the Kaiser Permanente Medical Care Program in Walnut Creek, California. The principal tool for accomplishing this objective is an automated multiphasic laboratory examination in which more than 17,000 women have been studied at least once. The long-range objective is to make longitudinal observations on various components of the automated multiphasic examination and to relate these to morbidity and mortality in the population.

Significance to Biomedical Research and Program of the Institute: This is a broad ranging program of surveillance of the possible effects of the use of various contraceptive methods with particular emphasis on the oral contraceptives. The particular value is the ability to monitor a large population in considerable depth and to be able to relate biochemical to clinical effects. It is relevant to the Center for Population Research program to monitor medical effects of oral contraceptives.

Proposed Course: A total of 17,000 women has been recruited into the Contraceptive Drug Study and further expansion of the cohort has been discontinued. Emphasis has been placed on longitudinal follow-up utilizing the automated multitest laboratory (AML), surveillance of hospital records, and an annual questionnaire. Cross-sectional analyses of the screening data have been virtually completed and as a result a modified version of the AML will be applied in the future to selected sub-populations in the cohort. Particular attention will be paid to monitoring for cardiovascular events especially hypertension as a model for developing and evaluating the system. Reports of progress are now regularly published in literature and a three volume monograph which will include both published work and supplementary tables is now in preparation.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Interaction of Contraceptive Steroids with Metabolic Functions of Vitamin B₆
Contractor: University of Pennsylvania School of Medicine
Money Allocated: \$137,183 (FY 1973 Funds)

Objectives: The purpose of the proposed study is to perform a statistically valid analysis of clinical and chemical disturbance of B₆ metabolism in women on oral contraceptive pills and in pregnant women. This should lead to the establishment of necessity of B₆ supplementation in the above two conditions and to the desirable dose level of supplementary pyridoxine. Accompanying clinical conditions, like depression, edema, rheumatoid manifestations, will receive special attention.

Significance to Biomedical Research and Program of the Institute: The proposed project may provide information on mechanism and consequences of altered vitamin B₆ metabolism in contraceptive users. In all published studies on abnormalities of vitamin B₆ status resulting from use of contraceptive steroids and pregnancy, primary or exclusive reliance was placed on the tryptophan load test or the determination of blood transaminases. This was due to lack of precise, reliable and reproducible analytical procedures for estimating pyridoxal phosphate (PLP) directly. In 1970 Chabner and Livingston described a method for the estimation of plasma PLP based on the measurement of ¹⁴CO₂ evolved from the decarboxylation of l-tyrosine-1-¹⁴C by tyrosine decarboxylase. The amount of ¹⁴CO₂ produced is proportional to the amount of PLP added to an excess of tyrosine apodecarboxylase. A modification of this procedure to be employed in the proposed project may offer new insight in the relationships between contraceptive use, primary manifestations of altered B₆ status and its secondary consequences, such as an abnormal tryptophan metabolism.

The dosage of pyridoxine supplementation is a pharmacological one and greatly above what is known as the requirement for this vitamin. Such a high dosage may be necessary to correct all abnormalities in tryptophan metabolism in all contraceptive users. This provides for simplicity of experimental design but limits reliable estimation and interpretation of altered dietary requirements of this nutrient. The experimental design underlying the project is considered outstanding both in regard to its epidemiological-clinical components and the nutritional-biochemical methodology.

Proposed Course: Three groups (35 in each group) of properly selected women will be studied to assess the metabolic interaction of contraceptive steroids and vitamin B₆. Group A has received previously oral contraceptives. Groups B and C have not used oral contraceptives before. After enrollment all women in all groups will undergo clinical examination. Simultaneously chemical studies will be carried out in the blood (plasma) for pyridoxal phosphate (PLP), in the erythrocytes for GPT, GOT, and in tryptophan-methionine load tests for xanthurenic acid and kynurenic acid in the urine and for homocysteine, cystathionine, and taurine in the blood. In patients showing signs

of depression, serotonin will be determined in the blood. Since the patients in Group A have been for at least six months before entrance in the study on contraceptive steroids, it is to be expected to find in them pathological disturbances of the B₆ metabolism, whereas Groups B and C never before receiving oral contraceptives should behave as normal controls. After completion of the initial clinical and chemical studies, Group A will continue and the second group will begin to take a combination oral contraceptive in the usual 21-7 day cycle. The third group will receive minidose progestogen alone. Groups A and B will receive 100 mg. pyridoxine HCl daily during the 7-day interval between active contraceptive medication. Repeated clinical and chemical studies will be made at 1, 3 and 6 months after entrance into the study. The probands for the three groups of contraceptive users are available from the Hahnemann Medical College and Hospital Family Planning Clinic. The two groups of pregnant subjects will be supplied through the private practice of the co-principal investigator. Within an initial operational period of 8 months, enrollment of patients in the three study groups has been essentially completed. Experimental efforts in the laboratory have been concentrated on determination of plasma pyridoxal phosphate (PLP) levels. These have been analyzed in a preliminary fashion and are presented in the progress report submitted in the renewal application. Measurement of more widely used criteria of pyridoxal phosphate status such as urinary excretion of xanthurenic acid and kynurenic acid after a tryptophan load or assay of PLP-sensitive glutamic-oxaloacetic acid and glutamic-pyruvic transaminases have also been done. This work appears behind schedule. Data and results are, however, to be submitted shortly. The wide scatter and variability of PLP levels and the prevailing uncertainty of a definitive normal range have so far precluded definitive conclusion as to the effects of oral contraceptives. In regard to Group A "old to Ovral", the possible reduction of PLP level appears to have become stabilized close to the normal range prior to enrollment in the study. A more remarkable finding than the uncertain PLP baseline levels were the response observed in subsequent B₆ therapy. In the case of patients on prolonged therapy (Group A, "old to Ovral") two cycles of B₆ administration did not cause a rise in subsequently determined PLP levels. In the absence of chronic contraceptive therapy (Group B, "new to Ovral", and Group C, "new to Norgestrel") B₆ administration led to a rise of PLP levels.

During the second year's contract period the investigation is contemplated to be continued as follows:

1. Patients presently on the study will be continued. A replacement of 15 patients is planned for each of the three groups in expectation that about that number from the original groups will be uninterested in remaining in the program.

Changes in experimental design and schedule are being worked out to optimize chances for obtaining definitive results. With regard to Group A, since there is so much interindividual variability, perhaps a third baseline reading before beginning the B₆ treatment cycles would be advantageous. Following the beginning of B₆ supplementation it is puzzling that the PLP levels did not respond nearly as well as those in Group B. If this trend is strengthened by further observations, it will be explored in more depth.

2. A group of new contraceptors (Group D) will be followed in the same fashion as other patients receiving the combination o.c. except that they will not receive the intermittent therapy with pyridoxine hydrochloride until some biochemical or clinical evidence suggests a stabilized deficiency which may be corrected by vitamin B6. This group will permit better correlation of findings.
3. As an additional control group, analysis of pyridoxal phosphate will be carried out on normal young women to observe "within day" and "within cycle" variations.
4. Comparison of the parameters of B6 metabolism and the relationship of plasma pyridoxal phosphate to abnormalities in tryptophan metabolism will be broadened by determination of 3-hydroxyanthranilic acid in urine. This test, simpler than the tryptophan load test, could be of value in a national survey.
5. Determination of plasma pyridoxal phosphate, erythrocyte glutamic-oxalacetic transaminase and 3-hydroxyanthranilic acid will also be made on the subjects of the study of Dr. Prasad at Wayne State University. This study encompasses women in higher and lower economic brackets and will enrich the data base in the goal of establishing normal and post-treatment values.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Interaction of Contraceptive Steroids with Essential Dietary Nutrients
Contractor: Wayne State University School of Medicine
Money Allocated: \$70,000 (FY 1973 Funds)

Objectives: The purpose of the epidemiological clinical study is to characterize nutritional profiles of selected and well-defined sub-populations of the Detroit area belonging to different socio-economic levels and users of oral contraceptives. Specifically, status of trace elements and vitamins will be investigated in this study. A correlation between the abnormal nutritional parameters and use of oral contraceptive pills will be sought by including proper controls of similar socio-economic backgrounds who are not using any oral contraceptives. Nutritional status of all subjects will be determined by proper physical examination and detailed dietary history. This will provide a basis for proper evaluation of changes due to use of oral contraceptives per se and interaction between the pills and poor nutrition on various abnormal parameters can be studied.

Significance to Biomedical Research and Program of the Institute: The scientific merit of this project is in its broad coverage of most of the important nutritional factors possibly interacting with oral contraceptive drugs. This study will produce useful information on the effects of contraceptives under the conditions of everyday life, against the background of a good dietary history in two socio-economic classes. The nutrients to be determined are well chosen. The dietary histories will be of value not only in the context of the contraceptive actions but also to nutrition in general. From a nutritional point of view, it is essential to assess any pharmacological effect against the background of dietary history and the conditions of every day life. Even the most sophisticated studies on a metabolic ward have to be extrapolated to conditions outside of the ward. The proposed project will produce a large amount of useful information. The *in depth* studies, as well as the animal experiments, are directed toward important questions. Consequently, the whole project is highly relevant to the objectives of the program.

Proposed Course: Eight hundred subjects are to be included in the epidemiological study. The subjects will be divided according to their socio-economic background into two major groups and each group will then be further subdivided into four subgroups, (i) those who are not taking any contraceptive pills, ii) those taking 1 mg progestogen + 50 µg mestranol, iii) those taking 0.5 mg progestogen + 50 µg ethinyl estradiol and, iv) those women who resume oral contraceptive pills after a pregnancy and during lactation. Women who become pregnant after discontinuation of contraceptive pills will also be included in this study as a special group.

Physical examination for signs of nutritional deficiencies, detailed dietary history, hematological examination and biochemical tests to include plasma--vitamin A, ascorbic acid, folic acid, vitamin B₁₂, zinc, copper, iron magnesium

calcium and cadmium, and red blood cells--folate, zinc, copper and magnesium will be carried out. These data will be correlated with whether or not the subjects are users of oral contraceptives, their nutritional status, socio-economic background and evidences for interaction of contraceptive steroids with essential dietary nutrients will be looked for.

Approximately 800 subjects will be investigated during the first two years of the project. In the third year 15 subjects from each of the groups will be investigated in depth with respect to the changes in trace elements, folic acid and vitamin B₆ metabolism. The physiological significance of a decrease in serum zinc level will be investigated further in order to correlate the total body store of zinc with the serum level. FIGLU excretion following histidine loading, oral absorption tests by using purified polyglutamate folic acid and tryptophane metabolites in urine after proper loading doses of tryptophane will be determined. These studies will provide additional data concerning the nature of abnormalities encountered in the metabolism of micronutrients due to the use of contraceptives.

During the initial operating period of this project the major effort of the investigators were directed to establish the methodology in the laboratory and the logistics and procedures in the clinic. The subsequent enrollment has yielded preliminary results on 150 subjects in the eighth study group of the experimental design. It is expected that by the end of June 1973, at least 250 more subjects will be enrolled, thus bringing the total to 400 within the first year of this contract. Limitations resulting from the short period of the project's operation have precluded a definitive statistical analysis of the accumulated data. Some trends however were noted and appeared remarkable. There appears to be a general trend towards a higher vitamin A and a lower iron binding capacity in patients using the contraceptive pill in all socio-economic groups. Other abnormalities were noted in regard to riboflavin, RBC count, zinc levels. These preliminary analyses suggest that both pill and nutritional status may be playing rolls in effecting the health status of contraceptive users. The continued investigation supported by this contract should provide a large number of useful information to elucidate these relationships.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Human Metabolism of Steroid Contraceptives
Contractor: University of Arizona
Money Allocated: \$114,000 (FY 1973 Funds)

Objectives: The purpose of the program is to evaluate the metabolism of contraceptive steroids given in women who have had complications while on oral contraceptives and also who are receiving concurrent therapy with other drugs. Specifically, it will evaluate whether there are unique steroid metabolic profiles in women having had serious side effects of the oral contraceptives. In addition, it will determine the effects of both acute and chronic administration of other drugs in women who are on chronic courses of oral contraceptive agents. Studies of the effects of contraceptive steroid therapy on the metabolism of vitamin D have also been initiated.

Significance to Biomedical Research and Program of the Institute: This program represents a unique opportunity to determine whether women who have had complications on the oral contraceptives might have some unusual metabolic pattern which might serve as a basis for the clinical effects. In addition, since women are taking oral contraceptives for long periods of time, it is likely that they will be using other potent and effective drugs during the course of this treatment. The possibility of a significant interaction between the use of concurrently used drugs and steroid contraceptives should be evaluated. This program is relevant to the objectives of the Center for Population Research's goal of determining special risks following acute and chronic administration of steroid contraceptive drugs.

Proposed Course: During the previous year inhibition of the metabolism of selected drugs was observed in oral contraceptive users; this effect appeared to be especially prominent in women who suffered thromboembolism. Studies of the effect of drug and steroid therapy on endogenous and exogenous steroid metabolism and the effects of these agents on metabolism of vitamin D are proceeding.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Relationships of Contraceptive Steroids to Nutritive State
Contractor: Tulane University
Money Allocated: \$100,398 (FY 1973 Funds)

Objectives: This project is based upon the hypothesis, that since contraceptive steroids produce physiologically a "pseudo pregnancy" state, nutritional complications of pregnancy may develop in persons using these steroids. The general purpose of this proposal is to examine the nutritional status of a number of individuals, who are potentially at high risk nutritionally and attending a family planning clinic. Some will be taking contraceptive steroids and others will serve as controls. It is felt that if contraceptive steroids create nutritional stresses, these can be most easily identified by studying women who received CS through family planning clinics. The specific objectives of this project are to examine the nutritional status of 50 new and 100 long-time users of oral contraceptives who are potentially a high risk nutritionally. Results shall be compared with two appropriately matched control groups who use other methods of contraception.

Significance to Biomedical Research and Program of the Institute: This project promises to provide detailed information on the broad nutritional profile of young women beginning contraceptive therapy immediately after pregnancy and of long time contraceptive users. The characteristics of the population to be studied offers several unique advantages: Past experience indicates a high prevalence of anemia, marginal or low serum vitamin A levels and possible folic acid deficiency. The experimental design of the project appears scientifically sound and clearly elaborated. It should provide a broad spectrum of data which characterize the influence of contraceptive steroids on the nutritional health status in a population from a low socioeconomic level.

Proposed Course: The basic design of this proposal is to determine the nutritional health status of women in each of four groups. The first two groups, of 50 women each, will consist of young individuals who are recruited during pregnancy into the family planning clinic for postpartal advice. These will be individuals who had never previously taken contraceptive steroids. Group I will consist of 50 women who, by their choice, select contraceptive steroids as the means of family planning. Many in this group will have the added stress of having a baby during their teenage years when they are still growing. Group II, a control group, will consist of women matched for age, parity, socioeconomic status, height and weight, and who choose methods of family planning other than steroids. Group III will consist of approximately 100 women attending the family planning clinics who have used contraceptive steroids for over 2 years. Group IV, a control-group, will consist of 100 women who choose methods of family planning other than steroids, but otherwise are comparable to Group III. Groups I and II will be examined at their 6 week postpartal visit to the clinic and again six months later. These women can be used to determine if nutritional effects are observed in addition to those related to pyridoxine and folic acid deficiencies which have been reported by Luby and others.

Groups III and IV will be used to determine whether there are consistent differences in the nutritional state of women who have received contraceptive steroids for a period of two years and those who have not. The increase in the size of these two groups (100 women each) is necessary because only one evaluation will be made with no prior baseline data. Five categories of data will be obtained in all women in each of the four groups: (1) clinical, (2) hematologic findings, (3) serum and urinary vitamin levels, (4) serum lipids and proteins, and (5) tryptophan load tests and blood glucose concentrations.

During the initial operating period of approximately 8 months approximately one-half of the individuals have been selected for Phase I which involves studies of women prior to and after their first six months of using contraceptive methods. The second examination period will begin in March 1973. Of the fifty women selected, nearly all tests have been completed as proposed with the exception of performance of assays to determine pyridoxine status. All other data are presented in the renewal report with the exception of dietary information which has not been calculated as yet. In phase II of this contract, individuals will be studied who have been using contraceptive methods for more than two years. The examination of these women has just been initiated and sufficient findings are not available to be reported. The available data is insufficient for even preliminary evaluation of the effect of contraceptive steroids on nutritive state. The nutritional abnormalities observed suggest that the population studied will be found to have some anemia due to low intake of iron and/or of folic acid. There is also an indication of low vitamin C intake. Ten percent of the women have cholesterol levels greater than 250 mg percent.

Before the end of the first year of this contract's lifetime all of the subjects should be selected in Phase I. In the Contraceptive Steroid Group recruitment will continue until approximately 100 individuals have been selected. This should provide a sufficient number of individuals so that a group of at least 50 can be examined six to twelve months later. There has been more difficulty in recruiting a group of non-steroid users. The mechanics of recruitment are such that it is not known until women arrive at the clinic which method of contraception will be chosen. The ratio of contraceptive steroid users to other forms in this group of women had not been as anticipated, namely seven to one rather than two to one. This will require a longer recruitment period for this group. It is felt that the initial sample selection of 50 to 75 women can be accomplished before the end of the first year's contract period. The six months evaluation, however, cannot be completed during this time.

The initial examination of at least 100 women using contraceptive steroids for a period of two years or more, and of a matched control group which has used an alternate method for a similar period, will have been completed by the end of the first year's contract period. It will require some additional time to process all data for this study. There does not appear to be a problem in this phase of the study in obtaining a sufficient number of women who have been using methods other than contraceptive steroids for two years. Additional time will be required for completion of the analyses of serum and whole blood, serum B₁₂ and pyridoxine assays for this study as well as samples obtained from Michigan and Texas. The evaluation of the women in Phase II of this proposal (those using contraceptive methods for more than two years) should

be nearly complete before the end of the first year's contract period. Additional time will be required, however, to complete the biochemical analyses and for the evaluation of the data.

Phase III of the study will consist of evaluation of data to determine the effects of the contraceptive steroids and to devise methods for nutritional intervention to correct any abnormalities observed. This phase of the program should be coordinated among the Michigan, Texas and Louisiana groups to determine which of many nutritional supplements used have an effect and whether or not the level used is sufficient to correct the nutritional problem. The detailed protocol of this phase will have to be worked out after initial evaluation of data to determine which of the nutrients need to be supplemented.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Study of Differential Effects of Oral Contraceptives on
Carbohydrate Metabolism
Contractor: New York University - School of Medicine
Money Allocated: \$20,000 (FY 1973 Funds)

Objectives: This study is designed to determine whether individual oral contraceptive formulations have unique effects on blood glucose, triglycerides, insulin and growth hormone in women who are obese, pre-diabetic or diabetic. Different preparations representing different dose levels of drugs will be studied in the same women to assess these changes.

Significance to Biomedical Research and Program of the Institute: It has been well established that oral contraceptive drugs cause decreased glucose tolerance and increase in circulating lipids. Whether these changes predispose, make worse or cause diabetes as well as arteriosclerotic cardiovascular disease is uncertain. It is not known whether women who are at risk of having diabetes mellitus are affected adversely by the use of these steroid drugs. The purpose of the program is to evaluate these potential risks in a population in special need of birth control. In addition, information of general value for contraceptive use in population would be made available.

Proposed Course: As noted in a previous report, there have been continuing difficulties in recruiting an adequate number of high risk patients and also concerning the accuracy and precision of the laboratory work. Since these problems were not adequately resolved in a submission from the principal investigator, a short concluding period of support was determined to be the best course of action. A final report of the study is now in preparation.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: An Epidemiologic Case-Control Study of Thromboembolism
Related to the Use of Oral Contraceptives
Contractor: Johns Hopkins University - School of Hygiene & Public Health
Money Allocated: \$40,698 (FY 1973 Funds, 6/22/73 - 12/22/73)

Objectives: The purposes of this study are: 1) to investigate further the association between oral contraceptive use and thromboembolism in order either to substantiate or to define further the risks of developing these phenomena among users; 2) to provide a continuing surveillance of the risks of thromboembolic disease associated with the usage of oral contraceptives and to monitor adverse reactions to new formulations as they come into wider use; 3) to define further any possible predisposing factors that may increase the risk of developing thromboembolic disease in oral contraceptive users; 4) to determine if there is a difference in the relative risk of developing thromboembolic disease associated with different formulations of oral contraceptives, e.g., sequentials and non-sequential; and to determine if a dose-response relationship occurs with increasing amounts of estrogen; and 5) to investigate further the reported proportional lesser incidence of thromboembolism in persons of blood group O.

Major Findings: By January 1, 1973 over 400 cases of thromboembolism among women of the child-bearing age group were collected. Three hospitalized controls were sought for each case. The investigators were in large measure successful in completing these sets. It is anticipated that by June 15, 1973 over 500 cases will be collected. Case-collection will be terminated at this time. The process has proceeded satisfactorily and the number of cases and controls will permit answering the research questions specified in the objectives of this contract.

Validity and reliability studies are in progress and suggest that these issues will present little problem for this investigation. Analysis of "missed" cases reveal no obvious biases leading to differential selection of the "found" cases.

Preliminary analysis of results suggest a lower risk of thromboembolism is associated with the lower estrogen dosage oral contraceptives. Plots of risk against dosage suggest a linear relationship.

The series of cases accumulated so far does not appear to show a higher frequency of most of the so-called "risk-factors" for the thromboembolism among the cases as compared to controls. The upcoming final analysis should shed further light on the importance or spuriousness of these postulated "predisposing conditions". However, the preliminary analysis suggests that no particular predisposing conditions will be uncovered by this study which might be used to predict (and thus label as contraindications) higher risk of thromboembolism should these women use the oral contraceptives. This will be

unfortunate but important to know lest such labelling give a false sense of safety.

Significance to Biomedical Research and Program of the Institute: Several studies have now established an association between oral contraceptive usage and the development of thromboembolic disease. Questions as yet unanswered concern the relative risk of developing thromboembolic disease as related to usage of different formulations of oral contraceptives containing various amounts of estrogenic compound or a progesterone. In addition, it is still unclear which medical conditions or predisposing factors increase the risk of developing thromboembolic disease among oral contraceptive users. Studies supported by this contract should provide essential information to establish or rule out potential contraindications to the use of oral contraceptives. It should also help to determine which formulations of these agents present the greatest hazards to users. This study is important, well-designed, and is being carried out by an experienced group of investigators. The significance of the study is re-emphasized by recent articles questioning the association of oral contraceptives and thromboembolism.

Proposed Course: This project is in the stage of approaching final conclusion of all anticipated and specified studies. It seems a reasonable expectation that all specified objectives will be reached within the continuation period of this contract, requested by the investigators.

Data analysis is proceeding according to schedule. Several difficult problems for further analyses are being approached at present: age-adjustment; classification of cases by certainty of diagnosis and severity of thromboembolic condition; and finally, separation of idiopathic cases.

Case-collection will end in June 1973 but an additional 6 months will be required for final analyses and reporting of findings and results. Final completion of all work related to this project is expected in December 1973. The support requested in this forthcoming final contract period is for these concluding activities such as data processing, analysis of data and final report preparation.

Abstracting of case-histories, re-interviewing for missing data, checking on incomplete data and for missed cases will all continue for a few months following cessation of case-collection. The final review of case material and editing, coding and entry onto tape will continue for several months (estimated completion, September 15). Validity and reliability checking will proceed until mid-September also.

After all processing is completed, and all data are edited and on tape, final analysis will be undertaken. This is expected to last from September 15 to November 15 and to involve a series of computer runs. As the data is analyzed, additional unplanned analysis will become apparent and carried out.

Because of the sensitive nature of this study and the possibility that any preliminary findings would receive wide press coverage, publications and presentations have deliberately been avoided. The final results will be communicated to the NICHD before presented, and, to the FDA so that responsible agencies

will be forewarned of any significant findings.

About six weeks (November 15 to December 30) will be needed to write the final report, prepare graphs, tables and other figures, obtain any needed consultant advice and thus complete the work.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effect of Oral Contraceptive and Conjugated Estrogens
on Renin Activity and Aldosterone
Contractor: Loma Linda University, Loma Linda, California
Money Allocated: \$28,077 (FY 1973 Funds)

Objectives: The investigations conducted under this project are aimed towards three principal goals. Firstly, to characterize the biochemical effect of various estrogenic and progestogenic compounds on the renin-angiotensin-aldosterone system as well as on sodium metabolism of normotensive contraceptive users. The second goal is to study these effects in persons developing hypertension either in young women on the oral contraceptive pill or in menopausal women receiving conjugated estrogen replacement therapy (Premarin). A new goal formulated on the basis of past results is to find an OCA preparation which lacks the mineralocorticoid effect of the presently available ones.

Major Findings: Ethinyl estradiol in normal subjects caused a marked increase in renin substrate with a slight increase in renin activity and aldosterone excretion rate under basal conditions. The responsiveness of the renin-angiotensin system to sodium restriction and standing was accentuated. Plasma levels of aldosterone, however, were unaffected. The administration of the estrogen was associated with about a 200 mEq increase in exchangeable sodium.

Other estrogenic compounds in normal subjects exhibited a similar stimulatory effect on the renin-angiotensin system with the production of a mild hyperaldosterone state and sodium retention. Two gestagens that were studied appear to cause sodium retention and suppress aldosterone excretion rate by a direct mineralocorticoid-like effect. Some gestagens have what appears to be an estrogenic effect with an increase in substrate concentration, but two out of the five that have been tested caused a slight decrease in renin substrate concentration. The addition of 25 mg of spironolactone with the usual dose of ethinyl estradiol was insufficient to counteract the sodium-retaining properties of the estrogen. A larger dose would appear to be needed.

Natural progesterone, on the other hand, causes a negative sodium balance with a secondary increase in renin activity and aldosterone excretion rate. A study of the data obtained from the patients who had developed hypertension after starting an estrogenic compound indicated the following points: When the estrogenic compound was discontinued plasma renin activity and aldosterone excretion rate decreased generally, and the exchangeable sodium decreased in the majority of the patients. Some of the patients continued to have a slight elevation in exchangeable sodium one month after the medication was discontinued.

Roughly 40% of those who had developed hypertension after starting Premarin are now normotensive without medication. It required up to 12 months after the medication was discontinued before the patients remained normotensive without spironolactone administration. Over 60% of the hypertensive patients who took spironolactone after they had discontinued the Premarin became normotensive in

one to three weeks on the spironolactone.

A few patients have been observed who developed hypertension after starting diethylstilbestrol. One of these has a blood pressure that now remains normal without medication. Seventy-five percent of them became normotensive during the administration of the spironolactone.

Ten patients have been studied during this contract period who had developed hypertension after starting an oral contraceptive. Over half of those who have been followed properly have become normotensive without medication. All of those who have had adequate followup have become either normotensive without medication or can be maintained normotensive with 200 mg or less of aldactone per day. In summary, synthetic estrogens, diethylstilbestrol, or the conjugated estrogen, Premarin, can all cause hypertension. The cause appears to be a result of a mild hypermineralocorticoid state produced by the medication coupled with the absence of the antimineralocorticoid effect of natural progesterone (because it is suppressed).

Significance to Biomedical Research and Program of the Institute: The scientific value of the proposed work lies in its promise to clarify pathophysiological relationships between steroid metabolism, electrolyte balance and hypertension. This knowledge is relevant to the program of the Center in order to assess risk and consequences of contraceptive induced hypertension and possibly devise an approach to its therapy and/or prevention.

Proposed Course: Four lines of work are contemplated for the next contract period, proceeding towards the following objectives: (1) To find more patients with hypertension who date the onset of their elevation in blood pressure after starting an estrogenic compound or an oral contraceptive agent, (2) to continue observations of exchangeable sodium, renin substrate, renin activity, and aldosterone levels in urine and blood in those patients who had developed hypertension on estrogenic compounds in an effort to determine the pathological physiology of this type of hypertension, (3) to find an orally effective gestational agent that has sodium diuretic properties similar to natural progesterone, and (4) to evaluate the effect on the renin-angiotensin-sodium system of a larger dose of spironolactone in combination with an estrogen and the effect of one or two gestational agents that promise to have sodium diuretic properties. The latter will include a study of the effect of these on exchangeable sodium, renin activity, and aldosterone concentration in plasma and urine.

The renin-angiotensin-aldosterone system and exchangeable sodium in patients who have developed hypertension on an oral contraceptive agent, on a conjugated estrogen, or on diethylstilbestrol is to be characterized. Initial studies will be made while they are on the estrogen and then repeated after one month off the medication. They will be followed to determine the incidence of return of pressure to normal and/or response to a spironolactone. The effect of a combination of ethinyl estradiol with a 100 mg per day dose of spironolactone and the effect of a new progestational agent, Norgestrol (Wyeth), on the renin-angiotensin-aldosterone system and exchangeable sodium of normal subjects are also to be investigated.

In the search for sodium diuretic, antihypertensive contraceptive agents, the following regimes are specified: (1) ethinyl estradiol with 50 mg of spironolactone twice daily, (2) Norgestrol (Wyeth Co.) one mg daily for 3 weeks. Seven to 10 normal subjects of reproductive age will be studied on each specified medication. Tests of the function of the renin-aldosterone system will be made in the second to third week of the menstrual cycle before medication is started and then again in the second to third week of the subsequent menstrual cycle during the administration of the medication.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Evaluation of Effects of Oral Contraceptives on Vitamin B6
Nutrition

Contractor: University of Wisconsin Medical School

Money Allocated: \$96,891 (FY 1973 Funds)

Objectives: The objectives of this study are to determine if the use of oral contraceptives enhances the rate of induction of vitamin B6 deficiency in women on a defined intake of vitamin B6 and folic acid, and to determine accurately the amount of vitamin B6 required to correct the metabolic alterations observed.

Significance to Biomedical Research and Program of the Institute: Altered tryptophan and vitamin B6 metabolism appears certain to occur in a number of women ingesting oral contraceptives whether these alterations have significant consequences to impaired health remains unclear. The studies outlined in this proposal represent an attempt to provide information concerning this problem. Eventually much larger population groups would need to be studied. Results of this project may be expected to delineate those aspects that would be of importance in such a population study. It is therefore highly relevant to the program. Since prior studies of vitamin B6 metabolism in pregnancy and conditions of illness have not considered or controlled intake of the vitamin, clarification can be expected through use of healthy subjects under conditions of depletion and repletion.

Proposed Course: It is proposed to study vitamin B6 metabolism in 24 healthy young women. Six would serve as control subjects who have not used oral contraceptives. The remaining 18 subjects would represent women who have regularly used oral contraceptives for at least 6 months. All subjects will be placed on a defined diet providing daily 75 gms of protein and 0.16 mg of vitamin B6. The nutritional and metabolic functions related to vitamin B6 and folic acid will be assessed initially and at intervals while consuming the deficient diet. The rate of induction of metabolic deficiency will be compared between the controls and those receiving the steroids. Following a deficiency period of 28 days, the subjects will be repleted for 28 days with known amounts of vitamin B6. The control subjects will receive 0.8 mg of additional pyridoxine daily, while the oral contraceptive users will be divided into 3 groups and receive either 0.8, 1.5 or 15 mg of additional pyridoxine daily. Upon completion of this period, all subjects will be supplemented with 20 mg of additional pyridoxine for 5 days. The metabolic studies will be conducted at comparable phases of each subject's menstrual cycle in order to avoid possible cycle-related metabolic variations. These studies will include measurement of tryptophan metabolites following a tryptophan load, measurement of cystathionine and other amino acids following a methionine load, measurement of urinary creatinine, 4-pyridoxic acid 5-hydroxy-indoleacetic acid, 3-methoxy-4-hydroxymandelic acid, measurement of plasma kynurenine, folic acid and pyridoxal phosphate and measurement of erythrocyte transaminases. Mental status

will be evaluated through the use of a self-administered test. Electroencephalographic examinations will be administered on each subject before depletion, during depletion, and after resupplementation with pyridoxine. Kynurenine load tests will be used to determine whether or not any altered tryptophan metabolism is due to a vitamin B6 deficiency or to elevated tryptophan pyrolase activity.

During the first six months the project study has progressed on schedule with no major problems encountered. As proposed, part of the number of subjects were recruited and studied during the period September - November 1972. Eight subjects (3 controls and 5 oral contraceptive users) were carried through the 65 day dietary period during which time they ingested only the basal, low-vitamin B6 diet described in the initial proposal. This diet was supplemented with 0.8 mg of pyridoxine per day for the initial 5 days during which time baseline urine and blood samples were collected and subjects became adjusted to the diet and to the routine of urine and blood collections. After this period the vitamin B6 supplement was withheld from all subjects for one complete menstrual cycle. For the second menstrual cycle the same diet was continued but control subjects were supplemented with 0.8 mg pyridoxine hydrochloride per day, and the oral contraceptive users supplemented with 0.8 (two subjects) 2.0 (two subjects) or 20 (one subject) mg per day.

Complete 24 hours urine samples were collected every day throughout the 65 day study period, and blood samples drawn weekly. Oral loading doses of L-tryptophan (2.0 g) or L-methionine (3.0 g) were given weekly and oral loads of L-kynurenine (200 mg) were given initially, at the end of the depletion period and at the end of the repletion period.

Results obtained so far suggest that women using oral contraceptives become depleted faster than non-users when fed a diet low in vitamin B6. This data is preliminary and is based on very few subjects. Final interpretations must await completion of data on additional subjects presently under study.

Fourteen additional pill users and 6 control subjects have been recruited and enrolled to complete the specified number of subjects to 24. This later group has started on the diet and will complete the dietary period before Spring Recess (April 14, 1973).

Those analyses necessary to monitor the extent of deficiency in each subject will be kept current. These include plasma pyridoxal phosphate, the erythrocyte aminotransferase, steam volatile assay for total kynurenines, and xanthurenic acid. Because of the increased number of subjects to be studied, more of the personnel are necessarily involved in the dietary logistics. Other assays will have to wait until completion of the dietary phase in April. It is believed that all contracted measurements will be completed by the end of the contract period with the exception of urinary cystathionine and other urinary amino acids.

On completion of the analyses, all data will be tabulated, correlated and reduced statistically and results reported to NICHD and papers prepared for publication. This should be completed within 9 months of completion of analyses.

The fundamental objectives of this contract, i.e., "Evaluation of Effects of Oral Contraceptives on Vitamin B6 Nutrition" will be achieved when the data are complete and papers published.

At the early date of preparing the renewal proposal, the outcome of the ongoing studies cannot be predicted. The proposal for renewal with continuation for a second year appeared, therefore, a logical decision of the investigators for completing present studies and extending them to related metabolic effects of oral contraceptives.

In view of the previously recognized alterations in glucose tolerance tests found in users of oral contraceptives, and particularly in view of the recent reports by Spellacy and by Rose that pyridoxine tends to normalize the abnormal glucose tolerance tests of oral contraceptive users, it was felt that the present study provides a unique opportunity to extend these observations to include the effects of vitamin B6 depletion on glucose tolerance tests. These tests are to be done on controls and oral contraceptive users at three intervals during this dietary study; (a) initially before depletion, (b) at the time of maximum depletion, and (c) after repletion with pyridoxine. The additional costs for this expanded investigation are small.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Interaction of the Contraceptive Steroids and Vitamin K
Absorption and Utilization
Contractor: Southern Illinois University, Edwardsville, Ill.
Money Allocated: \$34,031 (Contract was awarded in 1972 for a 16 month period.
No additional funds were committed in FY 1973)

Objectives: Thromboembolic accidents have been reported to occur at a significantly higher rate in women on oral contraceptives than they do in control subjects. Cause of this phenomenon is not well understood. It has been postulated that the contraceptive steroids may substitute for vitamin K or vice versa, or they may influence the gastrointestinal absorption of vitamin K. The objectives of this proposal are to: determine whether the molecular configuration of a particular estrogen or progestogen can be related to its vitamin K potentiating, or substituting effects, or its ability to facilitate vitamin K absorption.

Major Findings: Due to operational difficulties encountered at the onset of this project, it has not reached the stage of yielding major findings. Useful operational results are apparent in the establishment of differential baseline values for vitamin K depletion in male and female rats placed on a K-deficient diet. While the males showed an immediate prothrombin defect, vitamin K deficiency in the female, first induces a hypercoagulative state, followed in 5-8 days by a pronounced prothrombin deficit. Presumably this obvious sex difference in resistance to vitamin K deficiency is due to differences in the spectrum of sex hormones. In presently ongoing experiments, the regime of vitamin K depletion is combined with the administration of ethinyl estradiol.

Significance to Biomedical Research and Program of the Institute: The studies outlined hold considerable promise of providing basic information concerning the significance and mechanism of an apparent interaction between oral contraceptive steroids and vitamin K. In view of the possible implication of these effects in the pathogenesis of thromboembolic disease, the project is clearly relevant to the objectives of the program.

Proposed Course: The first phase of the study would be concerned with investigating the mechanism whereby estrogenic steroids have been reported to substitute for vitamin K in the maintenance of normal coagulability. Several approaches will be employed: (1) the relative rates of vitamin K depletion will be studied in normal female, male and castrate rats given either an estrogen or progestogen, both separately and in standard anovulatory combinations to establish the relative contribution of these several factors to the coagulation picture; (2) the estrogenic effects of vitamin K depletion will be studied in the normal and castrate females and the potentiation of testosterone on vitamin K deficiency or inhibition measured; (3) a wide variety of estrogens and progestogens will be assayed with the objective of evaluating chemical structural requirements for both vitamin K activity and estrogenic effects. It is hoped that the results of these studies to be conducted during

the first contract period will provide the basis and guidance for more specific enzymatic investigations. Moreover, the nutritionally oriented studies would provide information as to whether or not the steroids have an influence on the gastrointestinal absorption of vitamin K. If evidence is apparent of such an effect, studies will be conducted to evaluate its significance through the use of radioactively labelled vitamin K in rats with cannulated thoracic lymph ducts. Following the completion of these phases of the study, enzymatic experiments would be conducted to determine how and where the steroids fit into the vitamin K metabolism and how vitamin K can substitute for estrogen.

The operational start of this project was delayed by unexpected renovation problems and unforeseen operational difficulties. This led to a request for an extension of the contract period without additional funds. All difficulties had been overcome in December 1972. Progress since then has been orderly, encouraging and satisfactory. Nevertheless the status of the project has been estimated to be 3-4 months behind schedule. Projected goals for the first year's performance are hoped to be achieved by October 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Investigation of a Possible Interaction of Contraceptive Steroids with Vitamin E and Polyunsaturated Fatty Acids
Contractor: University of California - Los Angeles, California
Money Allocated: \$71,060 (Contract was awarded in 1972 for a 2-year period. No additional funds were committed to this project in 1973)

Objectives: It has been observed that in the rat the effects of administering oral contraceptive compounds resemble those resulting from vitamin-E deficiency. These include interference with reproduction, changes in lipoprotein distribution, and a decrease in polyunsaturated fatty acids in various tissues. The possibility exists that these anovulatory drugs enhance the requirement for vitamin E. Therefore, this investigation is designed to study the possible interrelationships between oral contraceptives and vitamin E. It is possible that oral contraceptive treatment results in situations where increased vitamin E and possibly increased polyunsaturated fatty acids are required for some essential physiological processes.

Major Findings: The hemolysis of RBC from rats kept on a E-deficient diet was almost 100%. Slightly less hemolysis was noticed in rats fed the E-deficient diet but with BHT. No effect of Enovid treatment on hemolysis was evident. While no vitamin E was present in plasma of the E-deficient groups, a somewhat lower than a control level of plasma vitamin E was found in the drug-treated rats. The difference was quite significant in rats dosed for 28 days. α -lipoproteins decreased and β -lipoproteins increased during treatment resulting in a significant lowering of the α/β ratio. Liver TBA values were significantly increased in the E-deficient groups. Adipose tissue TBA values showed an increase in the drug-treated, E-sufficient rats after 28 days of dosing. Plasma cholesterol levels were decreased or at least showed a tendency toward a decrease when the drug was used. These changes, as well as the increases in liver cholesterol, and decreases in adrenal cholesterol, were less significant when vitamin E was absent. Similarly, arachidonate decreased in plasma lipid fractions but less significantly in the absence of vitamin E.

Significance to Biomedical Research and Program of the Institute: The project addresses itself to the problem whether oral contraceptives affect the vitamin E status of young women. This is relevant to the objectives of this program.

Proposed Course: It is proposed to administer the oral contraceptive drug Enovid E, to adult female rats kept on diets either adequate or deficient in essential fatty acids, with or without dietary tocopherol or with an antioxidant BHT (butylated hydroxytoluene) since in some investigations the antioxidant, BHT, has been shown to alleviate some vitamin E deficiency symptoms. The possible effect of tocopherol repletion in vitamin E-deficient animals given the oral contraceptive drug will be examined as well. At the end of the experimental period, animals will be killed and cholesterol levels and fatty acid patterns of lipid fraction of various tissues will be determined along with alpha/beta lipoprotein ratios, serum tocopherol levels and the suscepti-

bility of erythrocytes to hemolysis, a test which has been used as an indication of vitamin E status.

In addition, a comparative survey of vitamin E status of young women given oral contraceptive drugs for various lengths of time, with untreated young women serving as controls, will be done. This will involve determinations of plasma tocopherol levels, plasma cholesterol and α/β lipoprotein ratios.

The study on the interrelationship of dietary content of vitamin E and contraceptive drugs is being completed in the first year of the contract (Experiment I). The project on the effect of essential fatty acid deficiency as an added variable in the tocopherol-steroid interrelationship has been progressing well. Definitive results of this line of investigation are expected in the near future. The comparative survey of vitamin E status in young women on oral contraceptives and appropriate control is progressing according to schedule and preliminary results are expected to be forthcoming shortly.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Effects of Contraceptive Steroids on Trace Elements
Contractor: University of California at Berkeley
Money Allocated: \$136,000 (Contract was awarded in 1972 for a 2-year period.
No additional funds were committed to this project in FY1973)

Objectives: Definitive information pertaining to the effect of oral contraceptive steroids on trace elements is missing. While many observations have been made pertaining to the influence of OCA on iron, copper and to a lesser extent, zinc metabolism for the most part these observations are directed towards determination of blood levels without regard to trace mineral balances. The objectives of the project are to investigate the effects of contraceptive drugs on trace elements, balance of iron, copper and zinc in women kept on controlled diets in a metabolic ward.

Major Findings: The project has not yet yielded significant results of major importance.

Significance to Biomedical Research and Program of the Institute: The proposed study of iron, copper, and zinc is justified and desirable. These three trace elements are known to be essential. For two of them (iron and zinc) we suspect or know that nutritional problems of suboptimal intake exist in some population groups, and all three are known to be in some way influenced by contraceptive drugs. The proposed balance studies will not only better define a number of metabolic changes, but they can be expected to furnish much needed information on the minimum daily requirement for these trace elements under normal conditions and with contraceptive medication. Only through carefully controlled metabolic studies within a metabolic ward can one accurately define the influence of contraceptive steroids upon mineral nutrition. The investigators possess one of the few unique capabilities in this country to conduct such detailed studies and the staff with the knowledge to provide adequate supervision for such a demanding project.

Proposed Course: It is proposed to conduct a rigidly controlled metabolic study of 6 young normal adult female graduate students who will be maintained under rigidly controlled conditions in a metabolic facility for 17 weeks. They will be fed a constant formula type diet containing all known essential nutrients. Observations to be made will be analysis for all losses including urinary, fecal, skin and menstrual losses; intakes will be determined by analyses of the diet. The subjects will receive in a randomized fashion two months of no steroidal agent, one month of a sequential type oral contraceptive and one month of a combined estrogen-progesterone agent. In addition to studies of trace elements, they will also further evaluate the effects of contraceptive steroids on calcium, magnesium, and nitrogen balances. These balances will be particularly observed in regard to cyclic variations. Blood samples will be obtained and analyzed three times per menstrual cycle. Basal metabolic rates and requirements to perform a fixed amount of work will be determined throughout

the cycle. Body weight variations will be observed. The trace mineral balance studies are primarily concerned with iron, copper, and zinc, employing conventional methods of analysis (absorption spectrometry for zinc and copper and calorimetric analysis for iron).

The initial period of the contract was consumed in preparing the human nutritional laboratory for trace mineral determinations. It required design, construction and installation of a recirculating water supply system to avoid undetected contaminations. Problems were also encountered in the laboratory with setting up optimal techniques for trace mineral analysis of human excrement. On account of these difficulties encountered enrollment of human subjects appeared not to be feasible until the end of March 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effect of Oral Contraceptive Steroids on Calcium, Phosphorus, Magnesium and Zinc Metabolism, with Emphasis on Bone Mineralization

Contractor: Veterans Administration Hospital, Washington, D. C.

Money Allocated: \$22,298 (Contract was awarded in 1972 for a 15 month period. No additional funds were committed in FY 1973)

Objectives: This proposal is designed to study the effect of steroidal contraceptive agents on mineral metabolism. The influence of a progestin, an estrogen, and a combination of these hormones on calcium, phosphorus, magnesium and zinc metabolism will be investigated. Particular attention will be focused on bone metabolism.

Major Findings: Major efforts were necessary to overcome technical and operational problems. The investigative phase of the project has been initiated in an orderly and systematic fashion but was of too short a duration to yield major results.

Significance to Biomedical Research and Program of the Institute: The effect of anovulatory steroids on calcium, phosphorus, magnesium and zinc has not been established in a definitive manner. All of these mineral nutrients are important in the continuous process of bone formation and resorption. No known calcium balance studies have been carried out in human subjects receiving the oral contraceptives. There is conflicting evidence in the literature regarding the effect of anovulatory steroids on serum calcium, phosphorus, magnesium and zinc. Some, but not other investigators, have seen decreases in serum calcium, phosphorus, and magnesium during steroid ingestion; only the decreases of serum zinc appears well established. None of the available information allows a conclusion as to the effect of steroids on the nutrition requirement for these minerals. It can be expected that the proposed study will show whether or not the contraceptive steroids produce an increased requirement, as expressed by negative balance. Particular emphasis is to be placed on calcium which will be studied at three dietary levels. The duration of the experiment is enough to allow for adaptation of the animals to low or high calcium takes, so that a valid interpretation can be made. It is important to assess the effect of the anovulatory agents on mineral metabolism since their prolonged use at a critical time (pre-pregnancy and pre-susceptibility to osteoporosis) may be detrimental to bone integrity.

Proposed Course: One hundred and twenty female rats will be equally allotted by weight into three principal groups receiving different amounts of calcium: A. Control diet (0.6% calcium), B. Optimal calcium diet (1.2%), C. Inadequate calcium diet (0.2%). These three groups of rats will simulate populations receiving a normal, high (such as during lactation), and low calcium intake. The three groups will be divided into four sub-groups with equal numbers of animals and will be fed the hormone(s) as follows: (i) no hormone, (ii) norethindrone, a progestin, (iii) mestranol, an estrogen, (iv) norethindrone and

mestranol simulating a combination oral contraceptive agent. The hormones will be fed in the diet for 120 days in amounts proportional to the body weight dosage used with human subjects. During the study two seven-day metabolic balance studies will be conducted to determine the retention of calcium, phosphorus, magnesium and zinc. At the termination of the investigation the concentration of these nutrients in serum, femur and liver will be determined. Bone length, density, cortical thickness, crystallization and histology will serve as additional parameters.

Considerable efforts during the first few months of the contract were required to prepare for a vigorous, orderly operation of the investigation. The preparatory work included: special training of technical personnel, formulation and analysis of the various diets and design and manufacture of special metabolism cages made of stainless steel and with zinc-free solder. Difficulties in preparation of quantitative zinc diets have been recognized, investigated and overcome.

On January 17, the animal experiment began with 120 rats fed the 12 different diets. The projected duration on the study as outlined in the contract is 120 days after the one week acclimation period. Thus, the animals will be sacrificed on May 30, 1973. The first collection period for the balance study (seven day period instead of only five days as required by the contract) was completed on February 14.

The investigators expect to have completed the animal experiment and collection of the samples from the two balance studies by the end of the current contract period. Analysis of the collected material, calculations and statistical evaluation will be in progress.

The work required to complete the contract objectives includes the following:

1. Complete analysis of all biological samples. With duplicates this will include 960 analyses on the blood samples and 960 analyses on the bone. These analyses will be for calcium, phosphorus, magnesium and zinc.
2. Determination of the fresh weight, fat free dry weight, ash weight, specific gravity, length and amount of organic material in the bone (femur).
3. Histological evaluation and cortical thickness of the femur.
4. Statistical computations and summarization of all data.

The original contract estimated the time required for completion of the analyses would be six months and statistical computations and summarization of data would be one month, or a total of seven months after completion of the animal experiment. This estimate, however, appears conservative and one year would be more realistic.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Biochemical Mechanisms of Contraceptive Steroid-Induced Abnormalities in Absorption, Metabolism and Function of Vitamin K
Contractor: University of Nebraska College of Medicine
Money Allocated: \$88,193 (Contract was awarded in 1972 for a 2-year period. No additional funds were committed to this project in FY 1973)

Objectives: The purpose of this project is to elucidate the mechanism of estrogen-induced changes in the requirement for vitamin K-dependent clotting proteins, and the decrease in sensitivity to oral anticoagulants. Specific objectives are to: (1) Determine the effect of estrogen and androgen on the quantitative response of deficient rats to vitamin K, (2) Characterize the effect of estrogen on oral anticoagulation in the rat, and (3) Ascertain the localization of vitamin K and the activity of vitamin K metabolizing enzymes in the liver of estrogen-treated animals.

Major Findings: Within the initial period of this project satisfactory progress has been reported in all lines of investigation directed towards the above objectives. The progress achieved suggests that all objectives, as specified above, will be achieved in the second contract period. Major findings have not yet been crystallized. Preliminary results have focused on an important tentative hypothesis, that a microsomal polypeptide precursor of prothrombin could be altered by estrogens and account for its coagulatory effect, and that of related synthetic compounds.

Significance to Biomedical Research and Program of the Institute: The most well documented serious non-contraceptive effects of the anovulatory steroids is the higher incidence of thromboembolic disease, resulting from such treatment. Vitamin K has been implicated in this syndrome more than any other nutrient. In general, there appears to be a positive interaction between vitamin K and the steroids which may be the result of "K"-like activity from the steroid or the steroid increasing the efficiency of K utilization. This study proposes to investigate this interaction by examining the effect of the steroid on K absorption, metabolism and utilization. The procedures outlined appear excellent for most of these tasks. Administration of the labelled and cold vitamin by intracardiac or tail vein injection do obviate the problems of absorption. Isolation of appropriate subcellular fractions should provide data to indicate if the steroid effect is on the receptor site. Concentration on the vitamin K metabolizing system should tell if activation is the principle point of attack.

Proposed Course: The scope of the project involves three lines of investigation. In the first part of the study, the requirement for vitamin K under various regimes of androgen and estrogen administration will be determined. In these studies, K-deficient rats with and without steroids will be given various levels of K in the diet. Blood samples will be assayed for K and for clotting factors such as prothrombin. The level of K in the liver will also

be determined. In the second line of investigation, the effect of androgen and estrogen on retention and distribution of vitamin K in subcellular fractions of liver will be assessed. The influence of steroids on the enzymes responsible for the interconversion of vitamin K and its 2, 3 epoxide will be characterized. For the kinetic analysis of K metabolism under the influence of hormones H³ and C¹⁴ labelled phylloquinone will be employed. A third line of work will attempt to characterize the mechanism of relative resistance to oral anticoagulants observed in pregnancy and under the influence of oral contraceptives. On the basis of his previous studies the applicant suggests that genetic resistance to anticoagulants may be due to an alteration in a vitamin K metabolizing system which is normally sensitive to warfarin and responsible for the anticoagulant effect. The experimental approach used to examine the warfarin resistant trait will be applied to the relative resistance to warfarin elicited in pregnancy and the estrogen-treated animal.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: . Endogenous and Exogenous Sex Steroids and Nutritional Status
Contractor: The University of Texas Medical Branch, Galveston, Texas
Money Allocated: \$110,925 (FY 72 funds - level of support from FY is expected to be in a similar magnitude, but has not yet been decided)

Objectives: This is one of three ongoing projects in the program of the FRMEB concerned with the broad characterization of the effects of OC's on the metabolism, requirements and status of micronutrients. The research program is designed to utilize cross-sectional and longitudinal approaches to elucidate the interrelationships between exogenous female sex hormones and a variety of nutritional-metabolic parameters. The investigative work has been aimed towards three circumscribed and specific objectives. (1) A baseline of the normal variations on nutritional status occurring during the menstrual and contraceptive treatment cycle will be established. (2) The effects of oral contraceptives on nutritional status of contraceptive users will be assessed in a cross-sectional comprehensive nutrition survey. (3) The time dependence of oral contraceptive-induced changes in nutritional status will be characterized.

Major Findings: The project is in the stage of data collection. No major conclusions have been crystallized.

Significance to Biomedical Research and the Program of the Institute: The investigative work carried out in this project consists of applied rather than basic research and should provide information on possible nutritional consequences and interrelations of contraceptive therapy. The research program is similar in content to two other projects (72-6-013-Prasad and 72-6-019-Smith) and is highly relevant to the objectives of the Institute carried out in the FRMEB, CPR.

Proposed Course: Four projects designed to achieve the specified objectives are involved in the project.

Project 1: Fifteen young females who are not using oral contraceptives and a comparable group of 15 contraceptive users were to be followed serially through two or three menstrual cycles to establish baseline parameters for nutritional metabolic data. All subjects have been enrolled. After an initial clinical and dietary evaluation, each woman has been serially assessed biochemically on day 3, 10, 17, and 24 of their endogenously or exogenously controlled menstrual cycle. After their first cycle each of their dietary intake was augmented with a mineral/vitamin supplement. Logistic and procedural aspects of the project have been completed and all data have been collected. Analysis of results is in a preliminary stage. A number of striking observations are mentioned in the renewal application but statistical analysis of results is still in a preliminary stage and not yet available.

Project 2: A cross-sectional nutrition survey and health examination is being conducted on 800 women at the time of their regular six-month follow-up visit to family planning clinics operated by the investigators. This sample is to include 70% contraceptive users; 20% IUD users and 10% women who use other methods of contraception. It will be attempted to relate observed variations of nutritional status to type, dose and time of contraceptive usage. The influence of contributing factors such as age, ethnic origin, socioeconomic status and dietary intake will be assessed. Correlations between all variables will be attempted.

Cross-sectional sampling of women currently actively enrolled in family planning clinics began in October 1972. Subjects were to be <25 years of age and nulligravidas and/or <20 years of age with only one prior pregnancy. A potential of 800 plus women who met these criteria were identified prior to the operational start of the project. It has taken longer than expected to enter them into the study. The logistics of working the evaluations into their normal return to Family Planning Care has been complicated with "failure to keep appointments" and the like. At present over 300 women have been enrolled with complete entry and sampling data. Preliminary analysis of this data is in progress.

Project 3: Three groups of 25 women each who are new enrollees in a family planning clinic will be prospectively followed to determine changes which may occur in their nutritional health status as a function of contraceptive use. The three groups are to be defined by their use of (a) common estrogen-progestin combinations; (b) low dose cyclic progestin; (c) depot injection of long-lasting contraceptive steroid combination. These three groups will be studied before contraceptive therapy and be reevaluated at 2-3 month intervals for at least one year.

Presently over 40 subjects have been enrolled and are being investigated. It is projected to have all subjects enrolled by mid April 1973 and that prospective observations will be completed within the next year of the contract.

Project 4: This project corresponds in experimental design and follow-up to the one outlined above. It involves, however, 25 subjects who have lost or never had an endogenous ovarian source of estrogen and/or progesterone. They will receive hormonal therapy. It has not been possible to enter more than five women who are congenital or surgical castrates and <25 years of age into this study. They will be serially followed for at least one year as they are provided cyclic hormonal substitution therapy.

By the end of the first contract year, in June 1973, the investigators anticipate having: (a) completed all aspects of Project I, (b) have entered about 500-600 women in Project II, (c) have enrolled from 30 to 40 in each of the sub-groups from study in Project III, and (d) will continue to enroll appropriate women in Project IV.

It is anticipated that it will take until late winter of 1974 before all data will be collected and subsequently subjected to a final comprehensive analysis. This is to be followed by the final steps of interpretation and reporting.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Relationship of Effect of Oral Contraceptives on Blood Lipids and Nutritional Status
Contractor: St. Louis University, School of Medicine, St. Louis, Mo.
Money Allocated: \$57,080 (FY 72 Funds)
\$58,707 (FY 73 Funds)

Objectives: The studies initiated under this contract were directed towards three principal objectives:

1. To relate the serum levels of copper, ceruloplasmin and ascorbic acid and presence of C-reactive protein to the serum lipid levels of females taking oral contraceptives, of pregnant women, and of men and women with elevated blood lipids as a consequence of either past diet or genetic variables.
2. To determine whether the elevations in blood lipids after therapy with oral contraceptives can be correlated with the rise in vitamins A and E in the serum.
3. To correlate the biochemical changes mentioned in 1 and 2 above with the results of the tryptophan load test to help evaluate whether the changes in lipid transport can be related to theoretical implications of the increase in liver tryptophan pyrrolase which is reported to occur during treatment with oral contraceptives.

Major Findings: The average serum cholesterol levels of the OCA group at 196 mg per dl are not much different than those obtained in the control group possibly because of an unfortunate selection of women who had high serum cholesterol to serve as controls. These data will have to be analyzed later on an individual basis with reference to age, blood pressure, and in a few cases, use of OCA in previous years. Of interest is the fact that the serum phospholipids (PL) of the OCA group (mean of 224 mg/dl) tend to be higher than in the control group (mean 205). It was noted that 76 percent in the OCA group have PL levels above 200 as compared to 46 percent for controls. In addition 20% of the OCA group had PL levels above 260 mg/dl whereas, to date, no subject in the control group has been that high. It may be noteworthy that in most of the other lipidemias, the PL levels were the least affected component of the lipids incorporated in the lipoproteins.

The data on serum vitamin A confirms the increase in those taking OCA that had been reported by Gal, et al. (Brit. Med. J. 2:436, 1971). The OCA group averaged 53.4 µg/dl and the control women averaged 34.4 µg/dl, to-date. There was only one subject higher than 42 in the control group whereas 21 subjects were higher than 42 in the group taking OCA. The different distribution of retinol between the blood and the tissues is probably a function of either an increase in retinol-binding protein or an increased transport of that part of the retinol not carried by this protein, or both.

The average serum vitamin E levels are only slightly higher in the OCA group. Data from pregnant women show marked elevations of vitamin E in the serum that correlates well with their increased total lipid levels. The average vitamin C blood levels have not shown any differences between the groups while serum copper and ceruloplasmin levels are definitely higher in the OCA group.

The average XA excretions after the 2 gm tryptophan load test were as expected much higher in the OCA group; 107 milliequivalents in the 8 hour collection vs. 24.8 mEq in the control group. The average XA excretion in the morning urine of subjects taking OCA is about twice as great as those not taking OCA; 8.12 vs. 4.09 mg XA per g creatinine (Cr.) These basal urine results were considered of greater possible importance than data obtained after the 2 g tryptophan load test.

Preliminary results on the HAA excretion levels in morning urine collections (also expressed as mg per g of creatinine) show that those subjects who were taking OCA had higher average results than the control subjects.

It was previously shown that pregnant women could convert tryptophan to NMe (N¹methyl nicotinamide) almost three times as effectively as in adult men. Now, one must consider whether women on OCA are also more efficient in converting tryptophan to niacin. The more than 20 year old fact discovered during nutritional studies that pregnant women excrete more NMe in their basal urine, makes it almost certain that women on OCA will to a lesser extent do the same. Accordingly, the investigators plan to study this facet of the problem more intensively if time is available in the future contract period.

The assessments of B₆ status, excessive tryptophan loads may give rise to misleading conclusions. Excessive loads of this amino acid may overwhelm levels of pyridoxal coenzyme that may be adequate for ordinary levels of protein consumption. The investigator considers the unexpected development of this realization as one of the most significant early rewards of this contract.

Summation of tryptophan metabolites in the absence of metabolic loads may offer distinguishing criteria of contraceptive usage. The fact that certain excretory products of tryptophan are increased in women is not per se an indication of vitamin B₆ inadequacy despite the fact that vitamin B₆ can decrease the level of excretion of some of these products.

Correlations are appearing between the blood lipid levels and the excretory products of tryptophan metabolism in the majority of the subjects.

Significance to Biomedical Research and the Program of the Institute: The project may provide better knowledge of the long-term medical effects of oral contraceptives. Consequently, it is relevant to this program. Although the plans were based on many hypotheses, they were considered scientifically sound and targeted towards providing relevant information.

Proposed Course: The principal investigator anticipates that all major investigative work and tasks specified in the workscope of the original contract will be completed by June 30, 1973 with the exception of the 6 month post partum females. This includes completion of the series of 50 women with and 30 without OCA during mid-month, 30 pregnant women in third trimester, 10

females after climacteric, 10 younger castrated females and 30 adult males half of whom have elevated serum lipids. The analyses to be performed include performance of 2 g tryptophan load test with measurement of xanthurenic acid excretion during the 8 hours after ingestion of the tryptophan. Analysis of the fasting blood for cholesterol, triglycerides, phospholipids, total lipids, ascorbic acid, total tocopherols, vitamin A and serum copper will be made.

In addition to the specifications in the work scope, the investigators have added analyses of the morning urine samples (6 to 8 hour collections) for tryptophan metabolites. Particular attention is being given to the determination of 3-hydroxyanthranilic acid during this contract year but procedural changes are being developed and contemplated for the future.

In the light of preliminary results obtained in this contract project, it is now reasonably certain, but not yet proved, that the serum lipid levels tend to change with the levels of copper compounds, vitamin A and vitamin E in the blood, as well as with the levels of some of the tryptophan metabolites excreted in the urine before and after a small tryptophan load. These changes appear to be related to levels of estrogen ingested or metabolically produced, extreme samples being provided by women in the third trimester of pregnancy. Except for the addition of the study of additional tryptophan metabolites, with special attention being directed toward morning urine samples, the basic plan for division of subjects remains the same as in the first year of the contract: (1) Women taking OCA will be compared with women not taking OCA, (2) Women in the third trimester of pregnancy will be compared with those taking and not taking OCA as well as with castrated females and women after the climacteric, and (3) Adult males with and without high blood lipids will be compared with all the above.

The tryptophan load will be limited to 750 mg and several additional end products of tryptophan metabolism will be analyzed. This preference for a smaller tryptophan load is based upon the assumption that no more tryptophan should be used in the load test than is expected to be ingested in a normal high protein meal in order to obtain a more practical evaluation of pyridoxal coenzyme requirements.

All serum samples will be analyzed for lipoprotein by the agarose-electrophoresis technique to attempt to classify the subjects according to Fredrickson. The collections and analyses of morning urine samples as well as 8 hour urine collections after the small tryptophan load will be performed. Based upon preliminary successes in combining the results of data on HAA and XA, the following end products of tryptophan metabolism will be determined in all the urines: XA, NMe, the pyridone of PYR and kynurenic acid (KA).

It is hoped that the results will eventually show that only the morning urine (post-absorptive) samples will prove necessary when the data from these end-products of tryptophan metabolism are properly interpreted but should this prove an incorrect assumption data on the smaller tryptophan load should cover any possible production loss.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Interaction of B-Vitamins with Contraceptive Steroids in Female Rats
Contractor: School of Pharmacy, University of Georgia, Athens, Georgia
Money Allocated: \$25,600 (FY 72 Funds)
\$26,000 (FY 73 Funds-estimated)

Objectives: The purpose of this project is to investigate in the rat the effects of vitamin B complex deficiencies and hypervitaminosis states on the metabolic hydroxylation of selected steroids, and the effects of contraceptive steroids on the function and disposition of thiamin, riboflavin and pyridoxine. Deficiency and hypervitaminosis B states have been shown to alter the activity of certain liver enzymes. Several of the B complex vitamins are constituents of liver enzyme systems involved in the metabolic disposition of steroids.

Major Findings: Thiamin and riboflavin deficiency states increase the rates of metabolism of certain drugs by hepatic microsomes. These states cannot be considered as inducers of drug metabolizing enzymes in the same sense as enzyme induction by drugs such as Phenobarbital, because no elevation of liver weight/body weight ratio, microsomal protein content, cytochrome c reductase activity or cytochrome P-450 is observed. Preliminary evidence from the investigator's laboratory (Biochemical Pharmacology--In Press) suggests that thiamin deficiency states may alter the character of the cytochrome P-450 which in turn may be responsible for these changes. It will be attempted to clarify this phenomenon during the later phases of this contract period and in subsequent experiments.

High levels of thiamin and riboflavin have profound effects on the drug metabolizing system of rat liver, depressing principally the metabolism of type II substrates with lesser effect on type I drug substrates. Associated with this decreased metabolic rate is decreased cytochrome c reductase activity and cytochrome P-450 content per unit of microsomal protein. More evidence is needed to support preliminary findings which suggest that high thiamin levels result in loss of the cytochrome P₁-450 constituent responsible for type II substrate metabolism while not affecting the concentration of the cytochrome P-450 constituent responsible for type I drug substrate metabolism. More data relative to this finding may be forthcoming during the current contract period.

The steroid contraceptives mestranol and norethindrone produce additional effects, the nature of which may be dependent upon the animals' diet. For example, the V_{max} for aniline hydroxylation is elevated by 0.1 mg mestranol/day in rats fed laboratory chow and high levels of riboflavin or thiamin. However, in rats fed thiamin or riboflavin deficient diets the rate of aniline metabolism is depressed. The V_{max} for ethylmorphine demethylase, on the other hand, is elevated by mestranol in rats fed laboratory chow, thiamin deficient diet, high thiamin and high riboflavin diet but not in rats fed riboflavin deficient diet. The effect of norethindrone is also seen to depend upon the dietary status of the rat. Whereas aniline metabolism may be depressed by

norethindrone in thiamin and riboflavin deficiency states, it is uniformly increased by 1.0 mg norethindrone in rats receiving laboratory chow, and high levels of thiamin and riboflavin. In general it appears that when enzyme activity has been depressed by high intakes of thiamin or riboflavin the administration of mestranol or norethindrone restores the activity of these enzymes toward normalcy. This is usually accompanied by elevations in cytochrome c reductase activity. Cytochrome P-450 levels do not clearly correlate with enzyme activity; however in light of other findings these drugs may be selectively depressing one component of the cytochrome P-450 system, i.e. P₁-450, with offsetting elevation of P-450. More experimentation is needed to clarify these complex actions and additional experiments should be designed to demonstrate the altered occurrence of these two forms of cytochrome P-450. Experiments designed to quantitate binding of ethyl isocyanide to microsomal cytochrome P-450 and the selective induction of cytochrome P-450 or P₁-450 in various dietary states and in the presence of the contraceptive agents will provide this evidence.

Significance to Biomedical Research and the Program of the Institute: Many reports have implicated contraceptive steroid preparations in the modification of B vitamin needs, but no evidence of the mechanism of this action has as yet been obtained. More importantly perhaps, the alternate problem has not been investigated, i.e., the effect of vitamin deficiency on steroid metabolism. It is apparent that the proper action of a drug such as these steroids depends on maintaining the balance between uptake and catabolism. Anything that modifies this pattern can result in either decreased efficiency or increasing deleterious side effects. The B vitamins are known to have a role in the oxidation and hydroxylation of steroids as well as maintaining the general metabolic integrity of the cell. This proposal is designed to explore this subject. The selection of appropriate vitamins and their levels is good, and the inclusion of hyper levels is very useful. The biochemical measurements are appropriate and will provide a basis for defining this interaction. Thus the project could potentially provide fundamental information concerning the safety of the use of oral contraceptives.

Proposed Course: Experiments are being concluded in which the effects of mestranol or norethindrone administration on the metabolism of aniline and ethyl morphine will be assessed in rats fed pyridoxine deficient or high pyridoxine containing diets.

Other experiments are in progress which should provide information on the effects of varying thiamin intake on the metabolism of mestranol and norethindrone. These will be followed by similar experiments in which variation in riboflavin and pyridoxine levels on the rate of metabolism of these two steroids will be studied. Anticipated completion date: April 13, 1973.

Localization of the subcellular site affected by dietary vitamin intake should be completed by May 17, and the study designed to determine the effects of B vitamins on the subcellular constituents of hepatic drug metabolizing enzyme systems will be completed in late June.

An attempt will be made to elucidate the mechanism by which contraceptive steroids alter mixed function oxidases of hepatic microsomes in the presence of

varying nutritional states. To accomplish this objective these enzymes will be examined for qualitative as well as quantitative changes in their biochemical composition and their pharmacologic reactivity. It has been established in the rat that ingestion of contraceptive steroids in varying nutritional states will selectively alter the rate of metabolic degradation of aniline and ethylmorphine. Experiments will be designed to establish correlations between these actions and (1) affinity of substrate binding to the terminal oxidase, cytochrome P-450, (2) rate of synthesis and (3) stability of this enzyme, (4) conformational changes in the environment of cytochrom P-450, (5) alteration in the heme or (6) lipoprotein moiety of the cytochrome, and (7) rate of the limiting step in drug hydroxylation reactions, i.e. P-450 reduction in presence of substrate. Investigation of the properties of cytochrome P-450 will be stressed since preliminary evidence from the laboratory suggests that qualitative changes in this cytochrome occur with increasing severity of the thiamin deficiency state.

Since multi-vitamin hypervitaminosis or deficiency states are more likely to occur in human populations, experiments designed to define the effects of these conditions on the drug hydroxylase system responsible for metabolizing oral contraceptives and other drugs will be undertaken. Drug hydroxylase activity, cytochrome P-450 content and NADPH cytochrome c reductase activity will be used to indicate alterations in this complex system.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Oral Contraceptive Agents and Thromboembolic Complications
Contractor: Washington University School of Medicine, St. Louis, Mo.
Money Allocated: \$85,815 (FY 72 funds. For FY 73 a six-month extension, for approximately \$40,000, is being negotiated.)

Objectives: The objectives of this contract are: 1) to develop, improve and standardize a new diagnostic method of fibrinogen chromatography; 2) to demonstrate the clinical usefulness of fibrinogen chromatography as a screening procedure for assessing the risk of thromboembolic complications in patients on various types of contraceptive therapy.

Major Accomplishments and Findings: At the beginning of the contract period, plasma fibrinogen chromatography appeared to offer an exciting new approach for a rigorous biochemical detection and characterization of hypercoagulability, risk and occurrence of thrombosis. A major drawback was the laboriousness and procedural difficulties of the manual methodology coupled with the necessity for subjective interpretation of chromatographic patterns. These technical difficulties with great demands on skilled technical manpower provided great obstacles for the clinical application of the methodology in screening programs. These obstacles have been seemingly overcome by methodological improvements in chromatographic procedures involving automation and objective computer analysis of the chromatograms. This permits the generation of assay results within two to three hours and allows extensive clinical screening programs. The initial clinical research projects on oral contraceptive users (in collaboration with Dr. Burstein in St. Louis) have been essentially completed. A manuscript is being prepared and expected to be ready in June 1973. It will report the following findings: A. That women receiving oral contraceptive agents demonstrate abnormalities in the plasma fibrinogen chromatogram, indicative of thrombosis, either clinically silent or overt, approximately 5-fold more frequently than do comparable unmedicated control subjects. B. That the abnormalities in the plasma fibrinogen chromatogram are characteristically transient, lasting for a 10-30 day period and terminating with the chromatographic pattern of thrombus resolution. C. That the thrombogenic effects of oral contraceptive agents increase with treatment duration over the first few months, but thereafter, remain essentially independent of treatment duration. D. That a substantial proportion of women, for genetic or other reasons, do not apparently incur the risks of complicating thromboembolism with oral contraceptive medication, but there is a smaller proportion of women who appear to be unduly susceptible to such consequences. E. That because of detection of a high incidence of transient plasma fibrinogen chromatographic abnormalities, indicative of usually clinically silent thrombosis, in subjects on oral contraceptive medication and a correspondingly low incidence of such abnormality in the control unmedicated group, the use of small populations (100-200 subjects) to study thrombogenic risk produced by various oral contraceptive formulations is wholly practical and finally, F. It was established that the chromatographic method was of a great utility in excluding the

presence of thromboembolic disease in oral contraceptive treated patients in whom the presence of such complications was suspected.

Significance to Biomedical Research and Program of the Institute: The development of a laboratory test capable to detect or predict intravascular coagulation is urgently needed in several fields of clinical and investigative medicine. It is of particular importance in contraceptive management, since unexpected thromboembolism appears to be a major well-documented side effect of contraceptive steroids.

Proposed Course: Findings and results obtained during the past contract period, as reported above, were obtained from a preliminary largely cross-sectional clinical screening program. It appeared not to be carried out under convincing "blind" condition and lacked other rigorous design features, such as appropriate controls which appear essential for a conclusive demonstration of the clinical value of fibrinogen chromatography in diagnosing clinical and sub-clinical thrombosis and identifying patients at risk. Accordingly, a major effort was to be directed toward performing a rigorous longitudinal clinical study on 150 student nurses through Dr. Newman at the St. Luke's Hospital in Kansas City. Thus the clinical aspects and evaluation of the study were physically and operationally completely removed from the principal investigator, and its blind character was assured. While subject recruitment and other clinical and biochemical aspects of the project in Kansas City proceeded satisfactorily, unexpected problems in plasma collection, storage and transport of the samples have impeded obtaining satisfactory data for analysis. In approximately 25% of the samples visual observations have revealed the presence of precipitates or clots. Fibrinogen chromatography of the clotted sample showed an unusually high incidence of abnormal patterns. The presence of precipitates indicates an unsatisfactory sample preparation and may invalidate the results and conclusions drawn from the analysis. This situation forced the decision to disregard possible misleading data and repeat the entire study according to the original protocol:

1. 150 student nurses will be recruited and enrolled in the study through Dr. Newman at the St. Luke's Hospital in Kansas City.
2. A detailed medical history will be obtained and a physical examination will be performed on all subjects. None shall have used contraceptive therapy prior to entering the study.
3. Seventy-five subjects will be treated with the oral contraceptive Ovulen (1 mg ethynodiol diacetate, 0.1 mg mestranol). The other 75 subjects will serve as control and use mechanical devices for contraception or abstain from sexual activity which may lead to pregnancy.
4. Blood samples will be obtained two weeks before therapy, at the start of therapy and 2, 4, 8, 16, 32 and 64 weeks afterwards.
5. Fibrinogen chromatography will be performed on all blood samples which will be shipped from Kansas City to the Principal Investigator's laboratory by Dr. Newman. Results will be interpreted under double-blind conditions.
6. Other biochemical examinations as specified in the protocol will be performed by Dr. Marjorie Sirridge in Kansas City. These tests

include: protamine and ethanol gelation, cryofibrinogen and antithrombin assays, and determination of triglyceride and cholesterol levels.

7. A brief medical examination will be performed during each subject's visit for obtaining blood samples. Emphasis of this examination will be to ascertain the absence or presence of suggestive symptoms which may be caused by subclinical thrombosis (e.g., pain or discomfort in legs or chest).

In order to permit completion of these most pressing requirements of the project, a six month extension of the contract period is being negotiated. Scientific review and reevaluation of the entire direction and course of this project is planned to take place at that time.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Metabolism and Metabolic Effects of Steroidal Oral Contraceptives
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: \$21,500 (FY 73 funds for contract period 11/1/72 - 10/31/73)

Objectives: The lines of research supported by this contract are aimed to:
1) measure the metabolic clearance, plasma half-life and rates of conversion of the various steroids; 2) define and identify metabolites of natural and synthetic estrogens and progestins.

Major Findings: The metabolic clearance rates for estradiol was distinctly greater in ethynyl estradiol users than in non-pill users and mestranol users. There were no appreciable differences in the metabolic clearance rates for estradiol between mestranol users and subjects not treated with synthetic steroids. The mean metabolic clearance rates (MCR) expressed in L/day M² for all non-pill users was 740+70, for mestranol users 720+40, and for ethynyl estradiol users 1090+50. These data indicate that mestranol has no effect on the metabolism of estradiol but that ethynyl estradiol increases the metabolism of estradiol. The findings are reminiscent of similar ones previously reported by Southren, that prolonged testosterone administration results in an increase in the metabolic clearance of ³H-testosterone, presumably by inducing enzymes responsible for this metabolism. The results obtained in this contract are compatible with a similar concept that ethynyl estradiol administration induces the enzymes responsible for estradiol metabolism.

The urinary metabolites of estradiol-17 β in the same three categories of subjects (non-pill users, mestranol users, ethynyl estradiol users) used for the kinetic analysis of metabolism were characterized. The following eight metabolites of the administered radioactive ³H estradiol were isolated with the carrier fractions: estrone, estradiol, estriol, 2-hydroxyestrone, 2-hydroxyestradiol, 16-hydroxyestrone, 2-methoxyestrone, and 2-hydroxyestrone 3-methyl ether. Metabolites of estrogens in some species such as the rat are predominately excreted in the bile. The data obtained in this study revealed that in the human female 60-87% of the administered dose of radioactivity in ³H estradiol appeared within 96 hours in the urine. Approximately 1% of the urinary estradiol metabolites were not conjugated and appeared in the "free" fraction, 60-70% appeared as glucuronides in the Ketodase fraction, 5-10% as sulfates in the pH 1-hydrolyzed fraction. The quantitative pattern of the eight metabolites, their distribution in glucuronide and sulfate fraction and their ratios to each other revealed no significant differences between non-users and users of synthetic estrogens. Thus the metabolic isolation work proved to be considerably less revealing and/or less sensitive for delineating effects of synthetic estrogen on the metabolism of natural estrogens than kinetic analysis.

The dynamic parameters of estrone metabolism were studied in five subjects using a mestranol containing oral contraceptive, in three subjects using a ethynyl estradiol containing drug, and in one woman not on medication. The metabolic clearance rates for estrone expressed in L/day/M² were 1050+60 for the mestranol

users, 1060 for the ethynyl estradiol users and 1320 for the non-pill users. These data suggest that chronic administration of synthetic estrogens effects the metabolism of estrone differently and in the opposite direction than that of estradiol. The chronic administration of both ethynyl estradiol and mestranol seems to decrease the rate of estrone metabolism. This tentative hypothesis, however, needs to be substantiated by additional dynamic studies especially on non-pill using controls.

Characterization of biliary metabolites of ethynyl estradiol and mestranol in rats--Female rats have been injected with double labeled mestranol and others with ^3H -ethynylestradiol; biliary metabolites have been isolated and characterized. The demethylation of mestranol appears to be completed before excretion into the bile; upwards of 90% of the ^3H appears in the bile in 24-36 hours following I.P. injection. Preliminary results indicate the presence of 4 major metabolites in the "glucuronide" fraction from mestranol: 2-hydroxyethynylestradiol, 2-methoxyethynyl estradiol, 2-hydroxyethynylestradiol 3-methyl ether and ethynylestradiol in ca. a 3:3:1:0.5 ratio. Of the total ^3H label in the bile, 11.3% represented the free fraction, 59.3% was in the form of conjugated material (butanol extractable) with a Ketodase treatment of the butanol extract releasing 42.1%. Carrier 2-hydroxyethynylestradiol was added to the conjugated fraction prior to hydrolysis with Ketodase. Chromatography of the hydrolysate on silica gel plates unpregnated with silver nitrate showed that these metabolites all retained the ethynyl group.

Significance to Biomedical Research and Program of the Institute: The proposed projects on metabolism and pharmacokinetics of natural and synthetic estrogens in users and non-users of contraceptive steroids are highly relevant. They may contribute towards providing a scientific base for the medical and metabolic assessment of contraceptive therapy by means of anovulatory drugs.

Proposed Course: Investigative work is following the workscope for the present contract period from 11/1/72 through 10/31/73. It was prepared during a project site visit with advice of scientific consultants.

A. Pharmacokinetic Studies:

1. Investigate the mechanism whereby long-term use of oral contraceptives containing ethynyl estradiol, but not those containing mestranol, increase the metabolism of the natural estradiol-17 β . The specific objective of this investigation is to determine whether the increased metabolic clearance rate of estradiol in ethynyl estradiol users represents a reversible or irreversible effect of chronic contraceptive usage.
2. Complete the ongoing work on the pharmacokinetic characterization of estrone metabolism in users and non-users of oral contraceptives. The goal of these studies is to substantiate findings made during the past contract period that chronic administration of synthetic estrogen affects the metabolism of estrone differently and in an opposite direction than that of estradiol. The hypothesis that chronic administration of the two synthetic estrogens, mestranol and ethynyl estradiol, both decrease the rate of estrone metabolism shall be confirmed or disproved.
3. Investigate the long-term effects of ethynyl estradiol administration on its own metabolism. This shall be done by determinations of metabolic clearance rates, volumes of distribution and half life of ethynyl estradiol

in five women before and after six to seven months of chronic administration of an oral contraceptive containing ethynyl estradiol.

4. Investigate the long-term effects of mestranol administration on its own metabolism. This shall be done by determinations of metabolic clearance rates, volumes of distribution and half life of mestranol in five women before and after six to seven months of chronic administration of an oral contraceptive containing mestranol.

5. Initiate studies to characterize the dynamic properties of mestranol metabolism and the metabolic effects and conversions of this synthetic estrogen in 3-5 sheep. These studies shall be aimed to elucidate whether mestranol has similar activities in sheep, where it is not demethylated by liver homogenate than in other species where such a demethylation occurs readily. In addition to the kinetic parameters of metabolic clearance, volume of distribution and half life, assays for the effects of mestranol on gonadotrophin levels and ovulatory behavior shall be performed.

B. Biochemical Characterization of Metabolites of Natural and Synthetic Estrogens

1. Complete the ongoing work on the characterization and distribution of eight estradiol-17 β metabolites in the urine of non-users and users of oral contraceptives. This analysis shall be concerned in particular with the questions whether chronic users of ethynyl estradiol reveal any differences in the peripheral metabolism of estradiol 17 β compared to non-users and mestranol users.

2. Characterize the distribution of eight metabolites of estradiol-17 β in the urine of 3-5 women who have used an ethynyl estradiol containing contraceptive for 2-3 years in the past but have discontinued the drug within the preceding 4-6 months.

3. In parallel with the pharmacokinetic studies specified under A-3 perform screening analyses for metabolites of ethynyl estradiol in the urine of five women before and after six to seven months of chronic administration of an oral contraceptive containing ethynyl estradiol.

4. In parallel with the pharmacokinetic studies specified under A-4, perform screening analyses for metabolites of mestranol before and after chronic administration of an oral contraceptive containing mestranol.

5. In parallel with the pharmacokinetic studies specified under A-5, identify and characterize the distribution of mestranol metabolites in bile, feces and urine of 3-5 sheep. These studies shall be aimed to elucidate the metabolic transformation of mestranol in a species apparently incapable of demethylation of this synthetic estrogen by the liver. Results shall be compared with those obtained in other species (rats and humans) where such demethylation readily occurs.

C. Immunoassays of Circulating Synthetic Estrogens

1. Develop an assay procedure for measuring the concentration of circulating ethynyl estradiol in users of oral contraceptives containing either ethynyl estradiol or mestranol.

2. Determine blood levels of ethynyl estradiol in 10 users of an ethynyl

estradiol-and in 10 users of a mestranol-containing oral contraceptive. Blood levels shall be determined serially, starting with the beginning of contraceptive therapy and at various time intervals afterwards. It is the purpose of this investigation to establish the relationship between acute and chronic administration of the steroids and the resulting blood levels.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Studies of Dose-Response Relationships Between Low Dose Steroid Contraceptive Drugs and Plasma Gonadotropin Levels
Contractor: University of Rochester School of Medicine and Dentistry
Money Allocated: None (The contract expired on December 31, 1972 and was not extended. No money was allocated in FY 1972 and FY 1973.)

Objectives: The purposes of this project were: to study systematically the plasma gonadotropin response to a number of combinations and dosages of these drugs with the expectation that one or more low-dose combinations can be found which consistently block ovulation with little or no effect on blood coagulation factors. Another specific aim of the project was to establish the lowest effective dose of several combinations of synthetic estrogens and progestogens which will reliably suppress the ovulatory surge in LH and FSH, and at the same time will be associated with either minimal or no measurable changes in blood coagulation factors II, VII, VIII and X, which are known to be elevated during oral contraceptive therapy. A secondary aim of the investigation was to identify which effective combinations have the least objectionable side effects of hyperestrogenism.

Major Findings: Four groups of women were investigated over a period of 5 treatment or menstrual cycles with appropriate control periods before and after drug treatments. The four experimental groups were: 1) women receiving a placebo; 2) women receiving 1.0 mg of megestrol alone; 3) women receiving 1.0 mg megestrol in combination with 50 mg ethynyl-estradiol and 4) women receiving 1.0 mg megestrol and 25 mg estradiol. Megestrol alone caused a complete suppression in the ovulatory surge of LH and FSH. The combination with the two dose levels of estrogen had no further effects. Consequently, no dose response data has been generated. None of the drug schedules had measurable effects on the coagulation parameters; side effects such as continuous spotting, irregular bleeding and abdominal cramps were pronounced. Altogether, 40 young, normal women were enrolled in the study, divided into four groups of 10 each. Of these, only 23 finished the study, the rest having dropped out because of various side effects notably irregular menses, intermenstrual bleeding, and nausea.

In spite of great efforts expended, the investigator became disenchanted with the project because of the apparent inability to demonstrate any significant difference in the plasma gonadotropin response between the experimental groups, although there was, of course, a significant difference between these and the control group which showed normal plasma gonadotropin patterns. Likewise, there were no clearcut differences between any of the groups in the coagulation studies and therefore it was not possible to correlate any endocrine responses with any alterations in blood coagulation factors.

Significance to Biomedical Research and Program of the Institute: A number of studies have indicated the possible importance of minimizing the dosage or

adjusting the proportion of estrogen and progestin in presently available contraceptive formulations. It appears likely that at least some of the undesirable side effects of these agents might be minimized by reducing the dose of drug to a level which is associated with adequate efficacy but minimal undesired effects. The study was expected to give useful insight into this matter at least for certain drugs and indicate the eventual utility of this particular type of approach.

Proposed Course: The project was discontinued when the principal investigator took leave for a sabbatical year. Interest was expressed for further analysis of the accumulated data and plans for a refined new initiative to the problem under investigation. None of these contemplated efforts presently require support through funding.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Postcontraceptive Reproduction in Man
Contractor: Albert Einstein College of Medicine
Money Allocated: \$126,688 (FY 1972 funds for 18 months)

Objectives: This project, which was previously supported by the Contraceptive Development Branch, was shifted to the Fertility Regulating Methods Evaluation Branch at the end of FY 1972 in line with changing objectives of the study. The contract now supports studies of the effects of prior usage of oral contraceptives on human female meiosis and reproductive wastage. The study of meiosis involves cytogenetic analysis of cultured ova obtained from 100-150 women and correlation of the results with contraceptive history. The effects of prior contraceptive usage are also studied in spontaneous and induced abortion and in newborn infants; data on chromosome constitution and physical and developmental characteristics will be correlated with maternal contraceptive history and a number of medical and socioeconomic parameters.

Major Findings: Chromosome studies of spontaneous and induced abortions and newborn infants are proceeding according to schedule using improved techniques developed under the contract. One of these techniques permits rapid processing of embryonic tissues for chromosome banding studies and increases the sensitivity of methods for detecting chromosome aberrations and polymorphic chromosome variations. A very large amount of individual chromosome polymorphism has been found, and the types and extent of variability are being studied to provide baseline data for this and other studies. A fluorescent staining technique allows accurate detection of both X- and Y-chromatin in interphase nuclei. Both abnormalities in numbers of sex chromosomes and apparently polymorphic variations in the size of Y bodies have been identified. The contractor had previously found a higher incidence of chromosome triploidy in spontaneously aborted fetuses from women who had used oral contraceptives, but in the present study no triploids have been identified in induced abortion material from either users or non-users of contraceptives. This may be related to the vacuum extraction technique used to induce abortion, and attempts are being made to determine DNA content in amnion or chorion as an alternative way of determining nuclear ploidy. Collection of data on newborn infants is progressing well, and data from both the abortion material and the newborns are being computerized.

Significance to Biomedical Research and the Program of the Institute: Oral contraceptives are the most widely used method of contraception but there is little information on genetic effects on children conceived after use of these agents. This study should contribute significant data on the incidence of congenital abnormalities and cytogenetic aberrations in fetuses and newborns after maternal use of steroid contraceptives.

Proposed Course: The collection of data is expected to be completed during FY 1974, but analysis of data may require additional time.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Chromosomal Breakage in Women Taking Oral Contraceptives
Contractor: AEC/Oak Ridge Associated Universities, Inc.
Money Allocated: \$117,900 (FY 73 funds)

Objectives: The purpose of this project is to confirm and extend earlier studies which suggested that use of oral contraceptives leads to an increased frequency of chromosome breaks in cultured lymphocytes and determine whether the increased chromosome breaks in lymphocyte cultures are representative of chromosome damage in other somatic cells.

Major Findings: Findings from preliminary hand analyses of a major part of the data in this study were reported last year. During FY 1973 cytogenetic analyses of lymphocyte cultures were completed and the data on 80,000 metaphases from 977 cultures were computerized for more complete statistical evaluation. The data were regrouped to consider history of oral contraceptive usage and pregnancy as well as oral contraceptive and pregnancy status at the time of each culture, and statistical adjustments were made for the effects of covariables. The finding of a small but statistically significant increase in chromosome breakage in oral contraceptive users is substantiated and the analysis also indicates a smaller increase in chromosome breakage associated with previous or current pregnancy.

Significance to Biomedical Research and the Program of the Institute: Oral contraceptives are the most widely used method of fertility control, but there is very little information on their possible genetic effects. Chromosome breaks in peripheral leukocytes are induced by many agents including carcinogens and LSD; the significance of such breaks is not known but they probably reflect damage to other somatic cells and perhaps also to gametes. Although the full biological significance of chromosomal breakage is not understood, it is important to evaluate this effect of an agent to which large numbers of women are exposed.

Proposed Course: Data analysis will be completed in FY 1973 and support for this project will be terminated at the end of the year.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title : A Study of the Metabolism of Steroidal Oral Contraceptives
Contractor : Medical College of Georgia
Money Allocated: \$13,000 (FY 73 funds)

Objectives: The purpose of the studies supported by this contract is to provide quantitative information on plasma half-life, metabolic clearance rates and metabolic products of synthetic estrogens and progestational agents in normal women. The drugs to be investigated are mestranol and norethindrone.

Major Findings: Standardized procedures for isolating labeled norethindrone and mestranol from plasma and for correcting for procedural losses have been developed. Using these procedures and acute intravenous injections and constant infusion of labeled contraceptive steroids, plasma half-lives and metabolic clearance rates of the parent drugs have been determined. Values obtained by both methods are in general agreement with MCR values of 500-800 liters per day for norethindrone and 1000-1600 liters per day for mestranol. Initial half-lives were 9-13 minutes for norethindrone and 3-9 minutes for mestranol. Previous determinations of MCR for mestranol in other laboratories have used the disappearance of total radioactivity from plasma rather than the disappearance of the labeled steroid. Results of studies under this contract indicate that both mestranol and norethindrone disappear rapidly from plasma but that metabolites of longer half life remain in the circulation for some time. Urinary excretion of radioactivity after administration of labeled norethindrone was 15% of the injected dose on the first day and 23% over a five-day period. Corresponding values for mestranol were 5% on the first day and 12% over a five-day period. Methods for separation of metabolites of norethindrone and mestranol in urine and blood are under development.

Significance to Biomedical Research and the Program of the Institute: Detailed knowledge of the metabolism of contraceptive drugs is necessary to understand the basic pharmacology of these agents and their possible long term effects. Studies conducted under this contract will provide metabolic data on two of the most commonly used contraceptive steroids in human subjects.

Proposed Course: This project is expected to continue for several additional years in order to provide complete metabolic data on mestranol and norethindrone.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Biochemical and Physiological Evaluation of an Oral
Chemical Contraceptive Agent
Contractor: Baylor College of Medicine
Money Allocated: \$13,000 (FY 1972 funds)

Objectives: The purpose of this program is to develop and use analytical methods of high resolution and high sensitivity for studying the absorption, metabolism and excretion of norethindrone. Packed and capillary column chromatography, gas chromatography and mass spectrometry techniques, with associated computer technology, are to be employed.

Major Findings: Work has been conducted on development of methods for isolating norethindrone and related steroids from blood in submicrogram amounts, detecting and estimating quantities of the drug, and separating steroids on capillary columns. Several methods for extracting tritiated norethindrone from plasma have been tested and a procedure has been developed which yields recoveries of 90% from plasma containing nanogram quantities of drug. The preparation of derivatives of 19-nortestosterone, 17 α -ethynyl testosterone, norethisterone and norethynodrel for electron capture detection has been investigated; reaction with heptafluorobutyric anhydride in benzene or isooctane and pyridine is satisfactory for microgram amounts of drug but less so when the sample size is reduced to a few nanograms. Picogram amounts of the HFB derivatives of norethindrone and norethynodrel can be detected by gas chromatographic separation and electron capture detection. Gas chromatography-mass spectrometry techniques with single ion detection were not satisfactory due to losses in the system. Tests of blood samples with the gas chromatography-electron capture method indicate that blood constituents will not interfere with detection of the drug. High resolution capillary column gas chromatography methods have been studied and methods of fabricating and coating capillary columns suitable for steroid separations have been developed. Preliminary studies with the plasma chromatograph suggest that this instrument may permit higher sensitivity of detection.

Significance to Biomedical Research and the Program of the Institute:

Detailed knowledge of the metabolism of contraceptive drugs is necessary in order to understand the basic pharmacology of these agents; high resolution, high sensitivity methods for detecting the drugs and their metabolites would be helpful for these metabolic studies.

Proposed Course: This project was terminated during FY 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Repository for Tissue Evaluation of Contraceptive Steroids
Contractor: Universities Associated for Research and Education in
 Pathology, Inc.
Money Allocated: \$65,500 (FY 1973 Funds)

Objectives: The purposes of this contract are to establish at the Armed Forces Institute of Pathology a central repository for tissue specimens from animals, particularly beagle dogs, receiving hormonal contraceptive agents and to conduct pathological evaluation of the tissues. Contribution of tissue specimens will be solicited from pharmaceutical companies, the Food and Drug Administration laboratories and its contractors, contractors conducting relevant research projects for the Center for Population Research, and others. Standardized procedures will be used in processing specimens, and interpretation of pathology will involve consultative circulation through appropriate divisions of the Armed Forces Institute of Pathology.

Major Findings: Excellent progress has been made in enlisting cooperation from pharmaceutical companies, the FDA and their major contract testing laboratory, and specimens of mammary nodules occurring in beagle dogs treated with contraceptive steroids have been obtained from several of these sources. Contacts have been made with life-span beagle colonies to obtain information and specimens on mammary lesions occurring in untreated dogs for comparative purposes. Two workshops involving representatives of the pharmaceutical industry, life-span colonies, AFIP, and NICHD have been held, one to work out arrangements for submission of specimens, and a second to consider problems of pathologic diagnosis and classification.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use. A major aspect of this testing is long term study in beagle dogs, a species which is unusually prone to development of breast nodules. Several promising contraceptive steroids have been removed from the market when breast nodules developed in beagles treated with these compounds, but there is little information on the pathology of the beagle lesions or their possible relationship to human cancer. This contract was awarded to meet the need for central, expert and unbiased review of tissues obtained from various laboratories and processed with standardized methods; it is expected that the nature of these lesions may thus be determined.

Proposed Course: This contract will probably be renewed annually for a number of years.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: **Pharmacopathologic Effects of a 17α -Hydroxyprogesterone Derivative in Beagle Bitches.**
Contractor: University of Rochester, School of Medicine and Dentistry
Money Allocated: \$144,770 (FY 1973 Funds)

Objectives: This contract supports studies of the effects of medroxyprogesterone acetate (MPA) on uterine and mammary gland structure and function in beagles in an attempt to explain the unusual sensitivity of this species to derivatives of 17α -hydroxyprogesterone. The minimum ovulation-suppressing dose of MPA will be determined, and the effects of this dosage and doses twice and half this amount on morphological, histochemical and biochemical characteristics of mammary glands and uteri will be investigated. Glands and uteri will be removed for study at intervals of 3-4 months for a period of two years, and complete necropsy examinations will be conducted after 12 and 24 months of treatment.

Major Findings: Problems were encountered in determining the minimum ovulation suppressing dose of MPA in the beagle since this steroid, administered in late proestrus or early estrus, does not suppress ovulation and may in fact stimulate it; extensive studies with drug administration at earlier cycle stages would be required to establish the minimal effective dose. Dosages of 3.0, 1.5 and 0.3 mg/kg/3 months were selected since the highest of these is known to inhibit ovulation consistently and a ten-fold range is provided. Test animals have been acquired and injected and histological and biochemical examinations conducted on tissue specimens obtained shortly after the first steroid injection.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Long-term studies in beagle dogs are part of the required testing, and several promising contraceptive agents have been removed from the market when mammary nodules occurred in the beagles, a species which is unusually prone to development of breast lesions. Work under this contract should help clarify the nature of the beagle nodules and their possible relationship to breast cancer in human subjects.

Proposed Course: Initiation of this contract was expected late in 1972 but was deferred until 1973 for administrative reasons. It is expected that the contract will be renewed for an additional year or two to complete these studies.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Circulating Polypeptide and Steroid Hormones in Beagle Dogs
Contractor: Cornell University College of Agriculture and Life Sciences
Money Allocated: \$80,000 (FY 1973 Funds)

Objectives: The purposes of this contract are to develop radioimmunoassay methods for LH, FSH, prolactin, estrone and estradiol and protein binding assays for measurement of progesterone and glucocorticoids in plasma samples from beagle dogs, and to use these assays to determine levels of polypeptide and steroid hormones in the normal reproductive cycle of the beagle.

Major Findings: Several heterologous radioimmunoassay systems for LH have been tested with dog pituitary and plasma samples and bovine and rat standards and systems have been developed which can measure relative changes in LH levels in beagle plasma. Problems with non-specific plasma interference remain to be resolved but this may not be necessary since a radioimmunoassay based on a purified canine LH preparation has recently been published by another group and is now being tested in the contractor's laboratory. Antibodies have been raised to purified bovine FSH and its α and β subunits for use in a heterologous FSH assay. Radioimmunoassays for estrone plus estradiol, and for estradiol specifically, are being adapted to avoid interference from a component of canine plasma. Competitive protein binding assays for progesterone and corticoids have been successfully used to measure levels of these hormones in beagle plasma drawn during the estrous cycle, pregnancy, and parturition.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. The present contract is designed to develop methods for use in subsequent studies of the effects of mestranol and norethindrone on endogenous hormones and a variety of other biological parameters in beagle dogs. The project is expected to contribute to understanding of the biological and pathological effects of two widely used contraceptive steroids in a species which is a required test animal for these drugs.

Proposed Course: When assay methods of appropriate specificity and sensitivity have been developed and levels of endogenous polypeptide and steroid hormones in the normal cycle of the beagle have been determined, this contract is expected to be extended to studies of the effects of mestranol and norethindrone on hormone levels, liver and kidney function, hematological profiles, fertility, and reproductive, endocrine and hepatic pathology.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Development of Radioimmunoassays for Norethindrone, Norgestrel, Ethynyl Estradiol and Mestranol
Contractor: Roswell Park Memorial Institute, Springville Laboratories
Money Allocated: \$50,000 (FY 1973 Funds)

Objectives: The purpose of this contract is to develop radioimmunoassay methods for determining the levels of two progestins (norethindrone and norgestrel) and two estrogens (ethynyl estradiol and mestranol) in human blood and urine and in blood, urine and tissues of monkeys and beagle dogs. The assays will subsequently be used in studies of the absorption, metabolism, excretion, and biological effects of these contraceptive steroids in these three species.

Major Findings: Substantial progress has been made toward the development of radioimmunoassays for norethindrone, norgestrel, ethynyl estradiol and mestranol. Intermediates of the two progestins and ethynyl estradiol (the 3-carboxymethoxyoxime of d,l-norgestrel and of norethindrone and the 3-hemisuccinate of 17 α -ethynyl estradiol) have been successfully synthesized and characterized, and coupling to bovine serum albumin has been completed with incorporation of 14-18 haptenic groups per albumin molecule. Difficulties have been encountered in the synthesis of an intermediate of mestranol for conjugation with albumin and several synthetic routes have been tried; the most recent synthesis has apparently been successful in producing 6-carboxymethoxyoximomestranol in good yield, although confirmatory analysis of the product is not yet complete. Synthesis of tritiated mestranol of high specific activity has been accomplished and ³H-ethynyl estradiol is expected to be prepared quite easily from the ³H-mestranol or from ³H-estrone acetate. Synthesis of tritiated norethindrone has not yet been attempted. The conjugates of norethindrone, norgestrel and ethynyl estradiol have been injected into sheep and goats over a six week period to raise antibodies, and the antibodies will be titrated when ten or more weekly post-immunization bloods have been drawn and the tritiated ligands are available in final form.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation but not relevant to man. The present study is a necessary preliminary to subsequent investigations which will provide information on the suitability of tests in beagles and monkeys for predicting the effects of four of the commonly used contraceptive steroids in man.

Proposed Course: During FY 1974 radioimmunoassays for plasma levels of the

steroids are expected to be established, and developmental work will be done on assays for steroid levels in tissues. The 6-carboxymethoxydine of 17-ethynyl estradiol will also be synthesized and coupled to bovine serum albumin for antibody production and the specificity of an assay using this antiserum compared with that using antiserum to the 3-hemisuccinate. When radioimmunoassays of appropriate selectivity and sensitivity have been developed, it is expected that this contract will be extended to studies of absorption, metabolism, urinary and biliary excretion of these contraceptive steroids and their effects on blood coagulation, the fibrinolytic system, liver and kidney function, mammary glands, fertility and reproductive wastage, and pathology.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Metabolism of Derivatives of 17 α -Acetoxypregesterone in Beagles and in Rhesus Monkeys
Contractor: University of Colorado Medical Center
Money Allocated: \$62,660 (FY 1972 Funds)

Objectives: This is an 18-month pilot study in which the metabolism of a derivative of 17 α -acetoxypregesterone will be studied in dogs and monkeys. The number and relative quantities of urinary and biliary or fecal metabolites of orally administered radioactive progestin will be determined by classical chromatographic methods, and qualitative identification will be obtained by gas chromatography-mass spectrometry techniques. Metabolic profiles in the two species will be compared and species differences in metabolism of the administered progestin will be identified.

Major Findings: This contract was initiated at the end of FY 1972 and studies of the urinary, biliary and fecal metabolites of chlormadinone in beagles and monkeys are in progress according to plan. Data on the identification of metabolites are not expected to be available until the end of the first contract period.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore can not be readily extrapolated to man. This study and the work to be conducted in subsequent contract periods should clarify the metabolism of an important class of contraceptive steroids in two widely used test species and establish the degree of relationship between effects in these species and in human subjects.

Proposed Course: The present contract is for an 18-month pilot study. It is expected that this study will subsequently be extended to determination of plasma levels of parent compounds and metabolites, to other 17 α -acetoxypregesterone derivatives, and to human subjects. Attempts will be made to identify metabolites unique to each species, and the effects of unique metabolites on the occurrence of breast nodules and on glucose tolerance will be investigated. Effects of the progestins on the renin-aldosterone-angiotensin system and interactions with other drugs will also be studied.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Biliary and Urinary Excretion, Enterohepatic Circulation,
and Hepatic Effects of Contraceptive Steroids
Contractor: Health Research, Inc., Roswell Park Division
Money Allocated: \$90,000 (FY 1973 Funds)

Objectives: The metabolism of six contraceptive steroids, including representatives of the 17 α -acetoxyprogesterone series and also of the 19-nor steroids are being studied in baboons and in human subjects by administering tracer amounts of radiolabeled steroids and determining urinary and biliary excretion of radioactivity and enterohepatic circulation and isolating and identifying urinary and biliary metabolites. In baboons, the effects of pharmacological doses of the steroids on biliary excretion, enterohepatic circulation, liver function, and damage to bile canaliculi and hepatic cells will also be determined.

Major Findings: In human studies, norethindrone has been administered to three cholecystectomy patients for studies of excretion of radioactivity and analysis of patterns of metabolites in bile and urine. Major peaks obtained by counter-current distribution of the urine of one of these patients have been further analyzed by secondary CCD, chromatographic methods, and enzymatic hydrolysis to determine types of conjugation. Tritiated dimethisterone has also been injected in two women of reproductive age for studies of urinary excretion patterns and rates of elimination.

Studies of steroid metabolism in the baboon have been conducted both at Roswell Park and also utilizing a baboon with an implanted duodenal cannula prepared at New York Hospital-Cornell Medical Center under another contract. After injection of labeled progesterone in the baboon, the distribution of radioactivity in bile and urine is very similar to that indicated in previously published human data; CCD indicates extensive metabolism and conjugation of injected progesterone in the baboon. Injected dimethisterone is eliminated in the baboon at rates generally comparable to those in the women studied, and CCD of the urines gives similarly uncomplex patterns. Specifically labeled norethindrone has been administered to baboons for study of metabolites in urine and bile and comparison with the studies in human subjects; urinary excretion patterns are complex in both species. Studies of the effects of chronic treatment with norethindrone on liver function and morphology are also underway in baboons, but results are still preliminary.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions may not be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species

under evaluation but not relevant to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically closer to man, may prove to be an excellent choice. This contract is one of several initiated last year to study the metabolism and effects of contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs.

Proposed Course: It is expected that this contract will be renewed for two additional years to complete metabolic studies of norethindrone, norgestrel, ethynodiol diacetate, dimethisterone, medroxyprogesterone acetate, and one other contraceptive steroid not yet identified.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: The Baboon: An Animal Model for the Study of Contraceptive Steroids
Contractor: New York Hospital-Cornell Medical Center
Money Allocated: \$36,000 (FY 1972 Funds)

Objectives: The purpose of this contract is to develop as an animal model the baboon with an implanted duodenal cannula and to use this model in studies of the effects of contraceptive steroids on biliary function. Several modifications in design of the Thomas cannula will be tested as possible improvements over a preliminary design which has already been implanted. A contraceptive steroid, probably chlormadinone acetate, will be administered over a wide dosage range to baboons with cannulas in place, and effects on hepatic excretory function, including bile salt metabolism and pool size, will be investigated. Tests of blood sugar, blood coagulation, serum lipoprotein patterns and liver function will also be performed. Some of the animals with implanted cannulas will be used in collaborative studies with other investigators, particularly in investigations of the metabolism of contraceptive steroids.

Major Findings: Problems have been encountered in mobilizing the baboon duodenum sufficiently to reduce tension on the cannula in the post-operative period, and post-operative complications have led to a high mortality rate. One baboon has made a healthy recovery after successful implantation of a duodenal cannula, and this animal has been used extensively by investigators studying the metabolism of contraceptive steroids under another contract.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically close to man, may prove to be an excellent choice. This contract is one of several initiated last year to study the metabolism and effects of the contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs. The studies supported by this contract should provide data on biliary effects of the steroids and also facilitate studies of their metabolism by other investigators.

Proposed Course: The initial contract has been extended without additional funds to provide further time for preparation of a group of experimental animals. It will probably then be renewed for one or more subsequent years for studies of effects of contraceptive steroids on biliary function and perhaps for preparation of additional animals.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Metabolism of Contraceptive Synthetic Estrogens
Contractor: Loyola University Medical Center
Money Allocated: \$36,700 (FY 1973 Funds)

Objectives: This contract supports studies of the metabolism of ethynyl estradiol and mestranol in baboons. Tracer doses of radiolabeled steroid are administered during the proliferative phase and again during the luteal phase of the estrous cycle, and blood and urine samples are obtained for metabolic studies. After an interval without medication, the animals will be given a combination type steroid contraceptive orally for two to three consecutive cycles, after which the tracer studies will be repeated. The disappearance of radioactivity and of the administered drug from blood will be measured and metabolic clearance rates and plasma half-lives will be calculated. Urinary metabolites will be isolated by chromatographic methods and hydrolysis of conjugates and identified by comparison with reference standards or by physical-chemical methods.

Major Findings: The studies proposed are in progress as planned, with minor modifications in protocol, but it will be at least another year before meaningful data can be expected.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to their approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation but not relevant to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically close to man, may prove to be an excellent choice. This contract is one of several initiated last year to study the metabolism and effects of contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs.

Proposed Course: It is expected that this contract will be renewed for one or more additional years to complete the proposed studies.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Peripheral Metabolic Effects of the Ethynyl Estrogens in
Subhuman Primates and Canines as Compared to Man.
Contractor: Southwest Foundation for Research and Education
Money Allocated: \$21,900 (FY 1973 Funds)

Objectives: Studies supported by this contract will determine the effects of two dosage levels of ethynyl estradiol and mestranol, alone and in combination with norethindrone, megestrol and norgestrel, on certain metabolic functions in baboons and beagles and compare the results with similar data being obtained for human subjects under support from other sources. The effects of the steroids on glucose tolerance, plasma lipoproteins, and plasma cortisol, testosterone and androstenedione will be determined in both species, and in addition, effects on serum bilirubin, fibrinogen, alkaline phosphatase, SGOT, tryptophan, ceruloplasmin, thyroxine, and steroid-binding protein will be determined in the baboons.

Major Findings: Preliminary data from early stages of the protocol suggest that there are significant differences between baboons and beagles in the effects of estrogen administration on carbohydrate and lipid metabolism.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval of these drugs for clinical use. In line with standard toxicologic practice, drugs are administered in arbitrary multiples of the likely or proven therapeutic dose in humans and the biologic endpoints measured are intended to represent the effect to be considered in humans. However, interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and, as a result, conclusions cannot readily be extrapolated to man. This study is expected to provide data on the effects of two dosages of the two estrogens most commonly used in contraceptive formulations, alone and in combination with three of the most commonly used progestins, in one of the required test animals (the beagle dog) and in the baboon which may prove to be more similar to man.

Proposed Course: During the first contract year studies are being conducted in 25 baboons and 25 beagles, and it may be necessary to replicate these studies in a second group of animals in the second year of study. Completion of laboratory tests in both groups of animals will probably require two additional years.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Alteration of Thrombogenic Potential by Oral Contraceptive Steroids in Rhesus Monkeys
Contractor: Litton Bionetics, Inc.
Money Allocated: \$30,841 (FY 73 funds)

Objectives: The purpose of this project is to determine whether administration of contraceptive steroids to rhesus monkeys leads to changes which may be related to the thromboembolic side effects seen in some women using oral contraceptive drugs. A wide range of hematologic tests have been conducted as well as ultrastructural studies of platelets and endothelial cells, and blood flow in mesenteric vessels was observed visually and recorded cinematographically. The drugs studied are mestranol alone and mestranol and norethynodrel in combination, at normal human dosage levels and at ten times this dosage.

Major Findings: Analysis of the data on blood coagulation and platelet function has been completed. The values for Factor II (Prothrombin), Prothrombin time, and partial thromboplastin time did not appear to differ from the control group consistently in any treatment group. Platelet aggregation results showed considerable variation in intensity and velocity using both ADP and Collagen. In some cycles, treatment groups had lower values than the control group, whereas in another cycle or in the replicate group, the same treatment group might have higher values. Overall, no significance except extreme variability could be drawn from analyses. Platelet counts were decreased during the first few cycles in all treatment groups after which they returned to normal. Minimal increases in concentration or activity were seen in fibrinogen and in Factor X, and a slight increase was seen in Prothrombin and Proconvertin (actually the interaction of Factors II, VII, and X). Minimal decreases were seen in Factors VIII and IX. Plasminogen values were increased early in animals given mestranol plus norethindrone, but apparently not in animals treated with mestranol alone. Later, values dropped. Animals treated with only mestranol had values below the control group, while those treated with mestranol plus norethindrone had values similar to the control group. Anti-thrombin III values were consistently and significantly elevated in all treatment groups over the control group. There was some slight suggestion of a dose-effect. Elevation in high-dose mestranol plus norethindrone and mestranol groups appeared slightly more marked than in animals treated with the corresponding low doses. In over-all summary of this portion of the study, it is well known that alteration of levels of individual procoagulants, anticoagulants, etc., are not directly mirrored in increased or decreased coagulability. Nevertheless, these findings suggest that the treated animals might have a decrease rather than an increase in coagulability and resulting thrombogenic tendency.

The morphological studies and analysis of blood flow films are being completed and results will be correlated with the hematological data.

Significance to Biomedical Research and the Program of the Institute: Thromboembolic disease in users of oral contraceptives is the most serious documented side effects of these agents. Little is known of the pathophysiological mechanisms which lead to this complication. Studies in animals, and particularly subhuman primates, may clarify these mechanisms and provide leads to prevention of thromboembolic complications in women.

Proposed Course: The experimental work in this project has been completed and analysis of data will be accomplished by the end of FY 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Drugs on Contraceptive Steroid Metabolism
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: \$21,500 (FY 1973 Funds)

Objectives: Studies will be conducted in rats and guinea pigs to determine the effects of acute and chronic treatment with commonly used drugs on the metabolism of contraceptive steroids. In vitro studies of steroid demethylation and 2-hydroxylation in liver preparations and in vivo studies of metabolic pathways and urinary and biliary metabolites will be conducted in animals treated with phenylbutazone, phenobarbital, chlorpromazine, aminopyrine, glutethimide, and aspirin. These studies will complement studies in untreated animals conducted under a separate contract.

Significance to Biomedical Research and the Program of the Institute: The problem of possible effects of commonly used drugs on the metabolism of steroidal contraceptives in humans is an important one, and these studies in drug-treated animals should contribute useful information on effects which may be expected in man.

Proposed Course: These studies are expected to require at least two years at the present relatively low level of funding.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Comparative Pharmacology of Steroid Contraceptive Drugs
Contractor: Institute of Pharmacological Research "Mario Negri"
Money Allocated: \$172,000 (FY 1972 funds for 18 months)

Objectives: This 18-month contract supports the first half of a 3-year program of studies in four major areas: 1) The effects of steroid contraceptive drugs on chemical mediators in the brain and in target organs; 2) effects on immunological responsiveness and the invasiveness of cancer; 3) effects on lipid metabolism and lipolysis in adipose tissue; and 4) the interaction of steroid contraceptive agents with other drugs. In all of these investigations, data will be correlated with levels of the steroid contraceptive agents in blood and tissue and with antifertility effects. Studies will be conducted in mice, rats, guinea pigs and rabbits. The animals will receive one of three drug combinations administered orally (norethynodrel plus mestranol, norethindrone plus mestranol, and lynestrenol plus mestranol), and in some experiments additional animals will receive the individual steroids alone. A range of dosages will be used in both acute and chronic studies, and dose-response effects will be determined.

Major Findings: A wide range of studies have been conducted in the initial months of this contract, primarily in rats chronically treated with lynestrenol plus mestranol. Preliminary data suggest a number of changes in catecholamine metabolism in brain, including increased synthesis of dopamine. Variations in levels of acetylcholine, choline and acetylcholine transferase in the cerebral hemispheres, diencephalon and mesencephalon have been measured in the estrous cycle and after treatment with the contraceptive steroids. Treatment with lynestrenol plus mestranol, even at low dosages which do not affect fertility, significantly reduces levels of homovanillic acid, the principal metabolite of dopamine, in the striatum of the rat. Carnitine acetyltransferase, an enzyme with high activity in brain and uterus, is increased more than four-fold in the rat uterus by the drug regimen. Lynestrenol plus mestranol at the antifertility and lower dosages have no effect on the dissemination and metastasis of cancer in three test systems; these studies are being extended to higher dosages. Over a wide range of dosages including levels well below the antifertility dosage, this steroid combination causes a marked elevation in plasma triglycerides, lowered plasma cholesterol, and reduced adrenal cholesterol; liver triglycerides are reduced at the higher dosages. Preliminary experiments in mice indicate that the effects of lynestrenol plus mestranol on lipid metabolism are not the same as those seen in rats.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use. In line with standard toxicologic practice, drugs are administered in arbitrary multiples of the likely or

proven therapeutic dose in humans, and the biologic endpoints to be measured are intended to represent the effect to be considered in humans. However, interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. This contract supports a wide range of dose-response studies of the effects of contraceptive steroids in laboratory animals; much of the work is imaginative and is likely to yield valuable new information on the effects of these widely used drugs.

Proposed Course: It is expected that the present contract will be renewed for an additional 18 months to complete the proposed work. If results in animal studies warrant, the studies of drug interactions may be extended to human subjects during the present or renewal contract period.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: A Comparative Study of Progestin Metabolism in Animals
Contractor: Pennsylvania State University
Money Allocated: \$96,100 (FY 1973 Funds)

Objectives: This contract supports comparative studies of the metabolism and biological effects of a series of progestational agents in monkeys, dogs, sheep, rats and mice. Investigations are being conducted on the effect of progestins on, 1) metabolic clearance rates of the progestins and of sex steroid hormones, 2) hepatic steroid metabolism, 3) extra-hepatic steroid clearance rates and steroid metabolism in reproductive and non-reproductive tissues, and 4) various target tissues as measured by gonadotrophin secretion, activities of hepatic drug metabolizing enzymes, synandrogenic actions in liver, kidney and preputial gland, synthesis of cholesterol and bile acids, plasma and liver non-specific esterase activities, and levels of epidermal growth factor in the submaxillary gland. Studies will subsequently be extended to man. The purpose of these studies is to develop methods for predicting the effects of chronic progestin administration in man by relatively short term studies in animals.

Major Findings: Studies of metabolic clearance rates for testosterone and progesterone have been conducted in rats, dogs, sheep and monkeys. Studies of testosterone-estradiol binding globulin revealed that the monkey resembles man in response to sex hormones but that the sheep had no response during pregnancy; this latter species was dropped from the project because of this different biological response. Studies of progestin metabolism as related to bile flow have proceeded and detailed studies of the extra-hepatic clearance and metabolism of various progestins have been conducted. Studies of the binding and metabolism of progestins using the preputial gland of the rat have revealed differences in effect between the uterus and the preputial gland. A stimulating effect of progestins on kidney enzymes has been carefully characterized. It appears that the effect of progestins on liver enzymes does not correlate well with progestational activity on the uterus. The action of a variety of progestins alone and in combination with testosterone was examined using three end organs. The progestins were found to exert independent, synandrogenic or antiandrogenic actions, depending on which chemical was selected and the nature of the end organ. Synthesis of radioactive medroxyprogesterone acetate and selected derivatives has proceeded.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species

under evaluation but not relevant to man. Studies supported by this contract will contribute much needed comparative information on the metabolism and biological effects of progestational agents in five species and could well lead to the development of new and more appropriate test systems.

Proposed Course: The FY 1973 renewal was funded for three months only due to limitation of available funds. It is expected that an additional \$200,000 will be provided in FY 1974 to complete the second contract year, and that the contract will be renewed for two additional years in order to conduct similar studies of other progestational agents.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Vasectomy on Testes, Epididymis and Vas Deferens
Contractor: University of Virginia School of Medicine
Money Allocated: \$26,000 (FY 1973 Funds)

Objectives: This contract supports two separate but related projects by two investigators in the same department. One study is examining the function of smooth muscle in the vas deferens and changes which may occur as a result of vasectomy; experiments are being conducted to measure mechanical properties of the vas, sperm transport, and histological changes in muscularis and connective tissue in animals and human subjects and the effects of vasectomy, age, hormones, drugs, and sexual activity on these parameters. The other study will utilize micropuncture techniques to study intraluminal pressure, sperm morphology, and sodium-potassium levels in the seminiferous tubules of normal, vasectomized and vasoligated rats.

Major Findings: Sperm granulomas have been found to occur without exception in rats vasectomized by a procedure involving mobilization of the vas deferens, but not in rabbits, guinea pigs or hamsters when the same technique is used. This finding indicates the importance of differentiating between the effects of vasectomy or vasoligation per se and immunological and other changes which may be due to granuloma formation in the rat. Granuloma formation can be avoided in a good proportion of rats by avoiding manipulation of the vas.

Micropuncture techniques for obtaining samples of fluid from the seminiferous tubules, caput and cauda epididymis of the rat have been developed and studies of sperm morphology in vasectomized animals are in progress. Preliminary evidence suggests continuous transport of sperm in the vas deferens in the non-ejaculating rabbit. Histological study of resected portions of the vas deferens from 50 men aged 27-82 indicates no significant variation in the amount of musculature and connective tissue with age although there appears to be some increase in fibrous tissue diffusely scattered throughout the muscle layers.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated last year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to provide new and much needed information on the physiological effects of vasectomy on organs of the male reproductive tract.

Proposed Course: It is expected at least an additional year will be required to complete the studies under this contract.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic and Morphologic Effects of Vasectomy in Monkeys
Contractor: Tulane University
Money Allocated: \$274,867 (FY 1973 Funds)

Objectives: The purposes of this contract are to study the immunologic responses and morphological changes in testis, epididymis and vas deferens in vasectomized monkeys and to determine the role of extravasation of sperm in the initiation of an immune response to vasectomy. The effects of three surgical techniques varying in degree of sperm spillage will be compared. Immunological tests include circulating antibodies to reproductive antigens, their immunoglobulin classes and cross reactivity with other tissues, and cell-mediated immune responses. Morphological changes in testis, epididymis and vas deferens will be sought by light and electron microscopy, and data will be correlated with results of immunological studies in the same animals.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there may be complex physiological and immunological effects as a result of this procedure. This project is one of a group initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in the development of these alterations, and their relation to the possible occurrence of systemic complications. This project will provide a thorough evaluation of morphological changes in the vasectomized monkey and correlation between morphologic and immune alterations, and should also provide information on the role of variations in surgical technique in the initiation of an immune reaction.

Proposed Course: This project was initiated at the end of FY 1973 and is expected to require two years to complete.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Sperm Immunology
Contractor: Netherlands Cancer Institute
Money Allocated: \$55,450 (FY 1973 Funds)

Objectives: This two-year contract supports studies in three areas. Sera from naturally infertile men which contain antibodies to spermatozoa will be used to detect autoantigens in sperm fractions, and immunochemical methods will be used to isolate and characterize the antigens. Sperm agglutinins in serum and seminal plasma of naturally infertile and vasectomized men will be characterized with respect to immunoglobulin class and the classes examined for specificity to certain parts of spermatozoa and association with various clinical types of infertility. A variety of modified and new techniques will be developed to measure antisperm antibodies, immunoglobulin classes, and cellular immunity against sperm.

Major Findings: An antigen different from those demonstrable by immunofluorescence on untreated sperm has been demonstrated in solubilized sperm head material. Antibodies to this antigen are present in about 70% of sera with high titers of sperm agglutinins. Purification of the antigen is in progress. Sperm tails have also been successfully separated from sperm heads and solubilized for analysis of specific tail antigens.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number supported by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to contribute significant practical improvements in assay methods for antibodies and cellular immunity to sperm. If defined antigens can be isolated, a radioimmunoassay for sperm antibodies would be possible and new contraceptive approaches may also be developed. The characterization of immunoglobulin classes of sperm agglutinins is of fundamental interest and also may have practical application in predicting the efficacy of vas reanastomosis after vasectomy.

Proposed Course: Initiation of the present two-year contract, originally planned for late in FY 1972, was postponed for administrative reasons until the middle of FY 1973. Studies to isolate sperm autoantigens are under way as reported above, and work on the development of a micro Kibrick assay will be started before the end of the year.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Immunochemical Studies of Sperm Antigens
Involved in the Autoimmune Response in Vasectomized Mammals
Contractor: University of Miami
Money Allocated: \$52,494 (FY 1972 Funds)

Objectives: The purposes of this contract are to study the effects of anti-sperm autoantibodies on sperm fertility, the localization of these antibodies in sperm and testicular tissue, and the role of permeability of the seminiferous tubule barrier in the development of an immune response to sperm. The effects of antisperm antibodies from immunized and vasectomized animals on sperm hyaluronidase activity and ability to disperse cumulus cells will be evaluated and the effects of vasectomy and immunoglobulins from vasectomized animals on sperm fertility will be tested in artificial insemination experiments. The localization of antisperm antibodies in sections of testicular tissue and in epididymal and ejaculated spermatozoa will be studied using light and electron microscopy and various antibody labelling techniques, and the permeability of the seminiferous tubule barrier to immunoglobulins and tracer molecules will be evaluated in immunized and vasectomized animals.

Major Findings: Preliminary data suggest that transitory lesions occur in the seminiferous epithelium of the guinea pig at short intervals after vasectomy and that in guinea pigs immunized with testicular homogenates, the permeability of the seminiferous tubule barrier to artificial tracers is increased.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated last year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies supported by this contract should help elucidate the mechanism of an autoimmune response to vasectomy and also contribute information on the possibility of subsequent restoration of fertility.

Proposed Course: This contract will probably be renewed for two additional years to complete these and related studies.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: An Immunogenetic Analysis of Sperm Autoantigens in the Mouse
Contractor: Cornell University Medical College
Money Allocated: \$52,500 (FY 1973 funds for 6 months)

Objectives: Under this contract the investigators are studying sperm surface antigens directed by genes at the T locus in the mouse to determine whether these surface antigens can act as autoantigens and whether they are represented on cells of non-testicular tissues. Experiments will be conducted to determine the occurrence of autoantibodies to these antigens following vasectomy and to identify the classes of antigens to which the antibodies correspond. The production of antibodies will be monitored using a sperm cytotoxicity test developed by these investigators.

Major Findings: During the first contract year the investigators have determined that the antigen specified by the gene T is autoantigenic, and preliminary evidence has been obtained indicating that wild-type alleles at the T locus, as well as lethal mutants, may specify sperm surface antigens which are also autoantigenic.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated last year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to lead to interesting and highly significant findings on the nature of gene products on the sperm surface which may act as autoantigens under such conditions as vasectomy.

Proposed Course: Substantial progress has been made in the first contract year but complete definition of the surface antigens on mouse spermatozoa specified by genes at the T locus and determination of their autoantigenicity and role in an immune response to vasectomy are expected to require three additional years.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic Studies on Steroid-Producing Cells
Contractor: State University of New York at Buffalo
Money Allocated: \$78,000 (FY 1972 funds for two years)

Objectives: This 2-year contract supports a study of steroid producing cells of the testis and the immunologic response of these cells to vasectomy. Sera from vasectomy patients will be tested for circulating antibodies to Leydig cells, adrenal and ovarian cells, using as controls sera from patients with Addison's disease which contain antibodies to these tissues. The antigens of the interstitial tissue of human and animal testes will be characterized by immunochemical and immunoenzyme methods. Experimental animals will be vasectomized, immunized with interstitial tissue and with steroid-protein conjugates for studies of antibodies and cell-mediated immunity, hormone levels, pathological examinations of the testes, and binding of gamma globulins to Leydig cells.

Major Findings: Preliminary data indicate that circulating antibodies to steroid-producing cells of the testis apparently are not present in sera of vasectomized men, or are present only in very low titers or at long intervals after the operation. Similar results have been obtained in vasectomized rabbits at short post-operative periods, but rabbits have been successfully immunized against testicular tissue to provide antisera for studies of testicular antigens.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated last year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. The studies under this contract should provide important information on possible autoimmune effects of vasectomy on Leydig cell function.

Proposed Course: This 2-year contract was initiated late in FY 1972 and will continue until the end of FY 1974. Plans for additional years of support will depend on the results obtained in the present studies.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Vasectomy and Autoimmune Disease
Contractor: Memorial Hospital, Worcester, Massachusetts
Money Allocated: \$46,592 (FY 1973 Funds)

Objectives: This contract supports studies of the immune sequelae of autoimmune aspermatogenesis and vasectomy in mice. Immunoglobulin profiles and cellular immune responses will be determined with a variety of techniques, evidence for autoimmune phenomena other than aspermatogenesis will be sought by such tests as antithyroid antibody levels and effects on LE preparations, and histopathologic studies will be conducted at autopsy with special attention to evidence for arthritis, endocrine and particularly adrenal tumors, and immune complex glomerulonephritis.

Major Findings: The experiments are in progress but it is too early to expect any significant results.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract provide for comparison between the effects of experimentally-induced aspermatogenesis and vasectomy and also should contribute important information on possible pathologic effects of vasectomy.

Proposed Course: This contract was initiated at the end of FY 1972 and the studies are underway according to plan. It is expected that the immunized and vasectomized mice will be followed for up to two years.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunologic and Morphologic Consequences of Vasectomy
Contractor: University of Missouri Medical Center
Money Allocated: \$86,378 (FY 1972 funds for 15 months)

Objectives: The purpose of this program is to determine the morphological and functional changes subsequent to vasectomy, the role of the immune response in the development of these changes, and the possibility of protection against immunologically mediated injury by certain immunoglobulin classes. Studies will be conducted in histocompatible strain 13 guinea pigs and in human patients. In the first year, tests of circulating antisperm antibody levels and their immunoglobulin classes, immediate and delayed hypersensitivity, macrophage migration inhibition, fertility, and pathologic and immunopathologic effects on reproductive and other tissues will be conducted in immunized, vasectomized and vasoligated guinea pigs. Studies in human subjects will include determination of antisperm antibody levels, immunoglobulin classes, and delayed hypersensitivity before and at intervals after vasectomy.

Major Findings: Work under this contract has been initiated according to plan but it is too early for any significant data from these studies.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number supported by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies supported by this contract should contribute significantly to defining immunological mechanisms in experimental allergic aspermatogenesis and following vasectomy, and provide for correlation of data between the guinea pig model and vasectomized patients. Studies planned for subsequent years should further clarify immunopathogenetic mechanisms in transfer experiments and test the protective capacity of passive immunization against the immunologic consequences of vasectomy.

Proposed Course: It is expected that this contract will be renewed for one or more additional years to support continuation of these and related studies.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immunopathologic Consequences of Vasectomy
Contractor: University of New Mexico
Money Allocated: \$59,900 (FY 1973 Funds)

Objectives: This contract provides for a two-year study of the effects of vasectomy in 500-1000 human subjects and in experimental animals. Sera will be monitored for antibodies to sperm; immunoglobulin classes of the antibodies, their cross-reactivity with other tissues and their ability to activate complement components will be determined; and leukocytes will be assayed for cell-mediated hypersensitivity. The patients will be followed, particularly for symptoms of a possible autoimmune response to sperm. Vasectomized animals will be autopsied at various intervals and pathological lesions will be sought in the testis and other organs, and attempts will be made to induce similar lesions by passive and adoptive transfer. The relationship between appearance of antisperm antibodies, degenerative changes in the testis, and sperm granuloma formation will be examined in vasectomized animals and the role of an immune component in the sperm granuloma reaction to α -chlorhydrin will be studied in rats with induced tolerance to sperm.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number supported by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract should provide valuable new information on autoimmune reactions to vasectomy and the pathogenetic mechanisms which may be involved.

Proposed Course: Plans for the initiation of this contract at St. Louis University in FY 1972 were deferred due to the investigator's move to the University of New Mexico. The project was actually started in January 1973, and it is too early for any significant results. Plans for support beyond the present two-year study will depend on the outcome of studies currently underway.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Autoimmune Phenomena and Mechanisms in Human Vasectomy
Contractor: University of Cincinnati Medical Center
Money Allocated: \$79,466 (FY 1973 Funds)

Objectives: The purpose of this contract is to evaluate alterations in immunologic responses, blood coagulation mechanisms, and medical status in 50 vasectomized men. Tests are conducted before and at intervals after vasectomy to assess the development of circulating antibodies and delayed hypersensitivity to sperm and seminal fluid antigens and of circulating immune complexes, alterations in humoral and cellular immune responsiveness, the occurrence of anti-nuclear antibodies, rheumatoid factor and other indicators of autoimmune disease, and changes in factors involved in blood coagulation. Clinical examinations and medical histories are also obtained at each visit.

Major Findings: The various tests have been standardized and patients are being enrolled in the study and subjected to the test battery according to plan. The study has not progressed to the point where significant findings may be expected.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. A number of contracts have been initiated by the Center to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. This contract is the first awarded for intensive clinical study of vasectomized men and it should provide important information on alterations in the immunologic and hemostatic systems and in medical status after surgical sterilization.

Proposed Course: It is expected that patients in this study will be followed for at least 2 years.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Immune Alterations Associated with Vasectomy
Contractor: Baylor College of Medicine
Money Allocated: \$133,000 (FY 1973 Funds)

Objectives: This contract supports prospective studies of the incidence, temporal onset and progress of immune alterations in vasectomized men. Studies will be conducted before and at intervals after vasectomy in 48 men; insofar as possible, the subjects will be individuals also under study for endocrine alterations under another contract. Tests will be conducted of sperm immobilizing and sperm agglutinating antibodies, HLA antigens, levels and types of serum immunoglobulins, autoimmune antibodies, cellular immunity and cellular autoimmunity to reproductive antigens. Data from three standardized sperm serology assays will be correlated with other data on the same subjects in an effort to determine the functional significance of positive serological reactions. Methods will be developed for measuring cellular immunity to sperm.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but complex physiological and immunological effects may result from this procedure. This contract is one of a number initiated by the Center to define the long term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. This project will provide a detailed definition of immunological changes in vasectomized men, with particular attention to cellular immunity and the HLA antigens, and also a careful evaluation of the significance of three commonly used serological assays for circulating antibodies to sperm.

Proposed Course: This project was initiated at the end of FY 1973. Plans for subsequent studies will depend on results obtained in the first year.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Endocrine Changes in Vasectomized Men
Contractor: University of Texas Medical School at Houston
Money Allocated: \$276,850 (Fy 1972 funds for two years)

Objectives: The purpose of this program is to conduct a prospective study of endocrine changes in men undergoing bilateral vasectomy. Plasma levels of testosterone, estradiol, 20 α -dihydroprogesterone, FSH, and LH will be determined in approximately 200 men prior to vasectomy and at 1 and 6 weeks and 3, 6, 12, and 24 months after vasectomy.

Major Findings: This project was initiated late in FY 1972 and in its first year of operation the enrollment of subjects and determination of hormone levels has progressed according to plan. It is too early to tell whether significant alterations in hormone levels will occur in the study patients.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated last year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. No data are presently available on the endocrine effects of vasectomy and such effects would not necessarily be expressed somatically or behaviorally. Accurate data on levels of testosterone, estradiol, FSH and LH are therefore required, and 20 α -dihydroprogesterone levels may be indicative of testicular damage if this should occur.

Proposed Course: This two-year contract was initiated late in FY 1972; plans for possible renewal for subsequent years will depend on the results obtained in the present studies.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: USC Collaborative Study of the Effects of Vasectomy
Contractor: University of Southern California Medical Center
Money Allocated: \$337,500 (FY 1973 funds for 18 months)

Objectives: The purpose of this study is to conduct a pilot evaluation of the effects of vasectomy in 50 patients and 50 matched controls who will be studied prospectively. The evaluation will include medical history, physical examination, psychological evaluation, clinical laboratory data, humoral and cellular immune alterations, and hormonal changes. The first contract period is intended to support a pilot study during which the administrative operations and laboratory data will be evaluated and plans will be made for appropriate modifications and possible expansion of the study in subsequent years.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but complex physiological and immunological effects may result from this procedure. This project is one of a number initiated by the Center to define the long term consequences of vasectomy, the mechanisms involved in these alterations, and their relation to the possible occurrence of systemic complications. This project will provide a comprehensive evaluation of medical and psychological alterations in a small group of men on a pilot basis. Data from this study and from other contracts in this program will provide needed practical information for planning subsequent prospective studies.

Proposed Course: This 18-month pilot project was initiated at the end of FY 1973 and will be concluded in mid-1975. Plans for continuation or expansion of the study will depend on data available toward the end of the first contract period.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Physiological Mechanisms Involved in Reactions to
Implanted Devices in Animal Uteri
Contractor: Agricultural Research Service, U.S.D.A.
Money Allocated: \$48,033 (FY 1973 Funds)

Objectives: This project was funded in 1973 for a terminal year to complete studies initiated in 1972 on (1) steroid and gonadotrophic hormone levels in plasma of sheep treated with synthetic progestins, (2) the effects of induction of hepatic microsomal enzymes on plasma levels of estrogen and progesterone and reproductive function in sheep, and (3) the effects of copper IUDs on developing rabbit fetuses.

Major Findings: Data from studies conducted this year are not yet available but will be reported at the completion of the study.

Significance to Biomedical Research and the Program of the Institute: Over the years this program has made significant contributions to knowledge of the varied effects of intrauterine devices and to more basic aspects of reproductive physiology. The studies conducted this year should provide useful data on the effects of synthetic contraceptive progestins on reproductive endocrinology in a new test animal; new information on the effects of commonly used drugs on the estrous cycle and corpus luteum function; and information on the possible toxic effects of copper IUDs on the developing fetus of a second species for comparison with findings last year on the lack of toxicity of these devices on the developing sheep embryo.

Proposed Course: This project will be terminated at the end of FY 1973.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract Title: Effects of Steroid Contraception on Cervical Dysplasia
Contractor: University of California, Los Angeles
Money Allocated: \$71,509 (FY 73)

Objectives: The contract is to study the effects of steroid contraception on cervical cancer by studying dysplasia of the cervix. This program has been in operation since June 1967. Patients from family planning clinics of the Los Angeles County Health Department are entered into the project for a prospective study of the relationship between progression of cervical dysplasia and the use of oral contraceptives.

During the first years of operation, the project was centered in the inner city where the racial composition of the target population and, consequently, of patients entering the study was predominantly black (82% of all eligible patients). The expansion of the project into East Los Angeles, Hollywood-Wilshire, Whittier West and Northeast, has led to changes in the demographic and socio-economic characteristics of the population under investigation. It now includes a larger proportion of Spanish Americans and Caucasians in higher income levels than the original populations. The aim of enrolling at least 200 patients in study and control groups has been achieved.

Major Findings: As a first step to evaluate a possible differential in carcinogenic development between pill users and controls, the characteristics of pill choosers and their controls (IUD Choosers) were investigated. Women choosing the pill differed from those choosing IUD's in having higher income and lower body weight, but no further differences were found over a wide range of demographic and biomedical characteristics, including religion, ethnic group, age at first intercourse, number of children, etc.

The major finding of the program so far has been published in Science, 31 July 1970, Vol. 169, pages 497-498: A significant association of dysplasia was found with choice of the contraceptive pill compared to choice of IUD, or of the IUD and other methods. These findings have been updated and extended from a predominantly black population of contraceptive users to other ethnic groups. The generalization of an increased prevalence of dysplasia associated with pill choosers seem to hold for all three ethnic groups examined; black, Caucasian and Spanish. More recent analysis (soon to be published in Contraception) has demonstrated that this difference has disappeared.

Most important has been the reaction that there may be difference in pill users and controls after 2 years of oral contraceptive use. This observation should be confirmed within a year.

Significance to Biomedical Research and Program of the Institute: This proposed work, if carried out successfully, could be of great value in assessing the risks of contraceptive therapy and is, therefore, clearly relevant to the program of the Center.

Proposed Course: The project should be completed within a year or two.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Fertility Regulating Methods Evaluation Branch
Contract and Collaborative Research

Contract and collaborative projects listed below are reported in detail in the Biometry Branch reports on pages indicated.

A Collaborative Study of Oral Contraception and Cerebrovascular Disease	BB 11
A Comparison of the Medical Effects of Induced Abortion by Two Methods, Curettage and Suction	BB 9
Oral Contraceptives and Tumors of the Breast	BB 6
A Retrospective Study of the Risks for Cancers of the Breast, Body of the Uterus, Ovary and Cervix Among Users of Oral Contraceptives	BB 8
A Study of the Outcome of Subsequent Pregnancies in Women Who Have Used Oral Contraceptives	BB 10
Epidemiologic Study of Breast Cancer and Benign Breast Lesions in Relation to the Use of Ovarian Hormones	BB 5



NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Office of the Associate Director for Extramural Programs

When the NICHD was established ten years ago, it brought to the National Institutes of Health a new concept of scientific and fiscal management of extramural programs. This concept was based on a conviction that extramural management should be on the basis of the problem to be solved rather than the mechanism to be used in providing the funds. At that time in the existing Institutes, research grants were managed by one group of scientist administrators and grants managers, training grants were managed by different groups, and the same was true of the Research Career Program and of fellowships. Research contracts were managed by a still different group.

The NICHD established a pattern based on the program concept wherein a group of problems are organized into a program for which scientist administrators who are experts in their fields are responsible, along with grants and contracts managers who serve only that program. Under this arrangement, a single scientist administrator can be responsible for all of the research grants, training grants, RCA's, RCDA's, fellowships and contracts in his area. He is in a position therefore to know in great detail exactly what is being supported by the NICHD in the area for which he is responsible. He can also keep abreast of other work in his field and can engage in active programming of new applications when gap areas are detected.

Each program is headed by a branch chief who, by keeping in close touch with all of his scientist administrators, is always in a position to know what research and training is being supported within his program. Similarly, the Associate Director for Extramural Programs and the Director of the Institute can be kept fully informed of progress on all aspects of the various problems for which responsibility has been assigned to the NICHD by the Congress.

This philosophy proved to be not only very successful in the scientific and fiscal management of grants and contracts but also exceptionally well adapted to planning for the future. Increasingly over the years, the Department and the Administration have stressed the crucial importance of planning. It soon became clear to the NIH that effective planning was much more difficult where extramural activities were administered strictly on a mechanism basis. Many of the Institutes were discovering this for themselves and, whether for this reason or for others, it has been interesting to observe that with regard to scientific management, the trend toward organizing along NICHD lines has accelerated until at the present time it is difficult to find within the NIH an organization based on mechanism of support.

The decision of the Administration, announced January 29, 1973, to phase out NIH training grants, fellowships and RCDA's will make it especially difficult for the NICHD to assist in the preparation of future investigators. Not only is the Institute a relatively new part of the NIH, but many of the

fields for which it is responsible (mental retardation, population, aging and neonatology) had very little research or training support prior to 1963. The Institute has emphasized the importance of training so that the individuals trained would eventually engage in vitally needed research in areas which had too long been neglected. It is not unlikely that some future investigators will still be trained on research grants but it is clear that the number who will be so fortunate will be very small.

The phase out of the support of training grants and fellowships will have an influence on our Research and Training Committees. Consideration is being given both to meeting less frequently and to some form of consolidation. On the other hand, the new requirement that all NICHD program projects and center grants must receive a full scientific review every three years will go into effect during the coming fiscal year. This will mean an added work load for the committees.

During fiscal year 1973, two valued staff members left the extramural programs: Dr. Florence Mayer of the Growth and Development Branch joined the staff of the National Heart and Lung Institute, and Dr. George Morgan of the Growth and Development staff joined the NICHD intramural program as a Senior Staff Fellow. We were very fortunate to find a replacement for Dr. Mayer in Dr. Gilman Grave who came to the NICHD from the NIMH intramural program. As this is written, recruitment efforts for a replacement for Dr. Morgan are very far along. Efforts to recruit a Medical Officer for the PBIM program have continued throughout the fiscal year, but this is proving to be an extraordinarily difficult task. We remain convinced, however, that an M.D. is essential in the program and the efforts are continuing. The program was greatly aided for four months by Miss Evelyn Hayes, a senior medical student from Louisiana State University.

The significant activities and accomplishments of the extramural branches are summarized in the reports which follow.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Growth and Development Branch

The Growth and Development Branch supported approximately 30% of the research grants in the NICHD in FY 1973. This proportion has remained constant for the past several years.

During this fiscal year the Branch supported three conferences and one small workshop. Two of these conferences were devoted to adolescence, which is a major new thrust for the Branch and for NICHD. The first was held in October 1972 and was entitled "The Control of the Onset of Puberty." The major focus for this large meeting was the hormonal mechanism triggering the physiological and neurological maturation that is the hallmark of adolescence. The proceedings of this conference are being prepared for publication by a private publisher in the fall of 1973. The second conference was on the subject of "Adolescent Nutrient Requirements"; it was held in early June. This invitational meeting reviewed and assessed current knowledge of nutrient needs in adolescence and the factors which influence nutrient requirements during this stage in life. A primary focus was on new ways to estimate and express nutrient needs during adolescence when individual differences in size, growth rate, etc., preclude the meaningful use of chronological age as a reference point. The proceedings of this conference also are to be published as a reference volume for scientists, physicians, and students.

The third NICHD-sponsored conference, in the area of developmental pharmacology was held in June, 1973. This conference was entitled "Chemical Pollutants: Susceptibility of the Fetus and Child," and was co-sponsored by the National Institute of Environmental Health Sciences and the National Institute of Child Health and Human Development and organized through the American Academy of Pediatrics. This conference facilitated detailed discussion of some of the problems that are unique to the fetus and child in handling pharmacologic agents and environmental pollutants, with special emphasis on the way in which a developing organism handles chemical agents at various age-dependent periods in the life cycle. The proceedings of this conference will be published.

The small workshop which was held was directed to evaluating the state of knowledge concerning the impact of a chronically ill family member or other persons in the family, particularly on the development of young children. This question becomes increasingly important as life sustaining techniques are being used more widely and as homecare is being recommended for people with chronic illnesses or long-term progressively debilitating illnesses. Other Institutes (NCI, NHLI, NIAMD, NINDS) participated in this workshop as the problem is of widespread concern. It would appear that there is a woeful lack of hard data in this critical area.

Two new publications were published this year. Nutrition, Growth, and Development of North American Indian Children, edited by staff members, appeared in the fall of 1972. This book presents new data on nutritional problems of American Indians and interprets these findings in terms of potential developmental or health problems for Indian children. A portion

of the book traces the past and present cultural heritage of our Indian population and suggests ways in which research and service can be intertwined to the benefit of the Indians themselves. Nutrition, Development, and Social Behavior, edited by a former staff member, summarized the papers and discussions during an interdisciplinary conference of medical and social scientists. The meeting was co-sponsored by the Pan American Health Organization; a Spanish language edition is planned. This volume reviews the current data on nutrition and mental development, with special emphasis on the social factors which surround malnutrition and influence behavioral development. Thoughtful critiques of alternate strategies for solving this complex problem are presented.

Training for Research

Until February of 1973, the Growth and Development Branch supported three types of research training programs: training grants, fellowships, and research career development awards. While these programs are being phased out and no new programs will be initiated, the support provided during FY'72 may be summarized as follows:

During FY'72, a total of \$3,667,000 was spent on growth and development training in support of approximately 350 trainees in these three programs. About 60% supported biomedical and 40% behavioral research training. Approximately 70% of the funds supported pre- and post-doctoral training grants, 5% supported fellowships, and 25% supported RCDAs.

In March of 1973 there were 35 training grants, 22 fellowships and 36 RCDAs.

Mechanisms of Biological Growth

The aims of this research program continue to be the unveiling and the understanding of the fundamental processes which initiate, control and promote orderly growth and differentiation at the cellular and subcellular levels. This area of research extends in scope from studies on the molecular structure of genomes to studies on hormonal effects on growth and maturation of children. One hundred and thirty-six research grants were supported bearing on various aspects of the mechanisms of biological growth.

We are presently supporting much crucial work on one of the most exciting areas in biological investigations, namely the transmission of information within cells and the transmission of information between the cell and the integrated organism. It is noteworthy that these timely topics were chosen to be the subjects of the IInd FASEB Conference held in Atlantic City in April, 1973. Control of transcription; control of translation; genetic control of cell differentiation; genetic control of mammalian enzymatic activity and genetic control of mammalian development are examples of ongoing work supported by the Branch at the basic level of intracellular and intercellular transfer of biologic information. Exciting progress has been made by one grantee in this area in an ambitious attempt to achieve the chemical synthesis of a gene. Two new grants awarded in this area during this fiscal year deal with the control of the orderly proliferation of muscle cells and the evolution of enzyme systems and their control points.

At the more complex level of information transfer within an integrated organism, the Branch supports much exciting work on the generation of pattern and form in biological systems. Studies on hormonal and neural mechanisms that control the onset of puberty and studies on the mechanisms of hormonal regulation of growth and development are examples of such work at this level of complexity. Another grant awarded during FY'73 deals with the development of sensitivity of mammalian cells to growth hormone. These investigators have discovered that human growth hormone reverses the disintegration of muscle cells in certain types of muscular dystrophy, a finding that holds great promise for clinical application.

Developmental Immunology

Understanding of the mechanisms involved in the development of immune competence in humans has become possible through rapid development of new methodologies and technologies in this field as well as through training of scientists who have used unique approaches for answering important research questions. This represents basic science closely wed to an array of clinical diseases and health problems. Insight into basic immune mechanisms has been gained through use of both animal and human models. Two general strategies have been used in eliciting new data. The phylogenetic approach using mammals and lower animals has been extremely important since by studying the more primitive responses in simpler organisms, it is believed that a more comprehensive insight and a better understanding and perhaps control of the more complex mammalian systems will be forthcoming. The ontogenetic approach in humans is also used so as to unravel the regulatory factors which control time-linked changes as they occur with increasing age and maturation from the fetal period onward. The final objective is to better understand the ability of the human host to combat infection and disease. Although there is no categorical disease orientation, efforts are focused on studies of basic mechanisms involved in humoral and cellular immunity as well as an understanding of the local secretory immunologic system and the various non-specific protective mechanisms of the human host.

During the past year 19 grants and contracts have been directed toward immune processes. Among these, several important immunologic investigations currently being funded through NICHD should be mentioned. Studies of human neutrophils (white blood cells with phagocytic function) have revealed several disorders of neutrophil function. This has resulted in marked susceptibility to infecting bacteria in the patients involved. The defects include a dysfunction in the terminal enzymatic steps responsible for bacteriolysis by these cells, as well as problems of attachment and opsonization of the particle and failure of lysosomal fusion with the phagosome. There has also been lack of random and directed neutrophil migration which is necessary to maximize neutrophil-particle interaction. In some patients the phagocytic process has involved deficient killing of only one specific organism whereas in others it is a more general defect. At present this group of clinical conditions is known as the "phagocytic dysfunction syndrome" and further studies are indicated before meaningful nosological terms can be applied. These investigations have made possible the development of appropriate therapy that has increased survival in some patients and has been life-saving in others.

Other studies have resulted in the recent publication of regimens for treatment of immune deficiency diseases, including such conditions as deficiencies in humoral immunity, phagocytic function, and cellular immunity. Another investigation has produced a method of treating the Wiskott-Aldrich Syndrome, a known genetic immune disease, with the use of "transfer factor," a product of lymphocytes. Other studies of cellular immunity have revealed information on the development of cellular immunocompetence in very early life, and a method has been proposed which appears to have the potential for use as a marker of thymus-derived cells which are responsible for cellular immunity. All of these studies demonstrate the need to know more about normal human development in order to better understand and treat the abnormal.

Continued efforts will be directed toward a holistic approach to the development of immunologic competence. Attempts will be made to look at all the various human defenses against foreign substances, infection, and disease so that the totality of the mechanisms involved can be better understood, and then a picture of immunologic balance or imbalance in health or disease states will emerge.

There also will be a continued effort to study the immunologic system in relation to changes in both the internal and external environments which may influence the host defense system in coping with pharmacologic and foreign agents. There is also continued interest in determining the effects of malnutrition on the immunologic process in order to better understand the relationships between nutrition and infection.

Developmental Pharmacology

Our society has given increased emphasis to the area of developmental pharmacology in recent years because of more awareness of such problems as adverse reactions and environmental pollutants. Just as earlier biochemical studies in this area have emphasized the pharmacokinetics of endogenous or natural occurring substrates, today the emphasis is more on maturational deficiencies in the developing human from the fetal period on through the aging process. Much of past drug therapy has been on an empirical basis and now more concern is evident for the way a developing fetus, infant, child, adolescent, adult, and aged individual handles a given drug based upon characteristics due to that particular developmental period in his life cycle. Nonetheless, much too little emphasis has been given to studies of pharmacologic agents and the age-dependent variations in the way the human body handles these agents. There is considerable concern over use of the appropriate dosage of medication in children as well as adults with a possibility of error being on too small as well as too large a dose.

For these reasons, the Growth and Development Branch is seeking to expand support for studies of pharmacokinetics of foreign substances and drugs which are most commonly a problem or in frequent use in treating infants and children. Only five studies were supported in this area but concentrated programming by staff should help increase this number.

A recent study funded by NICHD gives evidence of the needs and research possibilities within pediatric pharmacology. This investigator has developed

a new, rapid, sensitive, clinically useful radioimmunoassay procedure for determination of serum and urine digoxin concentrations. Digoxin is an invaluable drug for a variety of cardiovascular diseases in children, but it has a low margin of safety. Complications from use of this drug have resulted in the past in both children and adult populations. The availability of this new assay procedure can lead to a rational use of this invaluable agent in children with cardiovascular disease.

Future research must allow proper investigation of pharmaceutical agents as well as environmental pollutants in order to provide adequate patient safety and proper use and better understanding of important therapeutic agents as well as hazards to which our populations are exposed. Efforts must be made to provide protection to the public against environmental hazards and adverse drug effects, but at the same time we must recognize the need for the development of new drugs to keep pace with therapeutic needs as well as advances in knowledge of disease states.

Nutrition

Nutrition research supported by the Growth and Development Branch reflects the multidisciplinary implications of adequate or improper nutrient intake and utilization. Emphasis continues to be placed on the role of nutrition in physiological and intellectual development, and the important interactions among nutrition, behavior and environment. Studies of the metabolic, cellular and behavioral correlates of the initiation of obesity in infancy, childhood or adolescence are another area of program interest. Several investigators supported by the Branch are conducting diverse studies with the aim of more clearly defining the role of nutritional factors in cell growth, organ development and function, body composition, metabolism of nutrients in early life, and the physiology of normal and abnormal growth. A total of 32 grants and contracts have nutrition as their primary focus; many others include nutrition as an important but secondary component.

In human studies, special attention is being given to appropriate methods for assessing impaired intellectual and behavioral development and for evaluating the sociocultural environment of malnutrition--which in turn may influence mental development. Although emphasis has been placed on elucidation of the developmental consequences of severe maternal and child nutritional deprivation, possible adverse effects of the lesser degrees of malnutrition more commonly found in the United States are also being considered. The Branch continues to support a number of projects seeking to better define growth, body composition and nutrient requirements of normal individuals.

Definition of growth patterns, cellularity and metabolic processes of individual fat cells and adipose tissues in children and adolescents is the main thrust of several obesity studies funded by the Branch. Others are directed toward identification of the behavioral, genetic, endocrine and nutritional factors associated with overeating and weight reduction. The relationships of obesity with degenerative diseases in animals is being studied, as is the effect on body composition of weight reduction through

diet or exercise.

The Branch has implemented, through grants and contracts, a coordinated series of animal studies investigating the cellular and functional consequences for the central nervous system of prenatal and postnatal malnutrition. Rat studies are seeking confirmation of preliminary data indicating that severe pre- and early postnatal malnutrition may influence subsequent nutrient utilization and the possibility that the consequences of malnutrition may be additive over generations. The subhuman primate is utilized as a model for more direct correlation of malnutrition effects with those in man. One study is measuring the effects of closely quantified protein and caloric restriction of the rhesus monkey during pregnancy on the histological, biochemical and endocrine characteristics, cellularity and cell function of several vital tissues, including brain, of the infant. Another investigator is pursuing a multidisciplinary study of the physiological and behavioral effects of maternal prenatal protein restriction on the infant rhesus. Another researcher is seeking to differentiate the effects of early social deprivation from protein restriction early in life on later social behavior of the rhesus monkey.

Somewhat parallel studies are underway in human populations as well. A village level supplementation program in Guatemala has shown that maternal caloric intake during pregnancy is more directly correlated with birth weight than is protein intake. The latter appears more correlated with postnatal mortality, morbidity, and growth. Neurological and behavioral development are also being assessed. In another study, maternal supplementation during pregnancy and lactation not only produced a larger, more rapidly growing infant but also one who was physically much more active and demanding of maternal attention. These findings suggest that the active undernourished infant demands less and gets less attention, leading to worsened nutritional status and retarded development.

During the past year, preliminary data from the maternal nutrition studies indicate that protein requirements during pregnancy may be considerably lower for the rhesus monkey than heretofore considered, provided that the diet is adequate in other nutrients. Similar results were reported by an investigator maintaining monkeys on an extremely low protein diet from weaning to maturity.

One grantee has recently reported that in the rat, maternal caloric deprivation during early lactation produced transient reduction in fat cell numbers and size in the offspring, whereas maternal restriction of both protein and calories resulted in permanent reductions in depot fat cell number and size in the offspring. These results may have important implications in human obesity in light of a prior finding by the same grantee that obesity in childhood is a function of fat cell number, size, and age at which rapid increase in fat cell number/size is initiated.

Physical Growth

The 21 research grants and contracts supported by the Growth and Development Branch in the area of physical growth encompass developmental anatomy,

cranio-facial growth, skeletal growth and studies in population genetics relating to anatomical development.

Projects supported in the area of developmental anatomy include descriptions of postnatal development of human cerebral cortex, lung growth, ultrastructural morphology of bone, as well as the growth and hypertrophy of various tissues or organs in health or disease. Cranio-facial growth studies are concerned primarily with identifying the genetic, metabolic and endocrine mechanisms involved and examining interrelationships of the different variables of facial growth and dentition. Several investigators seek methods for the eventual prediction of individual cranio-facial growth rates and patterns, and early diagnosis of abnormal cranio-facial development.

Skeletal growth studies include projects developing normative data on the bone weight, density and ash content of normal children and animals. Other projects involve longitudinal study of skeletal growth of low birth weight and normal birth control children, and children whose development may be compromised by severe malnutrition or chronic disease. Still other investigators, utilizing anthropometric data and radiographs from completed and ongoing longitudinal child growth and development studies, are seeking improved methods for evaluation of skeletal development, assessment of skeletal maturity, and the prediction of mature stature.

Developmental Behavioral Biology

This area within the Growth and Development Branch is concerned with the biological substrate and mechanisms that mediate behavioral processes. Among the 30 grants, emphasis is given to studies that clarify the neural processes of sensory and perceptual functioning and how differences in early environments affect the development of sensory-neural and perceptual-behavioral functioning. Included in these areas of concern are the effects of malnutrition, anoxia, sensory deprivation, and the toxic characteristics of impoverished and traumatic environments upon the development of the brain and behavior. Additionally, attention is being given to clarifying the role of genetic and environmental factors and their interactions upon development and whether enriched early environments can alter the development of the individual.

Previous studies on the cortical evoked response to sensory stimulation have for the most part been concerned with nonpattern stimulation, i.e., flashes of light, pure tones, etc. Research programs have been established to investigate the cortical evoked responses to pattern stimulation, e.g., geometric figures and speech sounds. Pattern perceptions are dependent upon the functioning of higher brain processes and these phenomena are fundamental to more complex cognitive and learning abilities such as reading. An understanding of the developmental maturation of brain processes associated with pattern perception is an indispensable prerequisite to understanding abnormalities of pattern perception, e.g., learning disorders and dyslexia. Related to these issues is the finding that habituation of cortical evoked responses is related to its variability, i.e., a systematic decrease of responding is associated with an increase in variability of these responses. The neural

mechanisms underlying habituation and variation of responses to sensory stimulation are intimately related to the issues of selective attention, learning and memory which require additional research before application to perceptual disorders becomes possible. Additionally, the cortical evoked response has been found useful in neurological diagnosis of a brain-bisected child where a massive infarct on the right hemisphere was identified. Further progress in the clinical application of the cortical evoked response is in process where severely malnourished infants and children are being evaluated, utilizing cortical evoked response methodology. Preliminary results indicate gross abnormalities in the brain's functional ability to process sensory information in malnourished children.

Behavioral genetic studies have been initiated to: a) estimate and compare phenotypic and genetic factors on cognitive abilities; b) to assess the effects of inbreeding, assortative mating, ancestry, parental age, birth order, certain maternal-fetal risks and socio-economic variables upon cognitive development and functioning; c) to search for possible relationships between cognitive personality traits and genetic markers; and d) to search for nonadditive genetic effects in components of cognitive ability. The strength of the above studies is based upon the utilization of family groups (in contrast to twin studies) derived from distinct ethnic groups in a wide range of socio-economic environments. Progress reports from these newly initiated studies will be made annually.

Learning and Cognitive Development

Learning, usually thought of as relatively permanent behavioral changes associated with environmental interventions, and cognition, a term applied to the process of knowing, are not well understood or even adequately defined by investigators in the field. They are, however, conceded to be complicated processes which occur at any age in all living animal forms. In the past, investigators have been chiefly concerned with environmental factors affecting the rate and accuracy of learned performance. Within recent years, however, molecular biologists, such as geneticists and biochemists, have also concerned themselves with objective studies of learning at a relatively basic level. In fact, it has become increasingly apparent that many phenomena of life can be effectively studied at several different scientific levels and that it is, therefore, quite appropriate to look for an understanding of the biology of learning as well as its psychological determinants. This is particularly true because to date no one behavior theory, or learning theory, has been able to assimilate or integrate all the data that has been obtained from the wide variety of learning situations studied. Indeed, one of the major strengths of the support program provided by the Growth and Development Branch, NICHD, in the area of human learning and cognitive development has been its breadth and lack of theoretical bias. The Branch has supported about an equal number of investigators who have looked at learning from an environmental point of view and those who have looked at it from a biological or genetically based mechanism.

As of December, 1972, the Growth and Development Branch supported 59 research and training grants, fellowships, and career development awards in the area

of learning and cognitive development. This is approximately the same number of grants supported in this area during FY'71. While the specific research projects conducted by our grantees represent virtually all aspects of the learning and cognition area, several topics appear to represent concentrations of effort. For example, discrimination learning in children has attracted the attention of a number of Growth and Development sponsored investigators. In one group of studies, investigators are examining the developmental aspects of discrimination learning within the special framework of attentional theories of learning. Reinforcement contingencies are varied along different dimensions of the stimulus array including the discriminability of the stimuli. It is hoped that the data will provide statements about the developmental aspects of discrimination learning. The attentiveness of children is also under study by other investigators who are using measures of autonomic and central nervous system activity as research tools.

While other Growth and Development supported investigators are interested quite specifically in such areas as information selection and organization, the development of perceptual motor abilities, infant's memory for visual stimuli, and the development of logical operations, a number of researchers are broadly concerned with the general development of cognitive and perceptual processes in children. They are discovering that children are capable of much finer perceptual differentiation under certain conditions than the existing literature on perceptual processes would indicate. Aspects of children's perception are also dealt with in other studies focusing on the relationships between the level of cognitive organization and the child's perception of the intention of others. These studies are also examining imitated learning, self-reward and how models of inconsistency and hypocrisy influence the development of moral development and moral behavior in children.

A number of Growth and Development supported studies concerning the cognitive development of children are also being conducted in the tradition of Piaget. In one group of studies an attempt is being made to discover which logical operations are really available to children at different ages, the order in which these operations emerge, and the ways in which development is affected by training. Significant, practical implications should emerge from these studies.

While the majority of the Growth and Development Branch activities in the past have focused on infancy and early childhood, a new effort is currently under way to stimulate research in human learning and cognition during the adolescent period.

Human Communication

This section of the Growth and Development Branch focuses on the fundamental research problems associated with the communicative processes and the role these processes play in the growth and development of humans, viewed both as individuals and as members of a society. This area examines the biological, psychological and social processes which make it possible for infants and

children to receive both symbolic and non-symbolic stimulation, to store and/or associate this information with past experience, and to appropriately alter and transmit selected portions of this information to others. In other words, the primary concern is with studies of the nature of the acquisition and the development of speech and language including basic research related to the reading process.

During this reporting period, we have continued our interest in learning how children develop the language capabilities that they must later use as adults in speaking and in reading. Because some of this research was dependent upon the availability of specialized tape recordings, a research contract to provide these recordings has been continued. As a result of these contract-supported dichotic listening studies, a major breakthrough has occurred. There is evidence to indicate that infants are able to discriminate the acoustic cues underlying the adult phonemic distinctions between certain consonant sounds. Moreover, a Growth and Development supported grantee has now shown that the language hemisphere (left in most instances) of the cerebral cortex may be specialized to deal with grammatical coding, a conversion of information that distinguishes language from other perceptual and cognitive processes. Grammatical coding is unique in terms of its function, which is to restructure information so as to make it appropriate for long-term storage and (non-linguistic) cognitive processing at the one end of the system and for transmission via the vocal tract and the ear at the other. Furthermore, the very latest finding seems to indicate that anatomical measurements of the language-mediating areas of the superior surface of the temporal lobe (planum temporale) are significantly larger in the left cortical area of the neonate just as in the adult. Therefore, it is suggested that this anatomical asymmetry is present prior to any environmental effects such as "language learning" and unimanual hand preference, and may be an important factor in determining the typical pattern of left hemisphere speech lateralization found in the majority of adults. Furthermore, this anatomical asymmetry which is present in the neonate suggests that the human infant is born with a pre-programmed biological capacity to process speech sounds.

As of December, 1972, 25 research fellowships, career development awards, contracts, research and training grants supported work in the area of human communication research.

Personality and Social Development

The area of personality and social development is concerned with the ways children develop from immature, dependent organisms to fully participating members of society. The active portfolio of the Growth and Development Branch contained 20 research grants, one career award, two training grants, three fellowships, and three contracts in FY'73. During the past year investigators have made various new discoveries. One investigator found that vestibular stimulation, particularly in the context of soothing the infant, stimulates visual alertness and visual scanning. This finding has significant implications for early perceptual learning which are being actively pursued. Another investigator has discovered that there are six stages of moral thought which follow an invariant sequence in various individuals and cultures.

Confirmation of this finding from longitudinal data analysis is being carried out. Another investigator, supported in part by NICHD funds, has discovered that infants raised in relatively deprived environments in an isolated Guatemalan Indian village recover from retardation and attain age-norms of children in advanced countries if they are treated as normal infants by their cultural standards. This important finding indicates that we must have a more refined understanding of the effects of early deprivation in order to design effective intervention or remedial programs.

A total of 30 grants are supported by the Growth and Development Branch in this area.

Expanded Emphasis on Adolescence

In June, 1972, the Branch made a preliminary report to the NICHD Council on needed research in the area of adolescence. This report, based on extensive consultation with experts and intensive staff discussion, recommended research initiatives in both biomedical and behavioral areas:

- 1) The biological processes involved in puberty with emphasis on the hypothalamic-pituitary system, the extra-hypothalamic central nervous system, and the biochemical actions of hormones.
- 2) Adolescent nutrient needs to establish better dietary recommendations, especially within the context of rapid adolescent growth and idiosyncratic behavior patterns.
- 3) Intellectual development emphasizing the qualitative change which takes place during adolescence (formal operations) and the important interaction of cognitive processes with motives and attitudes.
- 4) Adolescent socialization looking at the way social patterns help or hinder the adolescent to engage in or disengage from appropriate role behavior during rapid transitions.
- 5) The relationship between changing hormonal levels and psychosocial development and behavior.

Council accepted the report with enthusiasm and recommended that an inter-agency task force on research in adolescence be established. Such a task force was subsequently established and now meets monthly with representation from all government agencies having a concern in this area. The task force, under the leadership of Dr. Edith Grotberg, is modeled on a similar one on early child development and is funded through the Office of Child Development with contributions from relevant agencies. The immediate goal of the task force is to summarize all adolescent research now in progress and to identify gap areas for program development.

At the same time, the Growth and Development Branch undertook the planning and execution of three conferences in the adolescent area to ascertain the state of the art and to guide future research. In October, 1972, a

conference was held on "Mechanisms that Control the Onset of Puberty." This conference is being published in book format by the John Wiley Company. The second conference, on adolescent nutrition, was held in June, 1973. The planning for the third conference concerning adolescent behavior, to be held in FY'74, is now completed.

The Director of NICHD has appointed a subcommittee of the NICHD Council to advise in the area of adolescent research. At the March '73 Council meeting this subcommittee convoked a panel of experts to help establish research priorities.

Staff are currently consulting with scientists and encouraging the submission of research grant applications bearing on adolescent development.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N22-02241
Contract Title: Electrophysiological Studies of Brain Function in
Malnutrition
Contractor: David B. Coursin, M.D.
Research Institute
St. Joseph Hospital
Lancaster, Pennsylvania
Money Allocated: None in FY 1973

The objective of this contract is to develop an appropriate research center facility and satellite operation for the acquisition of electrophysiological data from subjects in remote areas who had experienced malnutrition and where modern EEG data acquisition techniques for computer analysis are not readily available. Efforts were directed at two major objectives: a) determination of feasibility of utilization of the Barlow Curve Reader to transfer ink-trace EEG readings obtained in the field to magnetic tape for computer processing; and b) to develop a portable EEG acquisition instrument with stimulus program capabilities for the collection of electrophysiological data in the field.

Progress to date has resulted in a) the determination that the Barlow Curve Reader is not suitable for use in the meeting of contract objectives; and b) the development of a portable stimulus program unit with tape cassette recordings of two channels of EEG. The "front-end", i.e. the miniturized amplifier to record EEG signals has yet to be developed and integrated into a single complete portable EEG acquisition instrument. The innovative instrumentation developed to date on this contract has led to a unique patent and when completed as a total system will provide the "break-through" for field studies on the effects of malnutrition upon the developing brain that has not heretofore been possible.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 12419
Contract Title: Effects of Calorie and Protein Restriction in
Pregnancy and Newborn Rhesus Monkeys
Contractor: The Johns Hopkins University, Baltimore, Maryland
Donald B. Cheek, M.D.
Money Allocated: None in FY 1973

The research conducted under this contract represents the NICHD contribution to a collaborative NICHD-NINDS study to measure the possible effects of protein and calorie restriction in rhesus monkeys during pregnancy on selected histological, biochemical and endocrine characteristics of their infants. Four dietary regimes were imposed: normal protein and calorie; low protein-normal calorie; normal protein-low calorie; and low protein-low calorie. Care of the females and delivery of the infants by cesarian section was carried out by NINDS staff. The NICHD contractor has conducted a series of laboratory determinations on vital tissues of the infants to measure the degree of anthropometric, histologic, biochemical and endocrine damage, if any, imposed by the experimental maternal diets. Analyses have included estimates of cellularity and cell function of the brain, muscle, liver, and various endocrine glands.

The contractor has submitted his final report and the contract objectives have been met. This study has provided a better quantitative measure of protein and caloric requirements of the rhesus monkey during pregnancy. A large number of analyses were conducted to characterize the adverse effects of maternal nutritional deprivation on biochemical, histological and cytochemical development of the fetal brain, liver, lung, kidney, blood, muscle and other tissues. A number of key developmental parameters have been identified. Utilizing these data, NINDS has undertaken a follow-up study to develop critical criteria for establishing effects of maternal undernutrition on neuronal development.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 12417
Contract Title: Ecology of Malnutrition in Vulnerable Groups
Contractor: Massachusetts Institute of Technology, Cambridge,
Massachusetts
Nevin S. Scrimshaw, M.D., Ph.D.
Money Allocated: \$49,534 (FY 1973)

The goal of this contract was to determine what factors in the life of low income families in a U.S. urban area lead to the development of malnutrition in preschool children. Primary attention was given to developing screening techniques for the children and their families and to developing sociological test methods. A pilot sample of children were enrolled and followed for 6-12 months. These data are being prepared for publication. Grant applications are being reviewed by NIH to continue those portions of the work which appear most promising.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: NIH - NICHD 73-2069
Contract Title: Immunologic Responses of Normal and Malnourished Infants to Standard Antigens
Contractor: University of California, Los Angeles, California
Charlotte G. Neumann, M.D., M.P.H., and Richard Stiehm, M.D.
Money Allocated: \$94,700 (FY 1973)

The main objective of the project is to study humoral and cellular immune responses to standard antigens in normal and malnourished infants, with special emphasis on identifying changes due to nutritional deficiencies. The UCLA laboratory has developed methods for immunologic and nutritional assessment of malnourished populations in Ghana, Africa, which will be pre-tested on a small sample of North American children. Well nourished controls for the malnourished children will be selected on site in Ghana.

Four groups of subjects are studied:

1. Hospitalized Ghanaian children with severe malnutrition
2. Ambulatory Ghanaian children with moderate malnutrition
3. Age-matched Ghanaian children without malnutrition
4. Age-matched U.S.A. children without malnutrition

The testing to be employed consists of:

1. A battery of immunologic testing including studies of deficiencies in antibody immunity, cellular immunity, granulocyte function, and opsonic function.
2. Assessment of nutritional status to include clinical nutritional assessment, anthropometric measurements, and biochemical assessment of nutrients.
3. Other studies will include routine hematologic studies, thick blood smears for malaria, stool exam for ova and parasites, and sickle cell preparations.

During the first contract year, data was obtained on 117 children but analyses are not yet complete. Information on adequacy of cell-mediated immunity and immunocompetence in malnourished versus normal children suggests the possibility of impairment, but must await collection of further data from adequate age-matched controls. The most intriguing finding has been the suggestion of decreased granulocyte bacteriocidal capability in malnourished infants, and further studies should reveal defects in phagocytosis or killing, whether intrinsic granulocyte functions or deficiencies in serum opsonins are involved. This contract will continue for an additional 18 months so as to get definitive data on the relationship between the nutritive state and the immune response in man.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 12088
Contract Title: Non-Human Primate Colony
Contractor: University of California, Davis, California
Andrew G. Hendrickx, Ph.D.
Money Allocated: \$164,500 (FY 1973)

Under this contract, a colony of rhesus monkeys (*Macaca mulatta*) of known medical, reproductive and geneological history is being developed at the National Center for Primate Biology, 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.

Animals of many stages of development 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 supported by NICHD. Application for animals is competitive and allocation is 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 in visiting scientists' facilities on-site. Funds from the sale of animals are used to offset the costs of future contract years. As the colony is developed, biological and behavioral data profiles are being developed and made a part of each animal's computerized record. Such information is available to recipient investigators upon request.

During the third year of this contract, further stabilization of the outdoor families is being emphasized. Expansion of the breeding program will occur in accordance with requests received from investigators. Currently over 50% of the breeding colony is devoted to the production of pregnant females for studies requiring closely timed pregnancies.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 81512
Contract Title: A Study of Malnutrition and Mental Development
in Bogota, Colombia
Contractor: Harvard University, Boston, Massachusetts
Frederick J. Stare, M.D.
Money Allocated: \$165,900 (U.S.-Japan Cooperative Medical Science
Program Funds) FY 1973

This project was designed to explore the impact of a food supplementation program on the development of pairs of siblings living in the slums of Bogota. One sibling was to be malnourished, the other not malnourished, thereby minimizing genetic and family differences between comparison groups. A number of new psychological test systems have been developed and demonstrated reliable. However, it has proven impossible to enroll sufficient pairs of siblings in the four cells of the study design. Therefore, the contractor is preparing the tests, findings, and conclusions from the pilot studies for publication.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 22735
Contract Title: Prediction of Mature Stature in Growing Children
Contractor: Fels Research Institute, Yellow Springs, Ohio
Alexander F. Roche, M.D.
Money Allocated: None in FY 1973

This two-year contract, initiated during FY 1972, has the objective of achieving improved reliability in predicting mature stature of growing children. The prediction tables currently in use were developed from data collected 30-40 years ago and were validated against a very small sample. The contractor, utilizing radiographs and anthropometric data from the three largest longitudinal growth studies conducted in the United States, is developing and validating better parameterization of individual growth in stature from birth to eighteen years.

Collection, standardization, computerizing and analysis of the data have progressed in accordance with the original timetable. Several factors influencing the replicability of assessments of skeletal maturity with the current tables have been identified. A comparison of prediction errors based on sets of cross-sectional versus serial data is currently underway. Upon completion of the new parameterization of growth in stature, additional analyses leading to further reduction of prediction errors will be undertaken.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N22 22737
Contract Title: Analysis of Data from an Ecological Study of Infection, Malnutrition, and Growth of Children in a Guatemalan Indian Village
Contractor: Pan American Health Organization, Institute for Nutrition in Central America and Panama
Leonardo J. Mata, D.Sc.
Money Allocated: \$64,450 (U.S.-Japan Cooperative Medical Science Program Funds) FY 1973

The primary objective of this contract will be the analysis and publication of the data from a longitudinal study of nutrition, infection, and growth among pregnant and lactating women, infants, and young children in a small native village in Guatemala. Throughout the study attempts were made to interfere only minimally with the natural ecosystem of the village and the family. Approximately 45-50 babies have been born per year in this village, providing a population in excess of 400 newborns over the eight year study period (1964-1971). Growth and morbidity data have been obtained on all children. More detailed studies on neonatal infections, their nature, and origin have been done on 350 children. Within this latter group, 45 (representing 50% of those born during 1965-1966) were identified for intensive study from the prenatal period through the first seven years of life. Regular data were obtained on these children concerning height, weight, days of illness, dietary intake and nutritional status, and infections and colonization of the intestine as indicated by weekly stool samples. Twelve of these had more intensive work-up of patterns of development of indigenous microflora.

The contractor has completed most of the viral analyses, has established the data bank in Guatemala and in Seattle, and has completed preliminary analyses of the longitudinal data. The monograph has been outlined in detail and several chapters have been written in draft form.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 12420
Contract Title: Research Materials and Assistance for Studies of Language
Development in Children
Contractor: Haskins Laboratories, New Haven, Connecticut
Franklin S. Cooper, Ph.D.
Money Allocated: \$74,100 (December 30, 1972 to June 30, 1974)

The original purpose of this contract was to provide specialized facilities and expert assistance to research scientists at other institutions (user groups) who are engaged in language studies relevant to children. In December of 1972 the contract was renewed for another 18 month period to continue to provide these services to user groups. In general, three kinds of assistance are provided to research groups, most of whom are working on projects of direct interest to the NICHD.

1. Research materials. Stimulus tapes for a wide variety of experiments constitute the primary direct assistance provided under this contract. Several methods of generating recordings suitable for research on spoken language are available including natural speech, synthetic speech, and non-speech sounds and noises prepared for presentation on either single-track or dual-track.
2. Specialized assistance. Professional and technical staff of Haskins Laboratories make available their knowledge and skill in the preparation of synthetic and natural speech recordings and in the use of their unique computer facility for generating precisely controlled binaural tapes.
3. Research training. A by-product of the visits by user groups has been a training experience in the status and methodology of basic speech research and an opportunity to gain some familiarity with the laboratory's entire program of research in speech which is supported in part by the NICHD.

During the initial 13 months of this contract, requests for assistance were received from 37 users affiliated with 23 leading universities. While it is difficult to characterize the projects for which users have requested assistance, they have in general clustered around a few types of problems: auditory discrimination in infants, language discrimination as a function of the child's age, and lateralization and dichotic effects as indicators of language processes. Only a few studies have been concerned with clinical problems, one on deafness and one on temporal lobe involvement.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 50640
Contract Title: Study of Relationship Between Nutrition and
Behavioral Development
Contractor: Pan American Health Organization, Institute of
Nutrition for Central America and Panama
Robert Klein, Ph.D.
Money Allocated: \$543,685 (FY 1973)

This contract has been designed to contribute additional knowledge of the relationship between nutritional status and physical, psychological, and social growth. Two pairs of small villages (aldeas) of approximately 650 inhabitants each have been matched on a variety of sociocultural variables. In one village of each pair a dietary supplementation program is provided with special effort made to include in this supplementation program every preschool child and pregnant woman in the village. Incaparina, a high protein food supplement, is made available to every person in the village who wants it. In the control villages medical service is offered, but no food supplementation is provided. Thus a sample of well-nourished preschool age children can be compared on relevant dimensions with a sample of medically treated preschool children raised under the dietary conditions characteristic of the area. Children will be followed from birth until about six years of age, at which time effects of differences in nutrition should be clear.

Three years of enrollment of pregnant women and their offspring have been completed. This constitutes the basic population for long-term study of the effects of the supplementation program. It has been shown that birth weight is related to maternal height and weight at the onset of pregnancy; both are indicators of prior nutritional status. Days of illness during pregnancy also are directly related to decreased birth weight, apparently due to decreased food intake rather than infection per se. Most importantly maternal calorie intake is directly related to birth weight; maternal protein intake has little or no effect on birth weight but does appear to be related to infant morbidity, mortality, and growth. Pregnant women will continue to be supplemented in these villages to increase the number of infants under one year of age to confirm these relationships. Neurological and behavioral testing has not been completed on a sufficient number of children to give clear answers to other questions posed by this project.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Perinatal Biology and Infant Mortality Branch

The objective of the Perinatal Biology and Infant Mortality Branch is the promotion of a coordinated program of research and training which will enhance understanding and development of knowledge as related to pregnancy and maternal health, embryonic development, fetal growth, and infant well being through the first year of life. Efforts are also directed toward reducing this country's infant mortality rate, ameliorating infant morbidity, and narrowing the gap between the identification of new knowledge and its incorporation into the delivery of health care.

Program goals recognize the interrelationships of specific health and developmental problems encompassed in the prenatal, perinatal, and infant period of life, and the effects that these events may have upon subsequent development and well being of the child. Particularly relevant are those morbidity- and mortality-related maternal health problems which effect fetal and infant health status, problems with which the newly born infant must cope in his adaptation to extrauterine life and subsequent survival and well being, and events occurring during the period of hospitalization following birth which can influence the subsequent behavior and development of the baby.

The Branch supports research and training concerned with various aspects of the physiology of pregnancy, disorders of pregnancy, placental function, immunologic phenomena, developmental biology, biochemical processes of embryonic and fetal growth, biophysical and biochemical antenatal and intrapartum diagnostic procedures, the process of labor and delivery, and genetic, pharmacologic, toxic, and infectious processes and influences which can effect the mother and fetus. Primary attention in the area of infant survival and well being emphasizes respiratory function, metabolic processes, thermal regulation, immunology and blood dyscrasias, infectious diseases, congenital malformations, and sudden infant death syndrome.

Two major priority areas have been identified by the PBIM staff as a means of highlighting emphasis areas and guiding future program development. The first of these is to eradicate those maternal, fetal, and infant health problems which result in the highest incidence of infant morbidity and mortality. Chief among these problems are low birth weight, the sudden infant death syndrome, process of birth and extrauterine adaptation, abnormal fetal development, and complications of pregnancy.

The second major priority area is the identification of psychosocial factors in pregnancy and delivery which affect the fetus and outcome of pregnancy and contribute to infant morbidity and mortality. The emphasis in this priority area is on maternal stress, fears and conflicts and their influence on physiological functioning, post partum maternal behavior, and maternal-infant relationships.

The Perinatal Biology and Infant Mortality Branch is organized into four broad program areas: Pregnancy and Maternal Health, Developmental Biology, Fetal Health and Development, and Infant Survival and Well Being. These program areas are under the supervision of two Health Scientist Administrators responsible for the administration of research grants, training grants, career awards, and fellowships in each program area.

The Branch is responsible for the supervision and administration of approximately 21 percent of the Institute's extramural holdings, totalling about 310 grants and contracts and amounting to over 16.5 million dollars. Total program support has increased approximately 35 percent over that of fiscal year 1972. This increase in support is reflective of a 27 percent increase in the number of research grants and a doubling in the number of contracts.

The total program holdings, according to program sections, are shown in Table I. The percent changes in funding from fiscal year 1972 are indicated for the five different support mechanisms.

Approximately twenty-one percent of the grant holdings are concerned with training support. Seventeen of the training programs provide basic science research training in the field of developmental biology, six of which are closely allied to clinical departments. One training program is in a school of veterinary medicine. The remaining twelve programs are associated with clinical medicine and are conducted within departments of obstetrics and pediatrics. A serious gap continues in the PBIM training program in relation to the preparation of research neonatologists; of the twelve training programs in clinical medicine, only three are preparing trainees in the area of neonatal biology.

Areas of scientific responsibility and interest for each of the four program areas are highlighted in the following paragraphs; selected scientific accomplishments are included.

Pregnancy and Maternal Health

The Pregnancy and Maternal Health Section continues to support studies pertaining to normal and abnormal physiologic factors which influence the gravida state during the antepartum, intrapartum, and puerperium periods. Information is sought about the roles of the cardiovascular system, respiratory system, endocrine glands, and genitourinary tract in pregnancy and their contribution and/or involvement in abnormal conditions during pregnancy. These include such conditions as toxemia, diabetes, hypertension, malnutrition, isoimmunization, viral and bacterial infections, blood dyscrasias, anemia and other hemorrhagic phenomena associated with pregnancy. The program is also concerned with placental function, factors involving the maintenance of pregnancy, the initiation of labor, the impact of common pollutants, drugs, and anesthetics on the mother and intrauterine conditions, and the psychosocial dynamics of pregnancy.

Significant scientific accomplishments have been achieved as a result of NICHD grant support; these are highlighted in the following paragraphs.

TABLE I

PBIM Program Holdings According to Mechanism of Support

Numbers and Amounts, FY 1973

(amounts in thousands)

Mechanism of Support	No.	Amount	% Change FY '72	Pregnancy and Maternal Health		Developmental Biology		Fetal Health and Develop- ment		Infant Survival and Well Being	
				No.	Amount	No.	Amount	No.	Amount	No.	Amount
Total	330	\$16,568	34.2	93	\$4,010	81	\$5,177	74	\$3,200	82	\$4,185
Research Grants	224	12,120	35.2	76	3,564	56	3,251	54	2,428	58	2,876
Training Grants	30	2,391	10.5	3	109	18	1,795	5	215	4	271
Fellow- ship	23	249	25.1	8	83	2	23	5	59	8	85
RCP Awards	25	600	-4.2	4	100	5	102	9	216	7	181
Contracts	8	1,207	211.1	2	152	-	-	1	283	5	771

Note: This excludes one research grant for \$45,269 paid from Special Genetics Funds of NIGMS.
Dollar figures may not add to totals due to rounding.

Dr. William M. McCormack and a group of co-workers at Boston City Hospital have been investigating genital mycoplasma infections as to their presence, location, and pathogenicity. One study centered on the relationship of genital mycoplasmas to the occurrence of postpartum fever. Their clinical data failed to demonstrate any correlation between vaginal colonization with these organisms and postpartum fever. Invasion of the blood stream by these mycoplasmas was associated with less than ten percent of the cases of unexplained postpartum fever.

Dr. McCormack and his associates have also reported on a study of puerperal bacteremia and neonatal sepsis due to the same organism, Hemophilus parainfluenzae. Their observations suggest that this organism is capable of causing serious infections and that its pathogenicity should not be underestimated. Further suggested by this report is the clinical importance of specifying the specific Hemophilus group isolated in an infection.

Dr. Edward H. Kass of Harvard University continues a detailed follow-up of patients enrolled in a study concerned with the role of bacteriuria in the pathogenesis of pyelonephritis of pregnancy which was carried out more than ten years ago. Approximately twelve percent of the women who were bacteriuric during a pregnancy showed evidence of pyelonephritis in the follow-up. This gave a first approximation of the rate of renal disease associated with bacteriuria in pregnancy.

The placenta is an organ of major significance in the maintenance of the normal pregnant state and has, therefore, been receiving increased emphasis by scientific investigators. Although not the completely protective barrier it was once thought to be, the placenta is the site of many activities which cushion the developing fetus from the external environment. Dr. K. G. Symms and Dr. M. R. Juchau have been investigating placental enzyme systems in their work at the University of Washington in Seattle. They have carried out meticulous studies into the mechanisms of aromatic nitro group reduction and the components and requirements of its catalytic system. The importance of characterizing this system is evident when the nature of some of its substrates are considered: insecticides such as parathion; the fungicide pentachloronitrobenzene; toxic industrial chemicals such as nitrobenzene; the anticonvulsant agent nitrazepam; and the antibiotics chloramphenicol and azomycin.

Dr. Juchau, with co-investigators Dr. Q. H. Lee and Dr. P. H. Blake, has also investigated the hydrolase activity of the placenta. In studies of cell-free preparations of human placental tissues, a statistically negative correlation between aryl hydrocarbon hydroxylase (AHH) activities and enzymatic conversion of cholesterol to pregnenolone was observed. In view of previous reports that cigarette smoking appears to produce high levels of human placental AHH activity, this suggested to the investigators that cigarette smoking could also result in repression of the desmolase system where cholesterol is oxidized. The oxidation of cholesterol to pregnenolone is the rate-limiting step in the biosynthesis of progesterone. This is one of the placenta's important hormonal products which is necessary for the maintenance of pregnancy.

Ultrastructural study of the placenta has been carried out by Dr. R. M. Wynn of the University of Illinois Medical Center. He has clarified certain aspects of function of the rapidly growing placental tissue. His efforts have centered on the specialized cytotrophoblast and the junctional areas of the placenta in which they are found. Significant information is being obtained about the evolution, endocrinology, and immunology of human placentation. Only through a more complete elucidation of normal placentation will progress be made toward understanding the placenta in disease.

For a better understanding of the activities of the posterior lobe of the human pituitary gland, Drs. K. W. Cheng and H. G. Friesen of McGill University in Canada isolated and characterized neurophysin. Their aim was to obtain the pure form of neurophysin for chemical characterization and to generate antiserum to human neurophysin for the development of a radioimmunoassay for clinical studies. These investigators found that all different components of neurophysin from various species crossreact with one another immunologically. They pointed out that different components of neurophysin from one species or even from different species are structurally similar, sharing common antigenic determinants. These findings have important implications and potential clinical usefulness in the treatment and management of hormonal dysfunctions.

Dr. H. Friesen, also of McGill University, studied the prolactin secretion by means of stimulation and inhibition tests. With specific radioimmunoassay for human prolactin, it is now possible to examine the factors regulating prolactin secretion in health and disease in man. Thyrotropin-releasing hormone (TRH), which was thought to be the specific hypothalamic factor regulating thyroid-stimulating hormone (TSH) secretions, causes a rapid discharge of prolactin in man. His study demonstrated the potential values of increases or decreases in prolactin secretion under certain clinical circumstances. For example, it may provide a useful medical alternative to hypophysectomy in patients with metastatic breast carcinoma since there is considerable evidence to suggest that prolactin is the important pituitary hormone which facilitates mammary tumor growth in other species.

Developmental Biology

The Developmental Biology program seeks strategic approaches to prenatal and neonatal health problems through support of fundamental research concerned with events along a time vector. Problems at organ, cell and molecular levels relating to low birth weight, prematurity, susceptibility to infant death and congenital malformations in the human infant are being pursued. The emphasis is on development at increasing levels of complexity eukaryotic multicellular animals. Major areas of research include morphogenesis, pattern formation, developmental immunology, the genome in development, the organization of the cell, developmental endocrinology, and environmental impact on development. Investigators address fundamental questions and provide a broad research base for insight and derivation of clinical applications. Attention is directed both to investigations with mammals and to other model systems which afford distinct advantages for the study of development from conception through embryogenesis and into the first year of life. Scientific accomplishments by investigators whose research is supported by the Developmental Biology program area follow.

During embryonic development, the pattern of RNA synthesis changes markedly. Until recently, it was generally accepted that in several embryonic systems synthesis of transfer and ribosomal RNA did not occur during cleavage and became activated only about the time of gastrulation. By careful purification and labeling, Dr. Anne O'Melia of Harvard University has detected synthesis of tRNA and 5s RNA during cleavage. In contrast, when gastrula or later stages were labeled, newly synthesized tRNA and rRNA were readily detectable. Investigations of RNA regulatory mechanisms controlling tRNA synthesis relate to maturation and function of the newborn lung.

The ability of the thymidine analog, bromodeoxyuridine, to strikingly depress the synthesis of molecules which characterize a given differentiated cell type has been documented in a number of developing systems. The inhibition occurs in the context of only minimal changes in the capacity of these cells to divide and to synthesize or accumulate other macromolecules. Studies with the analog by Dr. Howard Holtzer of the University of Pennsylvania have suggested that normal differentiation of erythrocyte precursors involves the institution of a new program of synthetic activity resistant to bromodeoxyuridine. Further elucidation of the formation of hemoglobin bears on an understanding and treatment of hemolytic disease in the newborn.

Most of the work on embryonic muscle developing in tissue culture has concentrated on morphological and biochemical aspects, rather than on physiological properties. Individual muscle fibers grown in vitro contract spontaneously, but infrequently and at random, in contrast to an in vivo situation. Recent findings by Dr. John Coleman of Brown University of dramatic increases in contraction and propagation following methylxanthine theophyllin treatment afford new opportunities for investigations of the developmental physiology of muscle. These results suggest that this approach will permit an experimental dissection of the developmental sequence leading to physiologically mature and functioning muscle fibers.

Information on the regulation of protein biosynthesis during embryogenesis is necessary to gain a firm understanding of such developmental phenomena as differentiation and aberrant development. The work of Dr. J. Douglas Caston of Case Western Reserve University focuses on regulation at the translational level of protein synthesis. Comparisons of the tRNA associated with polysomes and cytoplasmic 4s RNA are underway both before and after fertilization. Isotopic and kinetic studies measurements have defined an extensive series of precursors of 28s and 18s RNA species in the experimental system.

Chicks with brain defects similar to those noted in human fetuses are employed in studies conducted by Dr. Doris B. Wilson of Stanford University to provide information on the mechanism by which abnormalities occur in the brain and its blood supply during prenatal development. Overgrowth in the embryos was induced experimentally by dislocation of the notocord beneath the anterior rhombomere. Behavioral characteristics observed in surviving overgrowth chicks are under further investigation.

Fetal Health and Development

This program area is concerned with basic problems related to normal and abnormal development at tissue and organ levels. Research support continues to focus on areas pertaining to normal embryonic development and to specific environmental hazards or events of a pathophysiologic nature detrimental to the growth of the fetus. Attention is given to nutritional, metabolic and other physiological sequences, immunologic factors, and pharmacological interrelationships between the mother and the developing fetus. Basic genetic, biophysical, and biochemical investigations are considered imperative, not only as a basis for assessing the fetal state, but also for meaningful antenatal diagnosis. As the placenta is the vital link between the mother and the fetus, investigations on the functional role of the placenta, particularly on feto-placental hemodynamics, feto-maternal gas exchange, and placental transfer of nutrients and drugs continue to be areas of interest.

Selective research accomplishments for the past fiscal year are highlighted in the following paragraphs.

In recent years, increasing attention has been placed on environmental hazards and their impact on both the fetus and the newborn. The unique role of the human placenta in the maintenance of normal fetal development and in its ability to act and interact with exogenously administered drugs and chemicals reveals its remarkable multiple functions. Transplacental passage of drugs, particularly the distribution and the fate of drugs in fetuses are of significant concern because of the increasing use of drugs by pregnant women. The potential harm from drug transfer to the developing fetuses continues to be investigated.

Dr. H. D. Mosier of the University of California in Irvine, used pregnant rats to study the distribution of nicotine and other metabolites in maternal plasma, brain, lungs, heart, liver, adrenals, kidneys, gonads and the stomach-intestine-spleen-pancreas en bloc. He demonstrated that nicotine readily crossed the placenta and that the fetal tissues were perfused with higher concentrations of nicotine than maternal tissues within 30 minutes after the entry of nicotine into the maternal circulation. He noted that the decidua may pump nicotine from the maternal circulation into the fetal circulation, and further, that amniotic fluid may constitute a reservoir of nicotine available for recycling through the fetus.

The survival of the fetus depends on adequate placental transfer of oxygen and carbon dioxide. The basic materno-fetal exchange process of carbon dioxide and oxygen, under normal and stress conditions, is influenced by a variety of physiological factors. Drs. G. G. Power and L. D. Longo of the Loma Linda University have described a mathematical model of carbon dioxide transfer in the placenta and its interaction with oxygen. The model calculates the time course of changes along the exchange vessels, overall rates of carbon dioxide and oxygen transfer, end-capillary values, and the meaning of interactions of oxygen, carbon dioxide and pH. Theoretical calculations concerning the equilibration in venous blood, gas exchange rate, and other factors on carbon dioxide exchange are supported by experimental findings. The mathematical model, as the investigators point out, is useful as a tool in elucidating the dynamic aspects of the exchange process.

Fetal nutritional requirements and their relationship to neonatal mortality and morbidity continue to be areas of high research priority. Dr. L. S. Hurley of the University of California studied the effect of magnesium deficiency in pregnancy and its impact on the offspring. No living fetuses were found at term from rats under severe dietary deficiency during pregnancy (day of finding sperm in vaginal smear to day 21 of gestation). Congenital malformations in rat fetuses from pregnant females receiving the magnesium deficient diet included cleft lip, hydrocephalus, micrognathia, clubbed feet, adactyly, polydactyly, short or curly tail, diaphragmatic hernia, heart, lung and urogenital abnormalities. This investigator pointed out that further study of magnesium is needed to ascertain the correlations between magnesium metabolism and such pathological conditions as low serum magnesium levels, toxemia in human pregnancy, persistent convulsions in newborn infants, and habitual miscarriage.

To better understand nutrient absorption and the basic biochemistry at the cellular level, animal models have been used to examine the enzymatic activities at the earliest fetal developments. The small intestinal epithelium of the fetus at the third month is known to possess a variety of enzymes and is capable of such functions as secretion and absorption. Dr. R. Lev and his associates at the New York Medical College made histoenzymatic studies of the developing human fetal small intestine (7 and 22 week ages). They demonstrated that alkaline phosphates, adenosine triphosphatase and leucine aminopeptidase are distributed in surface membranes; acid phosphatase in lysosomes; thiamine pryrophosphatase in Golgi apparatus and three enzymes similar to succinic dehydrogenase in mitochondria. Their findings indicate that the biochemical functions necessary for intestinal activity are well established in the human fetus by the sixth month of gestation. This is earlier than had previously been thought.

The survival of the developing fetus depends on either absent or depressed reactivity of both fetal and maternal cellular immune systems. To study the cellular immune aspects of the human fetal-maternal relationship, Dr. M. C. Carr of the University of California in San Francisco used phytohemagglutinin (PHA) to test the response of lymphocytes in human cord blood and adult peripheral blood. He reported that greater responsiveness of lymphocytes is seen from younger individuals and he suggested that the cellular immune function is well developed at birth. His findings are consistent with observations made by other investigators that human lymphocytes at term are regularly undergoing low level stimulation. The source of the stimulus and its nature needs to be studied further.

The need for developing animal model systems for clarifying the role of specific viral infections occurring in fetal and neonatal life remains a major concern; such findings can shed light on the assessor factors and pathologic potential in human transplacental infections. Dr. G. Margolis of the Dartmouth Medical School studied the fetal infections of hamsters, rats, and mice induced with the Minute Virus of Mice (MVM). Dr. Margolis described various congenital infections in the mouse. He indicated that MVM falls in the group of transplacental infections and that its mechanism of attack may hold true for all parvoviruses. After direct inoculation of some rat and hamster fetuses, generalized severe disease resulted. As has been previously observed in infections with other intrauterine parvoviruses, replicating tissues were the major viral targets.

Infant Survival and Well Being

This program area is concerned with the postnatal period from birth to one year of age. Problems of low birth weight, the major single factor contributing to the high infant death rate in the United States, are emphasized. So also is the prevention of deaths that occur soon after birth in newborns in association with faulty adaptation to the extrauterine environment.

Basic and clinical studies of the etiology, pathophysiology, therapy and follow-up of conditions and syndromes such as asphyxia, respiratory distress, hypoglycemia, hyperbilirubinemia, anemia, erythroblastosis fetalis are supported. Equally important are the physiological, biochemical and behavioral aspects of extrauterine adaptation including respiratory, cardiovascular, thermodynamic, hemotologic and metabolic changes associated with the crucial adjustments of the newborn at birth. Influences of maternal and environmental conditions and treatments affecting neonatal adaptation are studied to determine optimal nutritional, environmental, and metabolic requirements for both normal and high risk infants.

Of major interest are investigations on the effects of events and therapies during the early days of life on adaptation and subsequent development, particularly as they involve the high risk newborn and relate to such conditions as artificial ventilation, acidosis, hypoglycemia, hyperbilirubinemia, phototherapy and minimal handling. Research on etiology, pathophysiology and management of congenital malformations during the newborn period is an important part of this program area. Findings resulting from grants supported through this section are highlighted in the following paragraphs.

Infection during pregnancy is well recognized as a significant cause of fetal loss and morbidity. Although the mother may show little evidence of illness, infectious agents cross the placenta and invade the fetal tissue. Dr. Charles A. Alford of the University of Alabama is directing a prospective longitudinal study in pregnant women and their offspring on the pathogenesis of chronic intrauterine infections. While serologic changes occurred in a surprising number of pregnancies, congenital involvement in infants born to these women appeared to be rare. These data suggest an inherent protective mechanism on the part of the developing conceptus. The nature of the protective mechanism and a better definition of the nature of silent intrapartum infection and silent congenital infection are being investigated further.

Further efforts to achieve a simple safe and effective method of preventing hyperbilirubinemia in prematurely born neonates continue. Determination of minimal exposures to light necessary to achieve a reduction in serum bilirubin concentration in premature newborns with hyperbilirubinemia are being carried out by Dr. Jerold F. Lucey of the University of Vermont.. In other clinical investigations, sequelae to light therapy administered to neonates with hyperbilirubinemia continue to be monitored. Dr. Gerald E. Odell of Johns Hopkins University reported the occurrence of a "bronze-baby" syndrome as a complication of phototherapy in jaundiced, premature infants with liver disease. Clues to detect susceptible infants prior to exposure are sought. In another study, one of the most complete follow-ups to date on infants previously treated by phototherapy, Dr. Lucey found no untoward effects.

Pulmonary surfactant facilitates a physiologic function essential to extra-uterine survival and alveolar stability. When synthesis of surfactant is impaired, respiratory distress may occur in the newborn. A central and possibly etiologic role for the deficiency of surfactant in the pathogenesis of respiratory distress syndrome underscores the potential clinical importance of recently devised tests of surfactant components in amniotic fluid. Prospective studies by Dr. Peter A. Auld of Cornell University probed the diagnostic value of the amniotic fluid analyses and explored possible sources of abnormalities related to surfactant deficiency. In further examination of the correlation with idiopathic respiratory distress syndrome, Dr. Louis Gluck of the University of California has found additional parameters for lecithin and sphingomyelin synthesis at different gestational ages. Specific stresses in utero have been found to alter lecithin/sphingomyelin ratios. These include chronic toxemia, hypertensive cardiovascular disease, hypertensive renal disease, sickle cell disease, circumvallate placenta, narcotic addiction, and diabetes.

Neonatal hypoglycemia is a problem of major concern in the care of low birth weight infants. Dr. Marvin Cornblath of the University of Maryland has concentrated his efforts on the elucidation of the physiological and biochemical maturation of glucose homeostasis in the newborn. His report of the discovery of a Coenzyme A transferase deficiency as a cause for ketoacidosis presents a unique opportunity for establishing a clearer definition of the metabolic pathways functioning in maturation. Such information, in achieving a greater understanding of glucose metabolism, should provide a firmer basis for disease therapy in the newborn.

At the University of Minnesota, Dr. John W. Reynolds has been investigating postnatal adrenal cortical function in premature infants with respiratory distress syndrome of varying degrees of severity. By measuring serum cortisol levels in these infants, Dr. Reynolds has not observed associated postnatal adrenal hyposecretion in infants with fatal hyaline membrane disease. Substantiating other work in this area, the data suggested no valid rationale for hydrocortisone treatment of idiopathic respiratory distress syndrome.

As the newborn infant adapts to his new and changing environment, his ability to maintain a normal blood pH is constantly challenged. Of value to clinicians in the recognition and treatment of disturbances of acid-base equilibrium are the investigations being done by Dr. R. W. Winters at Columbia University. His studies included quantitation of acid-base displacement in small premature infants and infants with chronic diarrhea. By a detailed evaluation of computer-processed data, these researchers have established confidence bands for metabolic acidosis, chronic respiratory acidosis, and metabolic alkalosis. These standards are useful to the clinician in determining the acid-base status of the sick newborn.

Sudden Infant Death Syndrome

During the past year, increased emphasis has been placed on solving the mystery of the sudden infant death syndrome. This syndrome is a world-wide perplexing public health problem. Each year in the United States approximately 10,000 apparently healthy infants predominantly between the ages of one and six

die suddenly, quietly, and unexpectedly in their cribs or carriages. At autopsy no significant cause of death can be found. In the majority of cases, the baby is apparently in good health. He does not have a cold or infection and takes his feeding without difficulty. The infant is then placed in his crib for a nap or for the night; several hours later, or in the morning, he is found dead.

The Branch has embarked on a more broadly based program of research to further increase our understanding of underlying mechanisms of the syndrome, to discover its probable cause(s), to identify infants at risk of becoming its victims, to explore preventive approaches, to inform the scientific and lay community about the sudden infant death syndrome, and to stimulate scientists to direct their investigative efforts toward finding the solution to this complex biomedical problem. The Institute's program also includes (1) plans to learn more about the current status of management of SIDS cases in the United States; (2) development of guidelines for use by coroners, medical examiners, and pathologists in handling these cases; (3) support for interdisciplinary conferences and workshops concerned with the sudden infant death syndrome; and (4) the preparation and distribution of scientific publications and public information documents.

In August 1971, the Branch organized a program planning workshop to define future research directions which could elucidate the causes of the syndrome, and to identify the most fruitful hypotheses to be explored relevant to underlying mechanisms of the syndrome. Representatives from fields of neurophysiology, cardio-respiratory physiology, immunology, microbiology, veterinary medicine, pathology, and epidemiology participated. New approaches to study of the sudden infant death syndrome were explored and consideration was given to how developmental, physiological and environmental phenomena may interact to precipitate its occurrence. This workshop provided a foundation for expanded program activities in the sudden infant death syndrome.

Seven emphasis areas have been identified for investigation:

1. Developmental neurophysiology as it may relate to the SIDS, with particular emphasis on abnormal sleep patterns as associated with autonomic disturbances which may interfere with vital functions.
2. Respiratory-cardio-vascular responses to stimuli, such as hypercapnia and hypoxia, and unusual vagal effects in relation to susceptibility to the SIDS.
3. Problems related to the developmental aspects of heat production, thermal regulation and relevant ambient conditions which may be associated with the SIDS.
4. Abnormal perinatal development of immune response mechanisms, with emphasis on the relationship of deficits in immunologic competence as a predisposing factor in the occurrence of the SIDS.

5. Psychological processes experienced by parents, other relatives, and the community in association with the occurrence of a sudden infant death.
6. Epidemiology of the SIDS.
7. Anatomic pathology of the SIDS.

During the past year, the Branch has sponsored six research planning workshops relevant to these seven priority areas. Each of these workshops brought together groups of scientists knowledgeable in the specific area of the subject matter to be discussed, but not necessarily familiar with the phenomenon of SIDS. The purpose of these workshops was to consider the problem at hand, to identify new approaches to the study of SIDS, and to highlight specific research questions in need of in-depth study. As a result of these workshops, a number of specific researchable questions were raised; areas in need of further study were identified. A summary report for each workshop is being prepared for publication.

The results of these workshops are being used as part of the Institute's expansion of its SIDS research grant and contract program.

Dr. George Ray and associates of the University of Washington continue their studies concerned with the anatomic, epidemiologic, immunologic and infectious disease aspects related to the sudden infant death syndrome. They are also doing preliminary work on the ringtail monkey as an animal model of the syndrome.

Dr. Marie Valdes-Dapena of Temple University is completing her research project seeking answers as to whether lesions of the heart's conduction system play a significant role in the sudden infant death syndrome. Results to date are negative.

Dr. William Hall of Southwest Research Institute is beginning an investigation of possible causes of the sudden infant death syndrome through study of electrocardiograms, respiration, and body temperature in two groups of infants.

The development of certain autonomic reflexes during the first year of life in a group of premature and full term infants is being studied by Dr. Peter Katona of Case Western Reserve University. In this study it is hypothesized that infants who die of the sudden infant death syndrome have higher reflex sensitivity than normal, and that this can be identified soon after birth. Initial measures of reflex sensitivity have been developed. Approximately 200 newborns have been tested thus far.

Dr. Alfred Steinschneider of State University of New York at Syracuse is testing the hypothesis that apnea during sleep and its associated cardiac rate change, is part of the causal sequence resulting in the sudden infant death syndrome. Work on this project is just beginning.

The Institute has also let two contracts to the University of Southern California and the University of California at Los Angeles for study of developmental phenomena and the occurrence of SIDS. In these projects, investigation in developmental sleep neurophysiology as it may relate to the etiology of sudden infant death syndrome is underway. Various physiological measurements are taken on the infant during labor, delivery, in the early days of life, and again at monthly intervals until approximately six months of age. Specific measurements include heart rate, respiratory rate, expired levels of oxygen and carbon dioxide, blood pressure, and sleep state. The object of these studies is to describe the developmental sequence of various cardiopulmonary reflexes under the control of the autonomic nervous system which are, in turn, profoundly affected by sleep state.

The Branch is also supporting a survey of the current management practices of sudden infant death cases in a representative sample of standard metropolitan statistical areas in the United States. The study is designed to systematically obtain information in such areas as: laws regarding management of SIDS for the state, county, or city in which the standard metropolitan statistical area is found; percentage of babies receiving autopsies, who performs them, and the cost; procedures followed in notifying the family of autopsy findings; availability of counselling services to families.

An RFP for the development of animal models for the sudden infant death syndrome has been prepared and announced. Scientific review and evaluation of the proposals received are in progress. If the phenomena can be identified or simulated in an animal species, etiology and understanding of underlying mechanisms of the syndrome will be enhanced. In turn, approaches to prevention can be identified.

Three booklets relevant to the sudden infant death syndrome have been published. These include a selected annotated bibliography for 1960-1971 and two research planning workshop reports. There are seven additional publications in preparation. These include five workshop reports, one booklet for the rescue worker and one booklet for parents and the lay public. Work is also underway on a slide tape presentation for use by professional and community groups.

The Branch has continued to work with the National Center for Health Statistics for the inclusion of a specific category for sudden infant death syndrome in the Ninth International Classification of Diseases. The Branch has also continued with its liaison relationships with the Maternal and Child Health Program of the Health Service and Mental Health Administration on this problem.

Conferences and Publications

In addition to previously mentioned SIDS conferences and workshops, the program sponsored an interdisciplinary conference on Problems and Priorities in Perinatal Pharmacology in April 1973. Emphasis was placed upon problems such as differentiation, drug effects in tissue and organ culture, placental factors and environmental factors relative to nutrition and narcotics. Publication of the proceedings is planned.

Two conferences were also supported through the research grant mechanism: The Role of RNA in Reproduction and Development and a Symposium in Developmental Biology. Both were held during the annual meeting of the American Association for the Advancement of Science (December 1972). Publication of the proceedings is planned.

The Program provided travel funds to young U.S. scientists participating in the NATO Advanced Study Institute conference, Utilization of Mosaic Systems in Developmental Biology in Venice, Italy (September 1972). The meeting brought together investigators who use similar methods to ask developmental questions on quite different organisms. A summary has appeared in Science. The Branch also provided partial support for the Perinatal Research Society's annual interdisciplinary conference.

Plans are being completed for an interdisciplinary conference in the Spring of 1974 on the phototherapy of hyperbilirubinemia. State of the art, possible detrimental and long-term developmental effects of phototherapy and research needs will be emphasized.

A catalog of teratogenic agents, prepared by a pediatric consultant to NICHD, is in press. More than 600 agents which can produce congenital anomalies in man and experimental animals are described. By compilation in a single volume, data on dose, administration, defects and key references are readily available to clinicians, researchers and others seeking to prevent and alleviate the action of teratogenic compounds on human development.

The Program is also supporting the preparation of the proceedings of an "International Workshop of Nutritional Impact on the Human Fetal Development", held in November 1972, High Wycombe, Bucks, England. Publication of the workshop is anticipated in the Fall 1973.

"Respiratory Gas Exchange and Blood Flow in the Placenta" is the title of another book being prepared through Branch staff. This publication is based on reports from an International Symposium, August 1971, Hanover, Germany. Preparation of this book is under the direction of an NICHD consultant who is a pediatrician and physiologist with extensive experience in human placental studies. The book will serve obstetricians, pediatricians, and reproductive physiologists directly concerned with maternal-fetal interrelationships and problems concerned with the prevention and treatment of hazards associated with fetal development.

PBIM staff is also involved in the preparation of a book on "Psychological Aspects of a First Pregnancy and Early Postnatal Adaptation." This book is based on a project report of an NICHD-NIMH supported grant and contract which were under the auspices of the Group Health Association, Inc. of Washington, D.C. This book will provide useful information to pediatricians, obstetricians, psychologists and health workers who are concerned with the behavior problems and mental health of expecting parents and their relatives.

Foreign Country Activities

PL-480 Program: The PBIM Program administers one PL-480 research project in India (Dr. S. K. Arya), two in Yugoslavia (Dr. M. Skreb and S. Milkovic). Four proposals from Poland and one from Pakistan are under review. Continued efforts will be made to explore additional PL-480 collaborative research projects in Poland, Egypt and other countries subject to availability of funds.

INSERM: The PBIM Program has been participating in the NIH collaborative research activities and exchange program with the Institute National de la Sante et de la Recherche Medicale (INSERM) in the area of perinatology under the coordination of the Fogarty International Center. The United States coordinator for this area is Dr. Norman Kretchmer, Professor of Pediatrics and Human Development at Stanford University. During the past year, two scientists, Dr. Elizabeth Schwedersky and Dr. Alex Minkowski from Professor Minkowski's laboratory in Paris, spent three months in Dr. Kretchmer's laboratory. Dr. Nicholas Hoogenrood, an investigator in Dr. Kretchmer's laboratory, and Dr. Kretchmer worked in Dr. Minkowski's laboratory. An exchange of additional scientists is anticipated during the next year.

Personnel

The PBIM Program is assigned four professional positions; one clerk-stenographer position; two clerk typist positions; and a grants assistant position. Three of the professional positions are staffed with persons holding Ph.D. degrees. We are actively recruiting for a physician. The program is also assisted on a part-time basis by a professional staff person assigned to the Planning and Evaluation Program who serves as Executive Secretary to the PBIM Research and Training Committee and assists in handling other program activities.

In FY 73, three NIH grants Associates worked in the Branch. A thirteen week work experience in health science administration was provided for a fourth year student from Louisiana State University School of Medicine. One of her projects was the preparation of a report on High Risk Pregnancies: Their Contribution to Infant Mortality.

The Branch has actively participated in the NIH Upward Morbidity Program. One stay-in-school student has worked in the program for the past year. One secretary is currently enrolled in the NIH Federal City College Program. Two others wish to enroll in the program but must each complete a year of Federal employment first.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Perinatal Biology and Infant Mortality Branch
(Epidemiology Branch)
Contract and Collaborative Research

Contract Title : Analysis of State Integration And Related Cardiopulmonary Functions In Infants At High and Low Risk For The Sudden Infant Death Syndrome.

Contractor : University of California, Los Angeles

Money Allocated : \$101,285.00

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of Sudden Infant Death Syndrome (SIDS).

Summary: In four groups of infants at high and low risk for SIDS, Drs. Hodgman and Sterman propose to collect large amounts of physiological data which will be used to evaluate the early maturation of sleep "states" and cardiopulmonary function during the various "states". The four study groups will be selected as follows:

1. Subsequent siblings of SIDS cases
2. "Near miss for SIDS" cases
3. In utero autonomic instability cases
4. Healthy, "normal" newborns

All study subjects except the "near miss" cases will be monitored during labor and approximately once each month during the first 6 months of life. The subsequent-sibs and low-risk subjects will also be monitored for one 12-hour period during the last trimester of pregnancy. Each monitoring session will last 12 hours and about 12 physiologic variables will be continuously recorded throughout the session.

This contract will be oriented exclusively toward data analysis.

Significance to Biomedical Research and the Program of the Institute: At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make real progress in elucidating the etiology of SIDS, the Institute must open up promising new avenues of research. It is hoped that this project will do so.

Proposed Course: Renewal for planned 3-year effort is anticipated.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Perinatal Biology and Infant Mortality Branch
(Epidemiology Branch)
Contract and Collaborative Research

Contract Title : Development Of Sleep And Cardiopulmonary Regulation Within Sleep: Clinical Studies Of A Functional Mechanism For Risk Of Sudden Infant Death.

Contractor : University of Southern California

Money Allocated : \$99,050.00

Objectives: To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of Sudden Infant Death Syndrome (SIDS).

Summary: In four groups of infants at high and low risk for SIDS, Drs. Hodgman and Sterman propose to collect large amounts of physiological data which will be used to evaluate the early maturation of sleep "states" and cardiopulmonary function during the various "states". The four study groups will be selected as follows:

1. Subsequent siblings of SIDS cases.
2. "Near miss for SIDS" cases.
3. In utero autonomic instability cases.
4. Healthy, "normal" newborns.

All study subjects except the "near miss" cases will be monitored during labor and approximately once each month during the first 6 months of life. The subsequent-sibs and low-risk subjects will also be monitored for one 12-hour period during the last trimester of pregnancy. Each monitoring session will last 12 hours and about 12 physiologic variables will be continuously recorded throughout the session.

This contract will be oriented exclusively toward data collection

Significance to Biomedical Research and the Program of the Institute: At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make real progress in elucidating the etiology of SIDS, the Institute must open up promising new avenues of research. It is hoped that this project will do so.

Proposed Course: Renewal for planned 3-year effort is anticipated.

NICHD Annual Report
July 1, 1972, through June 30, 1973
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Current Management of Sudden Infant Death Syndrome in the United States

Contractor : The Children's Orthopedic Hospital and Medical Center, Seattle, Washington

Money Allocated : \$112,204.00

Objectives: The purpose of this contract is to assess current management practices regarding the sudden infant death syndrome using a representative sample of standard metropolitan statistical areas (SMSA) in the United States.

Significance to Biomedical Research and Program of the Institute: Between 7,500 and 10,000 infants die each year in the United States from the sudden infant death syndrome (SIDS). The syndrome is considered to be the major cause of death in infancy between the first and twelfth months of age. There continues to be considerable misinformation and confusion among the medical profession and the general public about the etiology and pathologic definition of SIDS. Management of the victims of this syndrome, including the baby's family, varies widely from community to community. Consequently, a number of families have been psychologically traumatized because of inappropriate management of the event.

The project was designed to systematically obtain information, through objective-type and focussed open-ended questionnaires, in the following areas: (1) laws regarding management of SIDS for the state, county, or city in which the standard metropolitan statistical area is found; (2) who handles cases of SIDS; (3) percentage of babies receiving autopsies, who performs them and the cost; (4) possible barriers, such as lack of funds, to obtaining an autopsy; (5) criteria used for performing an autopsy and criteria used to identify those victims who come under the jurisdiction of the coroner or medical examiner; (6) procedures followed in notifying the family of autopsy findings; (7) availability of counselling services to families; (8) state of knowledge about SIDS among professional lay groups; (9) type of newspaper stories which appear about infants who die suddenly and unexpectedly, i.e., is the word "suffocation" used in lieu of "sudden infant death syndrome." The information obtained will be categorized, coded and transferred to IBM cards for computer analysis. Results of the analysis, when published, should lead to a more humane approach to the management of SIDS cases.

Proposed Course: The contract will end on May 31, 1973. The final product of this study will be a profile of the various aspects of SIDS management for communities of different population densities, and for different regional areas.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Intrapartum Fetal Monitoring

Contractor : Professional Staff Association of the Los Angeles County/
University of Southern California Medical Center,
Los Angeles, California

Money Allocated : \$377,300

Objectives: The objective of this project is to characterize the changes and patterns associated with specific groups of high-risk obstetrical patients through detailed study and analyses of fetal heart rates (FHR), fetal electrocardiograms (FECG), uterine contractions (UC), and fetal acid-base status and respiratory gases. This work should help to clarify the value and merits of biochemical and biophysical methods of fetal surveillance in specific groups of high-risk obstetrical conditions. The high-risk obstetrical conditions to be studied include toxemia, chronic hypertension, Rh sensitization, abruptio placentae, maternal diabetes, premature rupture of membranes, premature labor, prolonged pregnancy, para 6 or greater, elderly primigravida, breech presentation, previous cesarean section and maternal hyperpyrexia.

Significance to Biomedical Research and Program of the Institute: As the result of applying higher standards of medical care to obstetrics during the past thirty years, maternal mortality has decreased tenfold. Over the same period, perinatal mortality has remained relatively unchanged even though similar high standards of medical care have been employed. In 1964 in the United States, from a total of about 3.7 million births a year, there were 28 perinatal deaths per 1,000 live births; the perinatal death rate in 1967 was 26.2 per 1,000 live births. This number of deaths is matched by a similar amount of perinatal morbidity which includes respiratory distress syndrome, mental retardation, congenital heart disease, and other malformations.

Since a large proportion of perinatal mortality is associated with the last week of gestation, it is pertinent to examine current methods of checking fetal condition during the course of labor. During the last decade, biochemical and biophysical techniques, separately and in combination, have been proposed as methods of fetal surveillance during labor and delivery. Since a sizable proportion of perinatal morbidity and mortality may be directly related to the stresses of labor and delivery, it is important that the relative merits of these fetal monitoring techniques for fetal and neonatal survival and well being be determined, particularly in relation to specific high-risk obstetrical conditions.

The complementary use of biochemical and biophysical methods to study the fetus, during labor and delivery, and the infant through the period of extrauterine

adaptation provides valuable information not previously available. Information obtained will aid in delineating the respective value of biochemical and biophysical techniques for fetal surveillance in specific types of high-risk pregnancies.

Proposed Course: Subjects are selected from "high-risk" patients who have diagnoses including toxemia, chronic hypertension, Rh sensitization, abruptio placenta, maternal diabetes, premature rupture of the membranes, premature labor, prolonged pregnancy, para 6 or greater, elderly primagravida, breech presentation, previous cesarean section, and maternal hyperpyrexia. High-risk patients are viewed from the standpoint of the Goodwin high-risk score and the specific high-risk indication, e.g., toxemia, and compared with patients without complicating obstetrical conditions.

Basic biophysical (FHR, FECG, UC) and biochemical (pH, pO₂, pCO₂) data are collected from detailed study of the mother and fetus during labor and from the newborn through the first hour of life. Maternal and neonatal conditions are assessed at 24 and 48 hours following delivery.

As of April 30, 1973, 398 patients have been monitored. Electronic data processing facilities are used to handle data accruing from these investigations. Data are being subjected to descriptive and statistical analyses (correlations, Chi-squares and multiple regression). A detailed profile is being developed on each group of high-risk indications. In this way it will be possible to consider the group as a whole or to compare one indication with another.

A clinical index of fetal risk is also being developed. This index takes into consideration the clinical maternal risk factors, baseline fetal heart rate, abnormal fetal heart rate patterns, acid base status of the fetus, and uterine activity.

NICHD Annual Report
July 1, 1972 through June 30, 1973
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Cooperative Hospital Study on the Respiratory Distress Syndrome in Infancy

Contractor : Case Western Reserve University

Money Allocated : \$6,506

Objectives: To undertake a collaborative study directed at comparing two regimens of early treatment of respiratory distress syndrome which differ only in the concentration of oxygen (80% versus 40%) in the environment beginning at one hour of age until six hours of age.

Significance to Biomedical Research and Program of the Institute: Idiopathic respiratory distress syndrome represents a major health problem throughout the world. In the United States of America, approximately twenty-five percent of infants born prematurely show signs of this disease within the first 24 hours of life. Twenty to forty percent of these babies succumb from it, accounting for about 25,000 infant deaths annually in this country.

Empiricism, rather than controlled clinical trials, have led to a variety of practices in the management of various aspects of the neonatal respiratory distress syndrome without any marked changes in mortality. It is now recognized that pulmonary hypoperfusion is an important factor in the pathogenesis of the respiratory distress syndrome, and that therapy should be directed to correction of pulmonary arterial flow. Methods which have been shown to be of value in normalizing pulmonary vascular perfusion are: the reduction of hydrogen ion concentration, increased oxygenation, and mechanical inflation of the lung. Because oxygen and pH can be more easily controlled, it is important to evaluate their effects before embarking on a study of mechanical ventilation. It is clear, however, from the findings of many investigators that correction of acidemia is imperative. There is controversy, however, with respect to the use of environmental oxygen supplementation because of the dangers of retrolental fibroplasia. To prevent retinal damage, the arterial oxygen concentration must be kept below the level that stimulates vasoconstriction. The arterial blood oxygen is crucial, not the ambient concentration. Thus, there is a need for a valid statistical design to evaluate the effects of oxygen therapy with careful monitoring of arterial oxygen tension to prevent the development of retrolental fibroplasia.

Proposed Course: Details of the protocol have been designed to provide high-grade intensive care from the earliest possible time; no experimental techniques or medications have been introduced. Each subject admitted to the study must weigh 1,000 grams or more at birth; be breathing without assistance by the age

of ten minutes; by sixty minutes of age more than 50% of his respiratory activity must be accompanied by grunting, whinnying or a complaining cry or groan on expiration; be free of any major congenital anomalies which could contribute to respiratory distress, be no older than one hour and fifteen minutes of age. Once a patient is admitted to the study, the assignment to one of the treatment regimens is made without bias so that the resulting data will show the relative merit of the two forms of early management.

Arterial blood oxygen concentrations are monitored through umbilical catheterization during the five hours following admission to the study. At six hours, the infant's clinical condition and laboratory values are evaluated using the scoring system developed for this study. This scoring system is recorded at 1, 6, 12, 24, 48, and 72 hours. All infants after six hours are treated according to the same regimen.

All data are submitted to the Department of Biometry at Case Western Reserve University for statistical evaluation.

Admission of subjects to the study ended on March 31, 1972; a total of 151 subjects were admitted and retained in the study. During the past year, efforts have been directed to intensive analysis of all data collected and preparation of the final report. It is expected that the final report will be available in Fall 1973.

NICHD Annual Report
 July 1, 1972 through June 30, 1973
 Adult Development and Aging Branch

The congressional discussion that preceded the establishment of NICHD stated that the Institute would be responsible for biological, medical, and behavioral aspects of aging.

Biological aging processes cause the deterioration that gradually changes young adults into old ones. They produce much disability in themselves and contribute to the development of many diseases of the middle-aged and elderly. The study of these biological processes is a central feature of NICHD programs in aging.

In general, the Institute does not support research on individual diseases since these are covered by the disease-oriented Institutes of NIH. NICHD's concern with the diseases of the elderly is much broader, covering morbidity and mortality of all the individuals who make up the population. In other words it is concerned with diseases and their interrelationships as they impinge on human development.

In addition to studies of the processes of cognitive change with age, the Institute is responsible for studies that bear on the problems of psychological adjustment that humans face as they pass from one life stage to another. It is also responsible for studies in the social sciences relating to the problems that the aging of our society pose for the individuals who are aging and for the society as a whole. There is much in this area of responsibility that also falls within the mission of other agencies. NICHD is particularly concerned with the development of a central body of sociological theory and fact that will make possible a comprehensive view of the problems of the aging and the elderly and an appropriate approach to the solution of those problems.

GROWTH OF EXTRAMURAL RESEARCH ON AGING

The growth of grant and contract programs in terms of budgetary expenditures (in thousands of dollars) is tabulated below.

<u>Fiscal</u> <u>Year</u>	<u>Research</u> <u>Grants</u>	<u>Contracts</u>	<u>Training</u> <u>Grants</u>	<u>Total</u>
1964	\$2,744	\$0	\$302	\$3,046
1965	2,625	141	568	3,334
1966	3,219	59	1,451	4,729
1967	3,267	102	2,089	5,458
1968	3,627	213	2,178	6,018
1969	3,559	325	2,286	6,170
1970	3,226	485	2,305	6,016
1971	3,801	240	2,232	6,273
1972	5,756	907	2,098	8,761
1973(Estimated)	5,972	700	1,565	8,237
1974(Estimated)	5,771	600	1,290	7,661

RESEARCH AND TRAINING PROGRAMS

This section will discuss various topics of importance in research and training.

Society, Aging, and Health

The control of infectious and nutritional diseases in this century has been a major factor in the striking alterations that have taken place in the size and age structure of the American population. The United States now has a much larger fraction of older persons in its population than it did at the turn of the century, and much of their morbidity and mortality comes from diseases that had relatively small impact on their ancestors.

Continuing social and medical advances may be expected to continue to change the age structure of the population and the relative importance of different diseases. Some efforts to foresee what may be expected have been made, but the efforts have been small, and there has been little systematic planning on the medical, psychological, and societal consequences of improved prevention and treatment of the diseases that are now important.

Eliminating cancer would add about 2.3 years to the expectation of life at birth in the U.S. Elimination of cardiovascular-renal diseases would increase the current expectation by 10.9 years. These increases would, of course, tend to increase the fraction of older persons in the population. Equally and perhaps more important in changing the age-structure of the population may be the birthrate. Mathematical models will be necessary to quantitatively project the age-structure that will be produced by alterations in fertility, mortality, and migration. Without such models one can only say that probably a larger fraction of the population will be older, that a different pattern of diseases will emerge, and that intrinsic aging processes will become more important.

NICHD is planning a program that will provide a broad conceptual background for persons concerned with the implications of changing population structure for health and other social problems. Currently it is funding two contracts to review, analyze, and evaluate past and current research in specified areas and to make recommendations for future research in those areas. The areas concern (1) mathematical models for predicting changes in population size and structure as a result of alterations in fertility, migration, and mortality rates, (2) estimates of possible future values for fertility, migration, and mortality, and (3) predictions of the impact that changes in size and structure of the population will have on health, needed health research, public health measures, and health services. A conference on these demographic problems was supported at Duke University this year.

Mental Function

One of the major problems of many old persons both from their own standpoint and from that of society is impaired mental function which may progress to

the point of dementia and completely incapacitate those affected. Much remains to be learned in this area. Impairment in cognitive ability probably begins in many persons at least by the middle years of life. In late life a significant number of persons are severely demented.

Whether these dementias are in part the end result of the cognitive decline that begins relatively early in life or whether they for the most part are due to other processes is unknown. It has been thought for many years that a large fraction of the senile dementias are due to atherosclerotic vascular disease. However, many neuropathologists are now beginning to believe that much of what was attributed to vascular disease is due to primary neuronal disease.

NICHHD supports a moderate amount of research on the cognitive defect of age and is supporting studies of the effect of the inhalation of 100 percent oxygen under increased pressure on the mental function of severely demented elderly persons. There is evidence that this may prove of value, but many more studies need to be done before this therapy can be considered anything other than experimental.

Aging in Women

Year before last NICHHD supported a conference to lay the foundations for a program to study the causes, manifestations, and treatment of the results of the menopause. At the conference the hormonal changes associated with the menopause, their possible relations to atherosclerosis, osteoporosis, and other diseases, and the possible advantages and disadvantages of replacement therapy were discussed. This year a retrospective study of the effects of replacement therapy, and a study of the hormonal alterations in the post-menopausal period were continued.

Aging and Immunology

There is considerable evidence that immunological competence declines with age and that the decrements that occur are important in some of the major manifestations of old age. The level of activity of both the cellular and humoral immune systems peaks during adolescence and thereafter declines. These decreases in immunological competence are very important in the diminished resistance of the elderly to infections and very possibly contribute to the high incidence of cancer in the elderly. An increased understanding of the processes involved might well make possible procedures to strengthen immunological competence in old age and thus to improve the health of the elderly. NICHHD is expanding its support of research in this area.

Cellular Aging

Changes at a cellular level are probably responsible for many of the aging changes that occur at the level of the whole person and are so easily apparent to layman and to physician. Thus the Institute has emphasized research on the cellular processes of aging.

Since the limited generation potential of certain mammalian cells appeared to be an important lead, emphasis was placed on understanding this phenomenon. Programmatic efforts in this area began in 1968.

At one time fibroblasts were thought to be potentially immortal in tissue culture, but this has proved to be not true of normal human fibroblasts. It is now known that they undergo only about fifty doublings in vitro before dying. It is also known from transplantation studies that at least one cell type, mouse mammary epithelium, will not continue to divide indefinitely when transferred serially from young mouse to young mouse.

NICHD has held a number of meetings of investigators in this field to develop an appropriate program dealing with this phenomenon. It supports the production of cells suitable for studying it. In the summer of 1972 it supported a course on the use of tissue culture in studies of aging. This course was given at the University of Vermont. A similar course will be supported in the summer of 1973. NICHD now supports a moderately large program of research in this area by the research grants mechanism.

Animals for Aging Research

A major limiting factor in the expansion of experimental work on aging has been the difficulties involved in getting old animals. This factor has created serious problems for investigators with a long-term commitment to aging research and has prevented investigators without great interest in aging from introducing age as a variable into their studies.

Most experimental work on aging has been carried out on rats and mice for what appear to be good reasons. They are mammals and therefore results obtained with them would appear to stand a better chance of being relevant to human aging than results obtained with non-mammalian species. Their relatively short lifespans are important both in the conduct of experimental work and in the cost of raising them into old age. Their small size makes it relatively inexpensive to raise them. They have been widely used in other types of biological studies and therefore are well-characterized in many ways.

However, the difficulties in raising rodents into old age are much greater than might be anticipated. The major problem has been acute and chronic infectious diseases. These impair health and shorten life. Their presence in colonies in the past led to animals being considered old when they were in middle age, and to their being studied for age changes when their functional status was deranged by disease.

Techniques are now available to raise rodents free of parasites and either free of bacteria or with a defined bacterial flora. However, the methodology requires a large investment of time and facilities. It is therefore not practical for most investigators to breed their own animals and raise them to old age. Therefore, NICHD has exerted much effort to make it possible for investigators to obtain well-characterized, healthy, old rats and mice from commercial breeders.

NICHD now has contracts with a commercial breeder to make this possible. It supports the characterization of inbred Fischer-344 rats, the inbred C57BL6 mouse, the inbred BALB/c mouse, an F1 hybrid mouse based on the two inbred mouse lines, and the outbred ICR mouse. The animals are free of bacterial

and mycoplasmal disease. Animals of various ages are periodically selected randomly and autopsied. Both gross and histological examinations are made. Appropriate bacteriological, serological examinations for viral infections, and parasitological examinations are carried out. A number of these animals will be kept through their lifespans so that investigators using these strains will have models whose natural histories and life tables are well characterized.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 73-2724

Contract Title: Production Colony of Aging Rats in an Isolator Environment

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$39,423 (FY 1973)

- Objectives:
1. Establish a production, rearing and maintenance system for aging rats that prevents introduction of pathogens prematurely shortening the natural lifespan of aging laboratory rats.
 2. Determine the commercial feasibility and per animal cost effectiveness of isolator rearing of aging laboratory rats compared with barrier and conventionally reared rats.
 3. To develop for research in aging:
 - a) a limited source of aging rats free of bacterial and detectable viral diseases.
 - b) a system of environmental control that is standardized and transferable to the research laboratory without unduly compromising the biological integrity of the animals.
 - c) laboratory rats that are known to survive to natural senescence as a natural population independent of bacterial diseases or detectable viral diseases
 4. Compare survival, pathology and degenerative change in isolator reared rats with barrier and conventionally reared animals.

Significance for Aging Research: The lack of aging experimental animals of defined quality is one of the major limiting factors to the study of aging, particularly animals that survive to natural senescence. Until recently aging studies in animals, especially rodents, were limited to those animals hardy enough to survive the stress of disease and a fluctuating physical environment. During the past several years several methods have evolved in the development, husbandry, maintenance and care of laboratory animals that permit routine cesarean derivation and rearing of laboratory rats and other experimental animals behind a barrier (SPF) or in the rigidly controlled environment of plastic isolators. The isolator excludes the introduction of bacterial and detectable viral agents. This methodology combined with genetically defined animals on a stabilized diet and a close monitoring system goes far toward establishing and providing aging animals that survive to natural senescence independent of the complications of infectious disease, parasitism, or wide variability in physical environment.

Basically the contract for an isolator reared colony of aging rats is concerned with developing an aging rat colony in a rigidly controlled isolator environment free of bacterial agents, except those purposefully introduced as normal bacterial flora. Changes in the aging process can most readily be determined when environmental conditions are uniformly controlled and sufficient numbers of animals representative of the total population of animals survive to an aged condition that can be studied as characteristic of the normal processes of aging. For this reason the contract requires uniformity in environment, diet, humidity and temperature and genetic quality of the animals in the isolator.

Once it is clearly established that laboratory rats can be maintained for their full lifespan independent of detectable microbial disease, and survival rates largely offset added costs for isolator maintenance, a resource of isolator reared animals can be provided to investigators in aging. The need for developing expensive and sophisticated environmentally controlled buildings to support the colony is unnecessary since the total environment of the animal is transferred within the isolator unit.

Studies of aging independent of environmental variables and disease can then be conducted to increase understanding of aging processes in animals and how these processes may apply to man or lead to experimentation in man. The major significance of the isolator system for rearing laboratory animals lies in the enhancement of detectable change in aging processes.

Proposed Course: The isolator contract is to be continued for a minimum of two years. Continuation will be dependent on commercial feasibility and colony survival rates.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 73-2720

Contract Title: Development of a Production Colony of Three Genotypes and
a Non-Inbred Strain of Laboratory Mouse for Aging Research

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$71,388.00 continued into FY 1974.

- Objectives:
1. Provide characterized genetically defined strains of laboratory mice reared in a defined environment for research in aging.
 2. Develop a ready commercial source of aging mice of three basic genotypes and an outbred strain to meet the demands for aging laboratory mice.
 3. Minimize lag time for the development of studies in aging requiring aged genetically defined laboratory mice from a controlled environment.
 4. Provide the minimum number of strains of mice necessary for cross comparison and extrapolation of experimental results to a broader natural population.
 5. Develop a colony of laboratory mouse strains in which pathological processes, degenerative change, morbidity and mortality to age 24 months are largely known and predictable.

Significance for Aging Research: A lack of aged genetically and biologically defined animals reared in a controlled environment has long hampered the development of aging research, particularly in the field of immunology. With increasing frequency studies in aging research require animals of known genetic background, biological characterization and environmental status. To meet this need for strains of genetic specificity, diversity and generalizability a colony of aging mice of the inbred strains C57/BL6, BALB/c, the inbred F₁ hybrid of the two inbred strains and the outbred strain ICR was established in a barrier enclosure (SPF) at Charles River Breeding Laboratories. Profile data will be acquired on the colony and strains of animals by periodic sacrifice and necropsy.

The major significance of this contract is the development of a readily available resource of aging, genetically defined and characterized strains of laboratory mice reared in a controlled environment. The standing colony of aging mice of the three genotypes and non-inbred strain proposed under this research contract will provide investigators in aging with basic genetically controlled model systems previously unavailable to most investigators in aging. This should moderate one of the primary constraining influences on

the development of aging research in animals by making available: 1) basic genetic model systems of the aging laboratory mouse for studies in aging requiring specific, diverse or general genetic control, 2) for study, one or several comparative animal model systems within a species, 3) an animal of known biological characterization and environmental status. In addition, once the colony is established the lag time from proposal to execution of the study will be substantially reduced.

Proposed Course: Contract will continue for a minimum of one year with the contract becoming increasingly self-sustaining in subsequent years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 73-2725

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: \$86,890 (FY 1973)

- Objectives:
1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.
 2. Establish a standing commercial resource of senescent rats on which investigators can immediately draw for aged laboratory rats.
 3. Develop baseline physiological and pathological characterization of the Fischer 344 rat over their full lifespan.
 4. Establish survival curves for laboratory rats reared specific pathogen-free behind a defined barrier system.
 5. Increase the numbers and ages of animals to be made available for studies in aging.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals that are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific causes of death.

Basic to the development of studies in aging research in animals is a characterization of expected physiological and pathological changes that occur over the animals' full lifespan as well as life tables that accurately reflect survival at specific ages. A primary aim of this contract is to acquire this data and make it available to investigators in aging. With this information, a reasonable comparative assessment can be made as to whether the animals, strain or stock is suitable for studies in aging. Also, within reasonable limits, numbers of animals needed for statistical significance of studies can be readily established, thus minimizing the likelihood of supporting excessive numbers of animals or too few animals for statistical significance of the study.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they could use in the studies they propose in aging.

Proposed Course: Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining, and until age-specific causes of death and life tables for the colony and strain can be established.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 72-2792

Contract Title: Prevention of Bone Loss in the Menopause

Contractor: Creighton University, Omaha, Nebraska

Money Allocated: \$47,000 (FY 1972)

Objectives: The purpose of this project is to study prospectively the effect on calcium metabolism and on bone mass of supplementing the diet with increased calcium or of administering sex hormones to women at or after the natural menopause. The study group consists of seventy five nuns varying in age from 50 to 65 years. They will be assigned at random to three equal groups. One group is given calcium carbonate, the second group premarin with methyltestosterone, and the third group is a control. At the end of the second year of the project, comparisons of regression of bone mass as a function of age in the three groups will be accomplished to examine differences in rate of loss of bone. The study may provide information on the mechanisms by which these differences occur.

Significance for Aging Research: This study represents one of the first reasonably accurate assessments prospectively of the effect of combined estrogen-androgen replacement therapy on bone resorption and formation in postmenopausal women. Data correlating the effects of treatment with estrogen-androgen replacement or oral calcium on bone in the menopause is highly significant to assessing the effectiveness and need for replacement therapy in postmenopausal women. Thus, the studies supported under this contract have significance for understanding the changes that occur in bone mass with aging and will provide important information on calcium metabolic changes that result from therapeutic intervention in postmenopausal women.

Proposed Course: The contract to study prevention of bone loss in the menopause will continue for two years. Continuation is dependent on results from the first two years study.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 72-2756

Contract Title: Origin and Action of Estrogen in the Postmenopausal Woman

Contractor: The University of Texas Southwestern Medical School, Dallas,
Texas

Money Allocated: \$104,411 (FY 1973)

Objectives: The long-range goal of this project is to determine qualitatively and quantitatively estrogen production in postmenopausal women and the mode of interaction of estrogens and their target tissues. Earlier studies have shown, in oophorectomized postmenopausal women, that the principal estrogen produced is estrone and that for the most part, this arises from the extragonadal, extra-adrenal aromatization of androstenedione. In normal postmenopausal women most of the androstenedione is secreted by the adrenal glands but in women with ovarian stromal hyperplasia it can be secreted by the ovary as well. Also, changes in estrone have been shown to be associated with a variety of factors including obesity, age, diabetes, hypertension, liver function and some ovarian neoplasms. In view of the acquisition of this earlier data, the primary objectives of the contract are aimed at:

1. Establishing the relative contributions of ovaries and adrenals to plasma androstenedione and the extent of conversion of androstenedione to estrone and estrone sulfate prior to and following oophorectomy.
2. Determining the chemical nature of the hormone product following aromatization of androstenedione.
3. Delineating the effect of obesity, aging, hypertension, diabetes, liver function and some ovarian neoplasms on the conversion of androstenedione to estrone and estrone sulfate.
4. Determining the capacity of a variety of non-endocrine tissues to achieve the conversion of androstenedione to estrone by in vitro incubation studies.
5. Evaluate the relative efficacy of substances potentially active as aromatase inhibitors in an in vitro system utilizing a placental aromatizing enzyme system to inhibit excessive conversion of androstenedione to estrone, that occurs in some postmenopausal women.
6. Examining the interaction of estrone and estradiol in target tissues such as human endometrium and immature rat uterus.

With the attainment of these contract objectives, the origin and action of estrogen in postmenopausal women can be clearly defined as well as quality

and quantity of estrogen production.

Significance for Aging Research: The results of this study should contribute to understanding of the physiology and pathophysiology of estrogen production after the menopause as well as its medical management. Neither the quantitative nor qualitative characteristics of estrogen production in the menopause have been fully elucidated. It is clearly evident that the data on levels of endogenous estrogen production, the chemical nature of estrogens produced and their biological activity as well as factors which may contribute to alterations in endogenous estrogen production are needed. The acquisition of data under this contract is critical to understanding the degree of decline in estrogen production and the alternative methods by which estrogen production occurs in postmenopausal women. Also, a rational approach to replacement therapy in postmenopausal women cannot be developed until quality and quantity of endogenous estrogens produced during the menopause are clearly delineated.

Proposed Course: The contract is to continue for a minimum of three years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 73-2762

Contract Title: A Retrospective Study of Postmenopausal Women with and Without Estrogen Replacement Therapy

Contractor: University of California at Irvine, Irvine, California

Money Allocated: \$97,508 (FY 1973)

Objectives: The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of stroke in a population of postmenopausal women living in a retirement community. The contract provides for the support of a retrospective study of the incidence of cerebrovascular disease, and other factors which predispose to cerebrovascular disease in a specific and uniquely discrete population of 7000 postmenopausal women. Cases of cerebrovascular disease occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified stroke cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting severity, or type of stroke.

Significance to Aging Research: Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risk of cerebrovascular disease in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two and one-half year period. At present the value of estrogen-like medication as therapy for postmenopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer and stroke. There are no data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the many millions of women over the age of 50 in the United States.

Proposed Course: The study is planned for a minimum of two years. Continuation is dependent on results from the first two years' study.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: 5th Annual Course on the Biology of Aging

CONTRACTOR: The University of Minnesota, Duluth, Minnesota
(Contract Officer: Dr. James J. Kafka)

MONEY ALLOCATED: \$30, 247 (FY 1973)

OBJECTIVES: This contract provides for a course in the biology of aging. The course is aimed at stimulating interest in the field of aging. The course will bring together a select group of faculty, whose contribution to the field of aging or to science in general is well recognized, and an equally select group of 30 advanced students, who have yet to make a substantial contribution to aging research, but who, given the proper stimulus, may well do so.

The format is designed to provide not one-way communication from faculty to student, but rather to stimulate mutual exchange of information and ideas, such that the student has the feeling from the outset that he is a participant rather than a spectator. This will be accomplished by a rather intensive lecture schedule, usually three a day, which will allow adequate but not unlimited time for questions and comments. In addition, panels will be held daily, usually in the evening, which will provide opportunity for an intensive and more open-ended dialogue. Further, and of at least equal importance, there will be ample opportunity for individual discussion. The Duluth campus provides good physical facilities in a setting which is tranquil, attractive, and yet which, as a university campus, is characterized by an atmosphere of serious scholarship.

SIGNIFICANCE FOR AGING RESEARCH: The many significant questions underlying the mechanisms of aging can now be formulated in such a way that there is some likelihood that some may be answered in the future. The biology of aging is an emerging field with a conspicuous lack of basic information and would benefit from the contributions of young scientists. This course provides the opportunity for scientists to gather for a week of intense discussion on the aging problem.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: (Reimbursable Agreement)

CONTRACT TITLE : Longitudinal Studies

CONTRACTOR : Veterans Administration, Boston, Massachusetts
(Contract Officer: Dr. Benjamin Bell)

MONEY ALLOCATED: \$6,601 (FY 1973)

OBJECTIVES: This contract supports several meetings involving investigators of the longitudinal studies of aging. The purpose of the meetings is to discuss topics of common concern to investigators in longitudinal studies, such as procedures and problems in collecting and utilizing longitudinal data. Each of the meetings involves a small number of working investigators who share a common interest and responsibility.

SIGNIFICANCE FOR AGING RESEARCH: Comparisons of procedures and data would do much to clarify the important differences and similarities among the various study populations. The conferences would serve as a coordinating mechanism for the longitudinal studies on aging.

PROPOSED COURSE: This contract should be maintained on a continued yearly basis.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 69-2091

CONTRACT TITLE: Production, Characterization, and Distribution of Human
Diploid Cell Strains

CONTRACTOR: Stanford University, Stanford, California
(Contract Officer: Dr. Leonard Hayflick)

MONEY ALLOCATED: \$117,000 (FY 1973)

OBJECTIVES: This resource is designed to encourage the development of high quality research in the field of cell aging by providing well-characterized cultured human cells to, as well as accelerated information exchange among, the community of investigators studying the phenomenon of aging in the human diploid cell. Starter cultures may be obtained on a noncompetitive basis whereas more extensive needs for material are subject to merit review and competitive allocation of the resource.

SIGNIFICANCE FOR AGING RESEARCH: This contract has been singularly effective in stimulating the development of a specialized field of research addressing cell aging phenomena in diploid human cells in culture. The contract has passed from the stage of being highly speculative, and of introducing new investigators to the field, to one where the major capacities of the resource are being consumed by investigators who are conducting long-term research on this cell strain and who depend upon this contract as a source of characterized and standardized cell materials.

Policy and substance of this contract have been subject to the review and deliberations of an ad hoc advisory panel which meets one or two times a year in addition to the formal contract review session. The three members of the panel are: Dr. Paul Kruse, Samuel Roberts Nobel Foundation, Route 1, Ardmore, Oklahoma 73401; Dr. George Martin, Department of Pathology, University of Washington, Seattle, Washington 98105, and Dr. David Axelrod, Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Cell Culture Procedures for Aging Research

CONTRACTOR: Atomic Energy Commission, Los Alamos, New Mexico
(Project Officer: Dr. C.R. Richmond)

MONEY ALLOCATED: \$50,000 (FY 1973)

OBJECTIVES: This course is designed to enhance NICHD program activities in the field of cellular aging by providing an optimum training experience in cell culture and cellular aging. The course will offer advanced lecture presentations and intensive laboratory instruction.

REMARKS: Participants and several auditors will be selected on a competitive basis. The course is the third in a series co-sponsored by the NICHD and the Tissue Culture Association (TCA). The 1973 course encompasses changes recognized as desirable from the experience of the previous courses and is responsive to current needs in the field.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-2767

CONTRACT TITLE : Review, Analysis, and Evaluation of Population Models

CONTRACTOR : Duke University, Durham, North Carolina
(Contract Officer: Dr. George Myers)
(Subcontract to Research Triangle Institute)

MONEY ALLOCATED: \$80,000 (FY 1973)

OBJECTIVES: This contract supports the review and critical evaluation of models and techniques for projecting future population trends. The objective is to provide a basis for the design and specification of a computerized mathematical model for projecting the future size and structure of the middle-aged and elderly population.

Mortality changes are often a neglected aspect of projection procedures and appropriate attention will be given to developments in this field. Considerable attention will be given to the notion of competing risks of death in a mathematical sense and models that can readily incorporate this element into various projective procedures.

SIGNIFICANCE FOR AGING RESEARCH: The information obtained from work supported by this contract will be important in planning future health research and services for the middle-aged and elderly.

NICHD Annual Report
July 1, 1972 through June 30, 1973

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: Unassigned

Contract Title: Two Gerontological Society Symposia: "Current Experimental Models in Biological Aging" and "Gene Regulation".

Contractor: Gerontological Society, Washington, D.C.

Money Allocated: \$3,775 (FY 1973)

Objectives: This contract supported two symposia at the 25th annual meeting of the Gerontological Society aimed at encouraging the development and refinement of research in aging.

The symposium on "Current Experimental Models in Biological Aging" was developed to provide a forum for the discussion and evaluation of biological models for research in aging. One of the primary purposes was to establish the relevance of various biological systems to research in aging irrespective of the level of simplicity or complexity of the model systems selected for study. In this respect, systems ranging from insects and man to cells and tissues and organs were considered.

The symposium on "Gene Regulation" provided for the participation of speakers who delivered major addresses at the Gerontological Society Meeting. Selected topics of interest to investigators in aging research were addressed by experts in the field of gene regulation. The purpose was two fold: (1) to provide an opportunity for Gerontologists to update their knowledge of the field of gene regulation; and (2) to interest the invited speakers in the field of aging research.

Significance for Aging Research: The recognition and development of biological models of aging relevant to the study of aging processes is crucial to the development of research in aging. The symposium provided a focus for consideration of several common and unique biological model systems currently utilized in aging research. Also, it established some of the criteria for their selection and utilization in aging studies.

The symposium on "Gene Regulation" served as a means of updating the knowledge in those areas relevant to current gerontological problems. It also provides an opportunity for gerontological investigators to meet with colleagues from outside the field in order to exchange ideas on current problems. Young scientists are also exposed to outstanding researchers.

Proposed Course: Non-continuation beyond fiscal year 1973.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-3-016

CONTRACT TITLE: Accelerated Immunological Aging: A Possible Model for
Research in Relation to Rejuvenation by Fetal Liver

CONTRACTOR: East Carolina School of Medicine, Greenville, North Carolina
(Contract Officer: Dr. A. Mason Smith, Department of Microbiology)

MONEY ALLOCATED: \$38,874 (FY 1972) (2 years)

OBJECTIVES: The contractor will exert its best efforts to:

- 1) Determine anti-sheep RBC responding capacities and levels of IgM, IgG, and IgA in the blood of several strains of mice at 0.5, 1, 2, 4, 8, and 12 months of age. Conduct a comparative study on the following strains of mice: SJL/J, BALB/C, mice congenic with BALB/C, and C57BL and their hybrids;
- 2) Perform autopsies on lymphoidal tissues for pathological diagnosis;
- 3) Attempt to impair the immune system by injecting at various ages heterologous anti-heavy chain sera and, if successful, determine if the rate of recovery is age-related;
- 4) Attempt to rejuvenate the immune system of aged mice by injecting fetal liver and thymus from syngeneic and congenic donors. The fate of the donor cells will be assessed; and
- 5) Determine the frequency of incidence of reticulum cell sarcoma, the levels of immunoglobulin classes, and the anti-sheep RBC responding capacity in mice injected with fetal liver and thymus.

SIGNIFICANCE FOR AGING RESEARCH: There is evidence that immunologic competence declines with age. Since immunologic diseases--neoplastic, autoimmune, and infectious--show increased incidence with age, it is important to understand the genesis of this age-related decrease in immune competence. The inability of the organism to control such diseases is likely associated with a functional change in immunologically reactive cells as well as with a decrease in ability to mount a rapid and intense immune response as would be required for infectious diseases. Studies should be encouraged to attempt to determine the mechanisms by which immunocompetence develops, the variables that modify the rates of its development, the significance of loss of competence, and the possible methods of therapeutic intervention.

PROPOSED COURSE: This contract should be maintained active at least two years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-3-008

CONTRACT TITLE: Analysis of Aging Associated Involution of Cellular Immunity
in Mice

CONTRACTOR: University of Florida, College of Medicine, Gainesville, Florida
(Contract Officer: Dr. Perry O. Teague, Department of Pathology)

MONEY ALLOCATED: \$100,787 (FY 1972) (2 years)

OBJECTIVES: The contractor will exert its best efforts to:

- 1) Determine whether or not thymic epithelial cells and/or uncommitted bone marrow lymphocyte stem cells decline in ability to function in normal cellular immune responses with aging;
- 2) Determine if an active thymus is required for retention of "normal thymus dependent cellular immune functions" during aging of thymectomized and of thymectomized and sublethally irradiated mice;
- 3) Determine whether thymic epithelial cells or bone marrow stem cells from young and old syngeneic donors will reconstitute thymectomized and lethally irradiated mice of three strains--A/J, C57B1/6J, and DBA/1J, and
- 4) Determine if spleen cells from each mouse strain respond to stimulation in vitro to PHA, SEB, and allogeneic mitomycin-C-treated spleen target cells.

SIGNIFICANCE FOR AGING RESEARCH: There is evidence that immunologic competence declines with age. Since immunologic diseases--neoplastic, autoimmune, and infectious--show increased incidence with age, it is important to understand the genesis of this age-related decrease in immune competence. The inability of the organism to control such diseases is likely associated with a functional change in immunologically reactive cells as well as with a decrease in ability to mount a rapid and intense immune response as would be required for infectious diseases. Studies should be encouraged to attempt to determine the mechanisms by which immunocompetence develops, the variables that modify the rates of its development, the significance of loss of competence, and possible methods of therapeutic intervention.

PROPOSED COURSE: This contract should be maintained active at least two years.

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Cell Culture Procedures for Aging Research

CONTRACTOR: University of Vermont, Department of Medical Microbiology
(Project Officer: Dr. Warren Stinebring)

MONEY ALLOCATED: \$54,567 (FY 1972)

OBJECTIVES: This course is designed to enhance NICHD program activities in the field of cellular aging by providing an optimum training experience in cell culture and cellular aging. The course is to be 3 weeks in duration (September 10-30, 1972) and offers advanced lecture presentations and intensive laboratory instruction.

REMARKS: Twenty-four participants and several auditors will be selected on a competitive basis. The course is the second in a series co-sponsored by the NICHD and the Tissue Culture Association (TCA). The 1972 course encompasses changes recognized as desirable from the experience of the previous course (held at the W.A.J. Cell Science Center, Lake Placid, New York) and is responsive to current needs in the field. A planning meeting for the 1973 course was held jointly by the NICHD and the TCA April 21, 1972 at Wistar Institute, Philadelphia. This third course in the series will be more advanced in subject matter and of shorter duration (2 weeks).

NICHD Annual Report
July 1, 1972 through June 30, 1973

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Mathematical Models for an Aging Population

CONTRACTOR: University of Chicago, Chicago, Illinois
(Contract Officer: Dr. David McFarland)

MONEY ALLOCATED: \$35,985 (FY 1972)

OBJECTIVES: Planning for future health research and services for an aging population depends heavily upon projections of the future size and age structure of that population. This contract supports the review, analysis and evaluation of existing mathematical population models, especially in regard to the feasibility of their use in connection with projection and planning of health research and services for the middle-aged and elderly. The review will include a number of currently unpublished works known to be in progress from the viewpoint of ascertaining the manner in which each model incorporates those members of the population in the upper age groups and the manner in which each model incorporates mortality from various causes.

Recommendations for new research to be conducted will be made.

SIGNIFICANCE FOR AGING RESEARCH: The contract should provide information on the current status of research on mathematical models for predicting population size and structure and an evaluation of the models in terms of their applicability for research relating to the elderly.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch

Perhaps no other single problem within the broad range of human development is more complex and challenging than that of mental retardation. No other problem more clearly reflects the broad mission of the Institute, for mental retardation is a life-span problem requiring for its solution study of the full range of developmental variables.

The inadequately developed intelligence which characterizes the retarded individual is present early in life and obtains through maturity. Throughout its course, mental retardation impacts heavily on the individual, his family, community and the economic life and resources of our nation. The complex phenomena subsumed under the term mental retardation stem from many different causes and are expressed most commonly as the product of multiple biological, behavioral and social variables in interaction.

The task of bringing this wide and diverse range of phenomena together as a coherent body of related elements is similarly complex. Central to this effort is the need to effect synthesis of the many separate but related variables underlying the retarded condition which can give direction toward solution of the problem. Within the Institute, this task is assigned to the Mental Retardation Branch.

The primary mission of the Mental Retardation Branch is to develop and support research aimed at the prevention of mental retardation and, when this is not possible, to effect its amelioration. With the Institute, the Mental Retardation Branch is now approaching the end of its first decade of mission directed effort. This annual report presents an opportune time for review and appraisal of the Branch's work.

The history of the Branch is one of steady growth. In its first year, the program held 59 grants and contracts for a total outlay of \$2,748,000. In January, 1973 the number of grants and contracts had increased to 147 at a total level of support of \$19,090,000. Because the Branch holds a large number of program projects, the number of grants and contracts reported for 1973 gives a misleading indication of the number of discrete research projects supported. Each program project consists of several different but related projects. Taking this feature into account then, tabulation of all discrete research projects supported shows the number to be 247.

Numbers of grants and funding levels are but two dimensions reflecting program growth and activity. Program growth is also reflected in an ever widening of research problem coverage. Early in this decade the program had narrow coverage with grants concerned with inborn errors of metabolism dominant in program holdings. Today, program supported grants and contracts cover virtually every area of mental retardation research concern.

Legislation was passed (P.L. 88-164) in 1963 authorizing \$26 million in construction grant awards for centers for research on mental retardation and related aspects of human development. These Mental Retardation Research Centers (MRRC's) were heralded as primary agents for the nation's major thrust in the attack on mental retardation. Responsibility for this construction grant program was assigned jointly to the Division of Research Resources and Facilities, NIH and the Institute. Continuing responsibility for center program development and implementation was given to the Institute and its MR Branch.

The center construction effort has occupied the full span of this decade. The first constructed center was completed and occupied in 1968, four years after initiation of the construction phase of the program, and the last center became operational this year, 1973. In anticipation of the lag between initiation and review of center construction grant applications and completed construction of centers, staff initiated program efforts to bring the centers into productive operation prior to completion of construction. This was accomplished through programing efforts with the "paper staffs" listed on the construction grant protocols and by organizational work with the designated center directors.

These early discussions resulted in the recommendation, approved by Council in June 1968, for establishment of the center grant. Center grants and the guidelines governing their award were conceived of as a means for strengthening center administration by adding to its functional identity and capabilities.

Establishment of the center grant mechanism carried with it the recommendation calling for establishment of the Mental Retardation Research and Training Committee (HDMR). Study of the problems of center administration indicated that a stable, continuing scientific review body committed to the center's successful accomplishment of mission was necessary.

The review charge made to the HDMR Committee was scientific review of all center grants and all program projects, training grants, and fellowships assigned to the MR Branch. In addition, the committee was charged with review and report to Council of the relevance of each center's research activities to their primary mission—"research on mental retardation and related aspects of human development."

The HDMR Committee, from its inception, has met its charge successfully and in accordance with the highest NIH standards for scientific excellence. Its growing experience with each center has provided in-depth awareness of each center's needs, capabilities, and limitations against which any one of its immediate review responsibilities can be discharged.

The program project grant has become increasingly a key element in building the MR Branch. These grants are particularly suited to the purpose of the MRRC's, for they provide a mechanism whereby multidisciplinary and interdisciplinary research can readily be forwarded. And, in particular, the program project offers the opportunity to effect a synthesis of related elements around a central problem or theme—an opportunity sorely needed in

mental retardation research because of the diverse and interacting variables that determine the developmental life course of retarded individuals. As a consequence of the advantages offered by the program project, program staff has turned increasingly to this grant mechanism for program development.

MENTAL RETARDATION RESEARCH CENTERS

The MRRC program represents a potentially unique resource for multidisciplinary and collaborative research between biomedical and behavioral scientists to better understand the complex causes, pathogenesis and modes of prevention, treatment, and amelioration of mental retardation. More than 600 investigators representing a broad range of basic, clinical, and applied sciences and nearly twice as many supportive personnel are engaged in this effort. This concentration of research talent in mental retardation and related aspects of human development is the nation's response to this major social problem.

An important adjunct to research is the testing of new ideas in practice, the clinical application of preventive and treatment modalities and the training of manpower to staff emerging service programs. The University-Affiliated Facility program is particularly well suited to this purpose. Ten of the research centers also have UAF's, frequently in the same building and occasionally under the same administrative direction. The two programs are generally closely integrated on most campuses, with potentially enriching benefits to both. The integration of research with service and training activities has the additional value of helping to direct the centers toward mission-oriented goals.

Concern with the human condition is further enhanced by center affiliation with public or private residential facilities for the retarded, school and day-care programs and university or community-based diagnostic clinics. In some instances (Washington, UCLA, Kansas; Fernald, Wisconsin, Peabody, North Carolina), the tie with state institutions is especially close, with investigators holding faculty appointments, occupying laboratories in the institution or working directly with retarded subjects in ward settings. These administrative arrangements have been encouraged by the Institute to bridge the traditional gaps between universities, institutions, and communities, to facilitate the dissemination of new knowledge and its translation into practice, and to expedite program relevance. While this goal is not yet fully realized, coordinating mechanisms and partial communication systems already in force, indicate that real progress is being made.

The Institute's development of innovative policies for the fiscal support of the centers has been a major contributing force to their growth and viability. The most significant policy, designed to help the centers carry out their legal commitment to conduct research in mental retardation and related aspects of human development for a minimum of 20 years, is the center grant.

The center grant, which includes support for administrative costs, common equipment and supplies and other supportive services of the research program effects certain economies through the sharing of resources (computer services, vivaria, high-priced scientific equipment, electronics, machine

shop, information services, support laboratories, etc). Many of these resources, which undergird center activities, are not readily obtained through regular grant mechanisms and can be justified only when they serve a large number of investigators.

The most significant aspect of center support, however, is the provision for support of new program development personnel for a limited time, and other costs related to the initiation of new program areas. Such support has greatly enhanced center recruitment capabilities and faculty appointment and has resulted in the commitment of many outstanding scientists to mental retardation research. Perhaps more than any other single category of support under the core grant, this category is largely responsible for helping the centers become centers of excellence and effective competitors for grant funds.

The spectrum of research studies being conducted in the MRRRC's embraces every known major dimension of the problem. Illustrative achievements are included in the section on research activities.

RESEARCH ACTIVITIES

Genetics and Inborn Errors of Metabolism

In institutions for the mentally retarded today, 5 percent of the patients have inborn errors of metabolism, 10 percent have Down's syndrome, and 25 percent have central nervous system defects, many of which are inherited. A survey of an unselected newborn population has demonstrated that 1 out of 200 babies has a major chromosomal abnormality. In addition, about 5 percent have minor chromosomal defects, the significance of which remains to be determined. Most of these genetically determined conditions are associated with mental retardation or defects which impair a child's ability to achieve his optimal development.

Investigators from the Cincinnati Children's Hospital MRRRC have developed an animal model for study of phenylketonuria. The model involves the combined feeding or injection of increased amounts of phenylalanine and an inhibitor of phenylalanine hydroxylase. Using this model, learning deficits have been shown to occur in offspring of female rats fed the PKU diet between days 10 and 20 of pregnancy, although the deficit exceeded and could not be distinguished from that resulting from maternal malnutrition produced by pair-feeding of control animals. Neither inhibitor nor excess phenylalanine alone fed to pregnant animals produced learning deficits in offspring.

Biochemical data derived from studies employing this animal model serve to confirm previous reports that the behavioral and biochemical aspects of experimentally induced phenylketonuria are similar to those of the human disease.

Galactosemia is an inborn error of metabolism characterized by inability of the individual to convert galactose to glucose. It is a recessively inherited condition which occurs once in every 30,000 to 40,000 births.

The manifestations of galactosemia, in surviving infants, vary greatly in severity. In the majority of cases, however, the symptoms have been severe, which emphasizes the urgency of prompt treatment. An important aspect of the disease is that untreated children surviving beyond the first few weeks almost always manifest signs of mental retardation.

Investigators at the City of Hope Medical Center in California have now carried out field tests to evaluate two new low-cost, simple methods for detecting this metabolic error in newborns. These tests can be combined easily with current screening programs for phenylketonuria to provide widespread early diagnosis of both diseases.

There is a need to map human genes, not only to provide useful general information, but also as a practical means of diagnosing disease. Gene mapping can be approached in at least three ways. The first involves classic linkage studies based on recombination in individuals who are heterozygous for both a common marker gene and a disease gene. The Institute is furthering a partial solution to the problem of linkage studies through the establishment of regional registries for a number of chromosomal and metabolic defects.

The second method of gene mapping involves cytogenetic localizations. By using cytogenetic variants, including marker chromosomes, translocation heterozygous, trisomies, and duplication-deficiency syndromes, gene identification and localization in the human chromosomes can be achieved. Linkage with marker chromosomes has been used successfully in determining gene loci on human chromosomes. The first case reports, which correlate deficiency of immunoglobulin IgA with abnormalities of chromosome No. 18, came from the MRRC at Fernald School and the Massachusetts General Hospital. Delineation of the clinical syndrome associating mental retardation and certain congenital malformations with deletion of the long arm of chromosome No. 18 was achieved by investigators from the Boston Children's Hospital MRRC.

The third method of gene mapping is through somatic cell hybridization: The development of techniques for cultivation and fusion in vitro of somatic cells has provided a powerful tool for the mapping of human chromosomes. The Institute supported a Conference on Somatic Cell Hybridization in March, 1973 to further work in this area.

It has been estimated that by 1980 there will be a backlog of over 40,000 cases in the United States and Canada which will require cytogenetic investigation. It is necessary, therefore, that a fast, accurate, and economical system of chromosome analysis should be developed if meaningful progress is to be achieved. To meet this problem, contract efforts are underway to develop an automated technique for chromosome analysis.

Teratology

Preliminary evidence obtained from experiments conducted at the Cincinnati Children's Hospital MRRC suggest that doses of acetylsalicylic acid too low to produce gross CNS malformations produce behavioral impairments. The learning impairment was attributed to functional brain damage resulting from

in-utero exposure to salicylates. The damage was not accompanied by anatomic abnormalities of the CNS although minor malformations of the skeleton and viscera were observed. This and other work in behavioral teratology points to need for expanded efforts in this area.

Malnutrition

Institute supported scientists at the Worcester Research Foundation for Biological Research collaborating with scientists at MIT and Harvard are studying the effect of maternal protein malnutrition predating mating on the behavior, neurophysiology, neuroendocrinology, neurochemistry and morphology of rat offspring. Pups of mothers deprived for six weeks prior to mating and during both gestation and lactation exhibit disturbances in recorded EEG patterns, proportion of REM sleep and changes in the associated neuro-endocrinological patterns, along with reductions in birth weight and in brain weight at birth and at ten days. Eight weeks deprivation results in fewer litters with fewer pups with more profound effects on brain weight at birth and ten days.

Early Diagnosis and Intervention

Evidence supporting the effectiveness of early interventions for improving the development of retarded children has recently been provided by investigators at the Experimental Education Unit of the University of Washington MRRC. Working with Down's syndrome children in age groups from birth to 18 months, 19-36 months and from 3 to 5 years of age, these investigators have been able to demonstrate significant developmental gains in cognitive, speech and language, motor and self-help performance at all age levels. These gains hold up under sustained programming to school age using a methodology for preschool classroom training and parental instruction in intervention procedures. The most marked gains were achieved in those children for whom intervention began early in their development.

Amelioration and Treatment

Growing disenchantment with the quality of residential care in the U.S. has led to administrative pronouncements to depopulate these facilities and to develop alternative patterns of care. Unfortunately, knowledge is not yet available as to the type of care most suitable for specific individuals and the program ingredients most likely to maximize development and adaptive behavior.

A large scale project at the Pacific State Hospital (a part of the MRRC at UCLA) is focusing on these issues. Efforts are underway to determine what criteria are applied in the selection of resources for placement, to specify the nature of the environment and to evaluate outcome behaviors. Through research of this kind, it is hoped that movement of individuals from institutional to community-based facilities and the selective placement of children and adults in a variety of care, training, and rehabilitative settings can proceed on a sounder basis than heretofore.

Further, epidemiological studies have examined public institution and community residents. Data which have been collected on more than 20,000 residents with respect to diagnoses, adaptive behavior, physiological traits, social relationship and psychological characteristics probably represent the most comprehensive information available on institution populations. This data has been related to mortality, life expectancy tables, and training outcomes and has been applied effectively to institutional management and programming. The community study at Riverside highlighted the discrepancy between measured intelligence and adaptive behavior for ethnic minority groups. Many children of Mexican-American descent and bilingual in speech tested poorly on standard intelligence tests because of deficiencies in the English language rather than intellectual impairment. These findings stimulated policy changes in the educational system and the decertification as mentally retarded of thousands of children.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH-71-2447

Contract Title : Diagnostic and Intervention Studies of High-Risk Infants

Contractor : Regents of the University of California, U.C.L.A.

Money Allocated: \$594,000, FY 72

Ojective : The purpose of this contract is: (a) to develop new diagnostic techniques and relate these to established ones, so as to best delineate those aspects of psychophysiological functioning in high-risk infants that indicate continuing risk for development, and (b) to ameliorate or modify this risk status through a program of specified interventions implemented from four or nine to twenty-four months of age.

Methodology : This prospective study is designed to specify those behaviors of high-risk neonates and infants which relate to performance on tests at two years of age. The study design is longitudinal, and correctional, covering a two-year period in each child's development.

The study sample of 200 subjects will be drawn from the UCLA Newborn Nursery and will consist of all premature infants of 37 weeks gestational age or less. The control subjects, full-term infants (39-41 weeks gestation) will also be drawn from the UCLA Newborn Nursery.

In the newborn period the infants will be rated on several behavioral and physiological diagnostic tests as well as on obstetrical and neonatal events. In addition, the infant's performance on other diagnostic tests will be assessed during the first four months. All infants and their parents will be provided with intensive medical, nursing, and social work support and anticipatory guidance in the nursery, well-baby clinic, and in the home.

When the infant is four months of age, the scores on all measures will be weighted and summed to derive a 4-Month Cumulative Risk Score (CRS). The CRS will then be used to classify the four-month old subjects as high or low risk. The infant will then be placed into one of the experimental or control groups. A randomly selected group of high-risk infants will begin to receive specialized 4 Month Intervention.

Additional diagnostic tests on behavioral and physiological measures will be administered during the four to nine months period. Medical, nursing, and social work support will continue for all babies both in the well-baby clinic and in the home. Scores on all of the diagnostic tests will be weighted and summed to yield a 9-month CRS. The 9-month CRS will be used to validate the risk and non-risk status of the 4-month CRS. At nine months of age, half of the high-risk-nonintervention group will receive 9-month specialized intervention. Intervention will continue with these infants and with the original HR-4-month specialized intervention group until the infants are two years of age. The diagnostic measures and the specialized intervention program, including age of intervention, will be evaluated by testing each child at two years of age on a variety of measures.

Results : The project is in its second year of operation. Pilot work is near completion and experimental subjects are being accepted into the formal research program.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NICHD-72-2794

Contract Title : Definition of a Behavioral Phenotype in the Cornelia DeLange Syndrome

Contractor : The Regents of the University of California, San Diego

Money Allocated: \$123,895 FY 72

Objectives : A description of the general behavioral repertoire of patients with Cornelia DeLange syndrome will be developed. This study will describe, codify, and quantify preliminary impressions that there is a characteristic behavioral pattern in this syndrome. Uniformity and consistency of observed movement phenomena will be examined and the distinctiveness of the behavioral phenomena to Cornelia DeLange syndrome will be assessed. Determination will also be made of the degree to which the developed quantitative indices of behavior are useful in discriminating between different clinical conditions.

Methodology : Nonverbal behavioral phenomena such as general body activity, body posture, gait, posturing of extremities, facial muscular movements, and social interactions, will be recorded on videotape in children with Cornelia DeLange syndrome. Subjects will be videotaped in two general settings: familiar to the subject, that is, home or institution of residence; Mailman Center in Miami or similar setting unfamiliar to the subject. In each setting the subject will be observed alone and in interpersonal interactions, the latter with a person familiar to the subject and with one he does not know. Subjects will be observed in small versus large rooms, in the presence and absence of manipulanda, and while engaged in activity which is mildly frustrating versus activity which is positively rewarding. Tapes will be digitally coded and analyzed using procedures and devices developed at the Studies of Nonverbal Behavior Laboratory, University of California, San Francisco.

Results : This project was initiated in June, 1972. To date, five patients from the Miami area have been videotaped in the Audiovisual center at the University of Miami. Coding of the facial-affective behavior and stereotypic behavior of 3 ambulatory patients has been completed. The social-interactive behavior coding is underway. Videotaping of additional patients with Cornelia DeLange syndrome is planned.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

- Contract Number: 1-Y01-HD-30001-00 Interagency Reimbursable Agreement with the National Aeronautics and Space Administration
- Contract Title : An Automated System for Chromosome Analysis
- Contractor : Jet Propulsion Laboratory, California Institute of Technology
- Money Allocated: \$342,710 FY 72
- Objectives : The purpose of this contract is to develop an automated system for analysis of conventionally stained as well as "banded" chromosomes. The following goals are to be accomplished in this contract: development of a semi-automated system for the preparation of metaphase chromosome spreads on microscope slides; development of an automated light microscope system for fast, reliable, and efficient location and digitization of metaphase spreads; development of computer programs for karyotype analysis and classification of the digitized spreads; development of an interactive data management and analysis system for a cytogenetic data file; and systems testing using slides obtained from different groups of subjects.
- Methodology : The contractor will perfect an automated light microscope system that they have developed. The system examines microscope slides of human metaphase spreads which includes an object locator technique employing a rotating mirror, optical scanner, automatic focus procedure, and necessary programs for analysis of the image of selected metaphase spreads stained conventionally and using various "banding" techniques. The signal received by the optical scanner is sent through an analog to digital converter to a computer where, under interactive control of an operator, digital images of selected metaphases are written on tape for computer analysis. The final computer-generated karyogram is presented in the form of a high quality, hard copy print. Corresponding numerical measurements are available from the line printer.
- Results : This project was initiated in February, 1973. To date, the investigators are in the process of procuring the necessary hardware needed for the study, and finalizing their systems design and operation. A tentative design has been accomplished including the development of the camera system, display device and an inexpensive hard copy equipment.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NICHD-71-2081

Contract Title : Intellectual and Behavioral Consequences of Severe Malnutrition in Infancy: A Collaborative Study

Contractor : Albert Einstein College of Medicine

Money Allocated: \$66,720, FY 72
Continuation for FY 73 without additional funds

Objectives : The aims of this study are to assess the relationship between severe malnutrition during the first two years of life in children and their subsequent intellectual development and behavior. Control groups of siblings and classmates are being compared on variables related to somatic, neurological, physiological, intellectual, educational and family characteristics.

Methodology : This is a retrospective study of children aged 6-10 years who were hospitalized for severe malnutrition before two years of age. Detailed clinical records were kept of these infants regarding clinical severity of illness, duration of stay and somatic measures. In the follow-up study information on the index children and controls was obtained on a wide range of developmental and behavioral indices, school behavior and achievement, child rearing and social environmental stimulation.

Results : This project has now completed its second and final year of data analysis. A number of papers have been published thus far or are in press. Findings thus far indicate that the index children are shorter, weigh less, have smaller head circumference, lower IQ's, lower school achievement and less satisfactory behavior in school. Malnutrition and lack of social stimulation both appear to play contributory roles. The age of hospitalization did not appear significant in affecting developmental outcomes. An extension of time without funds was approved to allow for complete analysis and write up of the voluminous data collected.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NO1 92180

Contract Title : Prevention of Prematurity and Developmental Disorders
Through Nutritional Supplementation and Laboratory Studies
on Prenatal Malnutrition

Contractor : Columbia University School of Public Health

Money Allocated: \$733,338, FY 72

Objective : The purpose of this contract is to carry out a controlled study on the effects of nutritional supplements to pregnant women in the socioeconomically deprived population of Harlem in New York City. Evaluation will be made of the impact of these supplements during various stages of pregnancy on birth weight, infant development, infant mortality and related disorders. Subjects will be drawn from a clinic population considered at high risk of giving birth to premature infants. This project is presumed to have great social and practical significance for improving physical and intellectual development in racial minority and poverty groups and for the prevention of mental retardation and related handicapping conditions.

The laboratory studies are designed to obtain a better understanding of the physiological parameters of growth and development and the potential role of infectious processes in low birth weight and retarded development. The correlation of data from these studies with pathological and clinical findings and data derived from the basic project will strengthen and extend the results and validity of the nutritional experiment.

Methodology : Experimental and control groups will be selected from a clinic population serving the Harlem area according to certain indices of high risk in producing low birth weight infants such as prepregnancy weight, previous history of prematurity, weight gain during pregnancy and age of mother and low protein intake. Other relevant factors including smoking habits, drug usage and trimester of entry into the project will be considered in the analysis. Infants will be assessed developmentally at birth, 26 and 40 weeks of age.

The laboratory studies include biochemical measurements of DNA, RNA and protein on placental and autopsy material and their relation to cell size and number. Morphometric measures will be made on by-products of pregnancy and infant tissues and studies of infection will be undertaken on maternal and infant serum and cultures.

Results : The project is now entering its fourth year and will complete its full subject enrollment of 1200 cases before the end of this calendar year. Approximately 600 babies have been delivered to date and recruitment rates have been highly successful and attrition rates low. Because the study is being conducted blind, data on outcomes are not yet available.

The supplementary laboratory work initiated in February, 1972 is being carried out successfully according to the original protocols presented.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NO 1-HD-1-2446

Contract Title : An Investigation of Certain Relationships Between Hearing Impairment and Language Disability

Contractor : University of Washington, Seattle, Washington

Money Allocated: \$160,841, FY 72

Objective : The objectives of this project are best described in two phases. The three specific objectives of Phase I of this contract are: (1) to determine the feasibility of conducting an extensive investigation of the relationships between hearing impairment and the speech and language status of children from birth to three years of age; (2) to formulate a test battery for assessing the speech, hearing and language abilities of children under the age of 36 months; and (3) to develop specific testable hypotheses regarding the relationships between hearing impairment and language function. The results of Phase I of this contract will serve as a base for Phase II, a subsequent project on the investigation of the relationships between hearing impairment and language in young children. Phase II will provide a base for the investigation of language disability of children with other problems, such as cultural-familial mental retardation, physically based mental retardation, and cerebral palsy.

Methodology : The research will be conducted in four major areas: (1) auditory perception, (2) receptive and expressive language, (3) speech production, and (4) communicative interaction. In the area of auditory perception four facets will be examined: (1) behavioral responses to different auditory stimuli will be examined utilizing a variety of play techniques and conjugately programmed stimuli with a specially constructed manipulanda; (2) speech sound discrimination of 2-3 year olds will be tested using a key-foil discrimination task; (3) auditory sensitivity, examining behavioral responses using a population of 6-24 month old infants; and (4) auditory sensitivity, temporal integration and discrimination between paired auditory stimuli will be examined in infants using modifications of averaged electroencephalic audiometry.

Receptive and expressive language will be examined in terms of phonological, morphological and syntactic development

using transcriptions of language samples recorded on video tape. Speech production will be studied by (1) describing the vocalizations of infants in terms of feature networks, (2) describing the prosodic skill development in both normal and hearing impaired children under the age of two, and (3) measuring such physiological aspects of speech control as lip movement, jaw movement and lung volume. Communicative interaction will be examined using video tapes obtained in the child's home and evaluated for communicative and interactive behaviors. The tapes will be studied for number of vocalization-verb-alization behaviors, communicative events, antecedent events, consequential events and behavioral events.

Results : This project is in its second year of operation. During this period substantial progress has been made toward accomplishing each of these goals. The contract team has not only developed a set of testable hypotheses concerning the relationship between hearing loss and language development, but is attempting to develop a common theoretical framework in which to study receptive and productive processes.

The contract team's work on the development of techniques for evaluating hearing and language have also shown adequate and positively accelerating progress. They have adapted behavioral techniques for evaluating the hearing of children down to about 12 months and now are adapting instrumental conditioning techniques to study auditory sensitivity, sound discrimination, and sound recognition of the younger infants. The electroencephalic audiometry research has produced several interesting findings, but it now appears that this procedure will not be included in Phase II. The research on phonological transcription and analysis of the acoustic output of young children also shows progress. The group has realistically restricted their study of language primarily to the study of phonology with only a limited amount of work on semantics and syntax. A broader study including semantics and syntax at this time would lead to diffusion of effort. These three aspects of language can be combined in Phase II. The contract team has already published several papers in the methodology area.

Several methodological problems have been solved in the communication interaction area and data have been collected in the home setting with a video-tape sampling procedure developed by the contract staff. These data are in the process of being analyzed.

Although the contract is still in the developmental phase, the work done is promising and unique insofar as it provides an intersection of several rather important areas of investigation. First, the group is making an attempt to integrate work on the development of auditory reception and vocal production within a common framework; namely, the framework of rhythm being an organizing principle for language development. Second, they are investigating these relationships with very young children. Third, they are introducing these sophisticated and promising techniques of study into the area of early language development of the hearing impaired and retarded populations.

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1973
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH-72-2016

Contract Title : The Development of Materials for Application of Behavior Modification Skills by Parents of Mentally Retarded Children

Contractor : Behavior Education Projects, Inc.

Money Allocated: \$67,600, FY 72

Objectives : The purpose of this contract is to test the efficacy of printed materials as used by parents, in shaping the behavior of their retarded children. Determination will also be made of the degree of supplementary training which may be necessary in the use of such materials and the relationships between child and parental characteristics and training effectiveness.

Methodology : This project involves several phases of operation. Initially, parents will identify problems and training needs posed by the behavior of their children and programmed texts will be developed responsive to these needs. After these materials have been pretested, controlled experiments will be undertaken varying the use of the texts and degrees and form of supplementary training. Parent and child characteristics will be included in the analysis of the data.

Results : This project was initiated in October, 1971. To date 13 programmed texts in the area of self-care skills, speech and language, and behavioral problems have been developed and pretested. Utilizing these texts, the experimental phase of the project has been initiated with the objective of evaluating under controlled conditions, the efficacy of the materials and the degree and form of supplementary training that may be required to achieve behavioral change goals. Preliminary data suggests that the written materials can be used independently for certain parents. Profiles of the characteristics of these parents are being described and will prove helpful in the practical application of behavior modification techniques. The experimental phase of the project is expected to be concluded in the fall of 1973.

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