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ABSTRACT

The 1988 Progress Report covers research activities of the five branches of the Center for Research for Mothers and Children of the National Institute of Child Health and Human Development. An introductory section briefly describes the Center, notes staff activities and Center sponsored conferences and workshops, and identifies highlights of activities of each of the Branches. A separate section is then given to each Branch's activities reported in terms of an overview, program activities, research highlights, and staff activities. The five branches are: the Genetics and Teratology Branch; the Pregnancy and Perinatology Branch; the Endocrinology, Nutrition and Growth Branch; the Mental Retardation and Developmental Disabilities Branch; and the Human Learning and Behavior Branch. Noted is a new Branch, the Pediatric, Adolescent, and Maternal AIDS Branch which is conducting research on Acquired Immune Deficiency Syndrome as it affects women and children. As an example, research highlights for the Mental Retardation Branch cover: genetics and genetic disorders; inborn errors of metabolism; prenatal diagnosis; exogenous and environmental factors; behavioral and biobehavioral factors; language and communication; treatment of behavior disorders; and adaptation in family, residential, vocational, and educational settings. Tables provide detail on the grants and contracts active during August, 1988. (DB)

1988 Progress Report

Center for Research for Mothers and Children

National Institute of Child Health and Human Development

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

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National Institute of Child Health and Human Development
1988 Progress Report

Center for Research for Mothers and Children

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INTRODUCTION

The Center for Research for Mothers and Children (CRMC) is the central focus for support of research and research training for maternal and child health at the National Institutes of Health. The Center is an integral part of the National Institute of Child Health and Human Development (NICHD). CRMC-supported research in the biomedical and behavioral sciences extends over the life span from conception to maturity. These research endeavors have advanced fundamental knowledge of maternal health and child development with improvements in consequent clinical care.

CRMC's research programs are designed to advance the goals of ensuring through research the birth of healthy babies and the opportunity for each infant to reach adulthood unimpaired by physical or mental handicap and able to achieve his or her full potential. The programs are carried out through the Center's Office of the Director and its six Branches.

The **Genetics and Teratology Branch** supports research to elucidate the underlying mechanisms controlling both normal and abnormal development including identification of the early stages at which defects may be triggered and the etiologies and mechanisms through which congenital defects are produced. Of special concern are the genetic and molecular aspects of embryogenesis.

The **Pregnancy and Perinatology Branch** supports research organized around maternal-infant health problems involving high-risk pregnancies, fetal pathophysiology, premature labor and birth, disorders of the newborn, and the sudden infant death syndrome in order to advance knowledge on pregnancy and maternal health, fetal growth and maturation, and newborn well-being. A highlight of this effort is the Perinatal Emphasis Research Centers (PERC) Program, an integrated multidisciplinary program addressing clinically important issues in perinatal medicine.

The **Pediatric, Adolescent, and Maternal AIDS Branch** although quite new, has taken a leading role in the study of human immunodeficiency virus (HIV) infection and disease as it affects women of childbearing age, pregnant women, mothers, fetuses, infants, children, adolescents and families. The research effort focuses on, but is not limited to, the epidemiology, natural history, pathogenesis, behavioral aspects, treatment, and prevention of this infection and disease as it affects the above-mentioned groups. This research was formerly a part of the Pregnancy and Perinatology Branch portfolio, and highlights of AIDS research in this reporting period are located under the Pregnancy and Perinatology Branch section.

The **Endocrinology, Nutrition, and Growth Branch** supports research on the roles played by nutrients and hormones in development during fetal life, infancy, childhood, and adolescence in order to attain a better understanding of the relationships between nutritional and hormonal factors during normal growth and development as well as in growth retardation and developmental disorders of the endocrine system.

The **Mental Retardation and Developmental Disabilities (MRDD) Branch** supports research concerned with the etiology and pathophysiology, diagnosis and evaluation, prevention and amelioration of mental retardation and related developmental disabilities. A major component of the MRDD Branch program is the 12 Mental Retardation Research Centers whose primary objective is to provide facilities for a cohesive, interdisciplinary program of research in MR and related aspects of human development.

The **Human Learning and Behavior Branch** supports research to ascertain how the interaction of biological, psychological, and socioenvironmental factors result in normal behavioral development and to identify those factors which interfere with such development. Included are learning problems, delayed or impaired speech, language development, and dyslexia.

More detailed descriptions of the research interests of these Branches and the names of their respective staff are included in the sections that follow. To maximize its effectiveness, the Center maintains a close liaison with all NICHD operating units including the Center for Population Research, the Prevention Research Program, and the Intramural Research Program. Close liaison is also maintained with other NIH Institutes, various Federal agencies, and with a variety of non-Governmental organizations concerned with children and their families.

HIGHLIGHTS OF CRMC RESEARCH DURING FY 1988

In August 1988, the CRMC supported 1,308 research and research training projects totaling more than $219 million (table 1). This represented a decrease of 252 projects funded, while increasing the funds expended by 35 million from August 1987.

Again this year, CRMC support of research on the biological and behavioral growth and development of children and on factors that may interfere with normal development comprised about 60 percent of all extramural funds expended by the NICHD.

The following highlights some of the research supported by the CRMC during FY 1988. The research in these areas is described in greater detail within the full descriptions of each of the CRMC Branches.

GENETICS AND TERATOLOGY BRANCH

- Clinical trials with human cytomegalovirus vaccines and vaccine challenge studies are being carried out in human populations.

Table 1.

NICHD GRANTS AND CONTRACTS ACTIVE DURING AUGUST 1988
CENTER FOR RESEARCH FOR MOTHERS AND CHILDREN

Funds (in thousands)

Branch	Total		Research Grants								National Research Service Awards		Research Contracts	
			Total Research		Research Projects (Incl. PO1)		Research Centers		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	1,308	$219,033	1,110	$191,407	1,011	$169,681	19	$16,586	80	$5,140	128	$10,032	70	$17,593
Genetics & Teratology	372	51,950	315	46,369	286	44,674	-	-	29	1,696	46	3,295	11	2,286
Pregnancy & Perinatology	304	57,936	256	46,862	233	40,791	6	4,577	17	1,494	20	1,856	28	9,218
Endocrinology, Nutrition & Growth	199	29,995	170	27,536	154	26,364	1	323	15	850	17	921	12	1,538
Mental Retardation & Developmental Disabilities	196	50,930	169	45,715	149	33,494	12	11,686	8	535	13	1,706	14	3,509
Human Learning & Behavior	237	28,221	200	24,924	189	24,358	-	-	11	566	32	2,255	5	1,043

Notes: 1) Excludes the scientific evaluation grants.
2) The Minority Biomedical Support Grants (S06) are included in the research projects.
3) Excludes thirty four grants and thirteen contracts funded from sources other than NICHD extramural funds.
4) Columns may not add to total due to rounding.

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- Molecular and cellular mechanisms involved in immune recognition and immune response to pathogenic organisms of newborns are being defined.

- The role of histocompatibility antigens in development and reproduction are being better defined.

- The region of the Y chromosome that encodes the testis-determining-factor gene that has been identified, cloned and sequenced.

- Studies of genomic imprinting in both human and nonhuman mammals suggest that methylation plays an important role.

- The role of homeobox-containing genes is being elucidated during early vertebrate development.

- The extensive use of transgenic mice has allowed the in vivo dissection of functional important sequences of DNA.

- The basic mechanisms that regulate how genetically identical cells become committed to different fates is being investigated.

- Cytoplasmic determinants that direct the subsequent differentiation of specific cell phenotypes have been isolated in several experimental systems.

- Molecules are being identified which generate pattern specificity in the developing nervous system.

- New noninvasive marking techniques are available which allow the direct observation of growing neurons in the living animal enabling us to view the establishment of nerve connections.

- The requirement for contractile activity on normal muscle development is being assessed.

- A new form of collagen has been described which appears to be critical for the process of bone ossification.

- Characterization of chondrocytes in both normal and chrondrodystrophic growth plates are providing insights into genetic bone diseases.

- The role of Vitamin A in both normal development and teratogenesis is being elucidated.

- Potencial bioeffects resulting from the use of diagnostic ultrasound during pregnancy are being evaluated for long range and subtle effects.

PREGNANCY AND PERINATOLOGY BRANCH

- In premature labor, onset is attributed to infection in 60 percent of the cases.

- The last major organ system to mature in utero, the fetal lung, synthesizes an increased amount of platelet-activating-factor which via the amniotic fluid to the amnion could be the signal to initiate human parturition.

- The placenta secretes corticotropin in a reproducible pattern suggesting the possible clinical value of sequential measurements.

- Using the knee height measuring device, it was documented that linear growth continues during adolescent pregnancy.

- The very-low-birth-weight infant has a marked decrease of bone mineral content at birth but catches up and, by 2 years, mineralization is adequate.

- In the intrauterine-growth-retarded infant, growth adheres to the sequential hypothesis; that is, allocation of nutrients follows the same pattern as in normally grown fetuses but at a slower rate.

- Only severe hypoxia causes fetal growth retardation in which growth of the brain is affected less than other body parts.

- Fetal hyperinsulinemia causes the production of a surfactant deficient in a major apoprotein, which may explain the increased incidence of respiratory distress syndrome in infants of diabetic mothers.

- Enkephalins are endogenous opiates co-secreted with catecholamines at birth and most likely play an important role in neonatal adaptation.

- Tin and zinc protoporphyrins inhibit heme oxygenase and thus bilirubin production, and could become the preventive treatment of hyperbilirubinemia of the newborn.

- Repeated exposure of lambs to decreased amounts of oxygen increases the time of arousal and decreases the arterial hemoglobin oxygen saturation at arousal during both quiet and active sleep.

- Preliminary data suggest that the rate of vertical transmission of HIV infection from mother to child may be as high as 40 percent.

- Cognitive dysfunction is a prominent and early sequela of perinatally acquired HIV infection.

- A new technique has been developed to test for HIV antibody in samples of blood collected on filter paper.

ENDOCRINOLOGY, NUTRITION AND GROWTH BRANCH

- A factor in human milk appears to stimulate the production of mucosal immune substances by nursing infants.

- Bile salt-stimulated lipase has been found to occur in ferret milk, providing a convenient animal model for studies of this key enzyme in the digestion of fats by nursing infants.

- A puff of air directed onto the face provokes reflex swallowing in subjects younger than 12 months and in older subjects with neurologic damage; this newly discovered reflex has practical applications in the insertion of nasogastric tubes, stimulation of swallowing during esophageal mobility studies, and facilitation of the administration of medications.

- In most infants with chronic diarrhea who fail to respond to a change to soy formula, symptoms will disappear within 7 days if lactose, the physiologic sugar of human milk, is substituted for sucrose in the soy formula.

- Marginal zinc deficiency in pregnant and lactating animals interferes with the formation of brain cell microtubules, important structures in the maintenance of cell shape and the process of cell division.

- When rats were fed B_6-restricted diets during gestation and lactation, profound metabolic changes, including the accumulation of a metabolite known to cause convulsions, were found in the brains of their progeny.

- Medium chain fatty acids can be absorbed directly from the stomach in small infants, thus providing a rapidly available energy source.

- Insulin administered to pregnant women with insulin-dependent diabetes can cross the placenta and appear in the fetal circulation, where it may account for the very large size of babies born to these women.

MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES BRANCH

- Five of the genes localized on human chromosome 21 have also been localized on mouse chromosome 16, considered to be homologous (in part) to the human 21 chromosome.

- By using restriction fragment length polymorphisms related to the phenylalanine hydroxylase gene, prenatal diagnosis of PKY homozygotes and PKU carriers has been achieved.

- Based on preliminary analysis of available data, chorionic villus sampling is an effective approach to early prenatal diagnosis but probably confers a slightly higher risk than amniocentesis.

- The fetus and young child may be adversely affected when exposed to blood lead concentrations below 25 μg/dl, the level defined by the Centers for Disease Control as the highest acceptable level for young children.

- Controlled studies of over 600 mentally retarded individuals show that the quality of their lives and developmental advances are related to the functional opportunities and social milieu of their residential environments rather than structural features, such as size, staffing patterns, location, categorical type, and funding sources.

- Preliminary results show that a new test battery designed to assess attentional factors in memory is particularly sensitive to the differential deficits associated with Down syndrome.

- A group of children from impoverished homes with mentally retarded mothers were nearly six times as likely as controls to function within the normal range after an intensive experimental educational program throughout the first 5 years of life.

- Functional assessments of self-injurious behavior suggests a variety of motivational factors for these life-threatening behaviors including: escape from, or avoidance of, demands made by others; inappropriate attention; or self-stimulation.

- Some individuals who exhibit self-injurious behavior are responsive to pharmacological treatment.

- Stimulus equivalence procedures have been used to improve performance in vocational settings.

- Individual differences in language skill acquisition in children with Down syndrome is influenced by the level of intelligibility of the child's speech.

HUMAN LEARNING AND BEHAVIOR BRANCH

- Local anesthesia administered to newborn infants undergoing invasive medical procedures attenuates fluctuations in physiologic, acoustic and behavioral responses.

- When their mothers were distracted, children with a history of injury had a higher level of hazard contact, disruptive behavior, and activity change than children without prior injury.

- A negative relationship between adolescent cigarette smoking and socioeconomic status is mediated by parent modeling, peer modeling and personality characteristics but not by beliefs concerning the effects of cigarette smoking.

- Four-month-old infants detect gestalt relationships in partly occluded objects. Their failure to use those relationships does not stem from a failure of perceptual discrimination.

- There are no differences between second and eighth graders' global desire for companionship and for intimate disclosure.

- Behavior genetics studies comparing twins reared apart reveal that, at 1 year of age, 8 percent of the variance in IQ is attributable to genetic factors. At age 7, genetic factors account for 35 percent and by adulthood some 45-50 percent of the variance can be shown to be related to inheritance.

- Motivation research in the perinatal rat pup demonstrates that behavioral activation is not necessary for reward or learning.

- Studies of reading disabilities in identical twins and fraternal twins leads to the conclusion that the heritable component of this deficit is 30 percent.

STAFF ACTIVITIES

PARTICIPATION IN CONFERENCES AND WORKSHOPS

Dr. Sumner J. Yaffe, pediatrician and pharmacologist, completed his seventh year as the Director of the CRMC. During FY 1988 he attended and participated in the meetings listed below.

Served on the Surgeon General's Panel on Smoking and Health, October 15, 1987, to present.

Presented Research in Maternal and Child Health at Children's Hospital of Philadelphia, PA, October 21, 1987.

Attended Ross Research Conference on Surfactant, Carefree, AZ, October 26-27, 1987.

Presented Grand Rounds at Schneider Children's Hospital, Long Island, NY, November 6, 1987.

Served on resource panel at biennial meeting of Clinical Infant Programs, Alexandria, VA, December 4, 1987.

Chaired annual meeting of the Directors of the Perinatal Emphasis Research Center, Denver, CO, March 14-15, 1988.

Attended the National Invitational Conference on "AIDS in Adolescents: Exploring the Challenge," New York, NY, March 27-28, 1988.

Organized an International Conference on Pediatric and Maternal AIDS, Bethesda, MD, March 29-30, 1988.

Attended "Clinical Application of Genetic Advances to Medicine: Social and Policy Implications," Arden House, Tuxedo Junction, NY, April 7-9, 1988.

Chaired Steering Committee Meeting on Vaginal Infection and Prematurity, Bethesda, MD, April 11, 1988.

Attended Perinatal Biology Symposium and the Congress of the European Society of Perinatal Medicine, Rome, Italy, April 14-16, 1988.

Met with officials of Indian Council on Medical Research to plan workshop on Perinatal Determinants of Child Survival, New Delhi, India, April 17-19, 1988.

Attended European Society of Developmental Pharmacology, Lausanne, Switzerland, April 20-23, 1988.

Attended Forum on Drug Development, Institute of Medicine of the National Academy of Sciences, Washington, DC, May 5, 1988.

Organizer and participant in "Interventions for the Prevention of Low Birth Weight," Chatham, MA, May 8-11, 1988.

Attended American Academy of Pediatrics Committee on Drugs Meeting, Chicago, IL, May 18-19, 1988.

Participant in symposium, "Biology of Down Syndrome--Insights from Prenatal Screening and Epidemiology," Portland, ME, May 23-25, 1988.

Keynote Speaker, European Society for Pediatric Research, Oslo, Norway, June 19-22, 1988.

Attended a symposium on "Neonatal Infections and the Role of Immunotherapy" sponsored by Sandoz Chemicals, Huron, OH, July 18-20, 1988.

Attended Perinatal Research Society Annual Meeting in San Diego, CA, September 25-27, 1988.

Dr. Kavanagh gave a major address at the Annual Meeting of the American Speech-Language-Hearing Association in New Orleans, LA, November 1987. He spoke on the activities of the Interagency Committee on Learning Disabilities and its Report to Congress.

Dr. Kavanagh helped the NINCDS plan a Consensus Development Conference on Cochlear Implants and attended the meeting on May 2-4, 1988.

Dr. Kavanagh made two presentations at the Fifteenth Annual Conference of the New York Branch of the Orton Dyslexia Society on March 4, 1988. His first speech was in a morning session entitled "Medical Symposium, the Brain and Behavior," where he discussed the new Federal Biological Definition of Learning Disabilities and Attention Disorders. His second speech was entitled "LD: Educational and Social Perspectives."

Dr. Kavanagh was the Keynote Speaker at the P'TACH Society First National Scientific Conference on Jewish Children and Young Adults with Learning Disabilities, held in New York City, March 24, 1988. His topic was "Learning Disabilities: The State of the Art."

Dr. Kavanagh attended a conference on Modularity and the Motor Theory of Speech Perception at Yale University, New Haven, CT, on June 5-7, 1988, and gave the "Banquet Talk."

Dr. Kavanagh conducted a 2-hour staff development workshop for Public School Psychologists and Social Workers of Alexandria, Virginia, August 31, 1988. He lectured and led a discussion concerning new legislation in the field of learning disabilities.

Dr. Kavanagh continued to serve on the Editorial Board of the journal, Topics in Language Disorders, and was a frequent reviewer of articles submitted to the journal. He participated in the discussions at the annual meeting of the Board.

The Institute was informed that the book Dr. Kavanagh edited in 1986 entitled Otitis Media and Child Development (Parkton, MD, York Press, Inc.) was selected as the MacMillan Science Book Club main selection for August 1988.

During this reporting period, Dr. Kavanagh continued to serve on the NIH Extramural Board, the Panel of Special Examiners for Health Scientist Administrators and Grants Associates, and the NIH Grants Appeals Board. He also continued to serve as a member of the National Child Abuse and Neglect Board, a position he has held since 1978.

PUBLICATIONS

Goldman, A.S., Zackai, E.H., and Yaffe, S.J. Fetal trimethadione syndrome. In: Sever, J.L. and Brent, R.L. (Eds.) Teratogen update: environmentally induced birth defect risks, New York: Alan R. Liss, 1986;35-38.

Kavanagh, J.F. The interagency committee on learning disabilities and its report to congress. In: Elstner, W. and Supple, M. deM. (Eds.) Proceedings of the Schools Committee Meeting on Language/Learning Disability of the International Association of Logopedics and Phoniatrics. Dublin, Ireland: 1987;52-61.

Kavanagh, J.F. Report to the U.S. Congress on Learning Disabilities. Their World, 1988;70-71.

Sonawane, B.R. and Yaffe, S.J. Drug exposure in utero: reproductive function in offspring. In: Kacew, S. and Lock, S. (Eds.) Toxicologic and pharmacologic principles in pediatrics; Washington: Hemisphere, 1988;41-66.

Yaffe, S.J. and Catz, C. Lo stress respiratorio neonatale intervento farmacologica. Federazione Medica, XL-9.1987;987-991.

Yaffe, S.J. Drug disposition in malnourished infants and children. In: Xanthou, M. (Ed.) New aspects of nutrition in pregnancy, infancy, and prematurity; Amsterdam: Elsevier, 1987;143-154.

CRMC-SPONSORED CONFERENCES AND WORKSHOPS

1. The Role of Cytoplasmic Factors in Development
2. The Onset of Labor: Cellular and Integrative Mechanisms
3. Nutritional Therapy of Inborn Errors of Metabolism
4. Future Needs in Human Milk Research
5. Development of Methods for Analysis of Human Colostrum and Milk
6. Pharmacology of Dichloroacetate
7. Initial Events in the Pathogenesis of Insulin-Dependent Diabetes Mellitus
8. The Molecular Basis of Human Growth Disorders
9. Assessment of Behavior Problems in Persons with Mental Retardation Living in the Community

10. Effects of Inborn Errors of Metabolism on Pregnancy Outcome

11. Biobehavioral Foundation of Language Development

12. Exchange of Information on Research Program Projects on Dyslexia

13. Generalizing from Experience: An Issue of Development

14. Issues of Reliability and Validity of Measurements of Risk Behaviors Associated with HIV Infection Among Adolescents

15. Socialization of Emotion

16. Social Influences on the Development of Children's Practical Intelligence

17. AIDS Research Planning Workshop

Genetics and Teratology Branch

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OVERVIEW

The Genetics and Teratology Branch of the National Institute of Child Health and Human Development coordinates a program assessing the mechanisms of both normal and abnormal development. The program is closely integrated with the Institute's overall efforts in maternal and child health and focuses on all stages of development, beginning with early embryogenesis, in order to define the underlying principles that direct normal and aberrant patterns of growth and differentiation. To achieve this goal, basic science studies not only in man but also in experimental model systems are supported. These basic studies are supplemented with clinical and epidemiological research. Research training in developmental biology and related biomedical areas is an important corollary activity of the program.

Human development, like all biological development, is a continuum. Beginning with the differentiation of male and female germ cells, followed by fertilization, the developing organism proceeds through a highly coordinated and orchestrated series of developmental events, which ultimately yield the mature adult. Any alterations in this complex series can result in developmental defects, which limit the potential of the mature individual. Such defects are broadly defined as structural, functional, or biochemical anomalies found in the human organism that are expressed prior to birth or shortly thereafter, causing immediate or delayed abnormality. Thus, in attempting to understand normal development and explain how developmental defects can occur, it is necessary to study developmental events at early stages of intrauterine life. To succeed in this task, studies which focus on normal biological development, abnormal development, and experimental teratology are all considered important areas of investigation. Such combinations of studies can successfully expand our understanding of congenital malformations. Faulty maternal or paternal health that would influence germ cell differentiation, the occurrence of specific mutant genes, chromosomal aberrations, environmental toxins, or other epigenetic events leading to both genetic and physiological abnormalities are all considered in our search for the causes of developmental defects.

The Genetics and Teratology Branch is divided into four overlapping but complementary component research areas. Therefore, the various activities of the program can be viewed together as an overall effort. These areas include (1) Developmental Genetics (to determine hereditary influences and identify developmentally regulated genes that direct normal human development and developmental disorders); (2) Developmental Biology (to determine underlying

mechanisms at the cellular and molecular levels against which aberrations of the process can be understood); (3) Teratology (to identify and assess mechanisms by which developmental aberrations are produced); and (4) Developmental Immunology (to understand the maturation of the developing immune system as well as adverse pregnancy and early postnatal outcome produced by immunologic responses or immaturity of the defense system).

PROGRAM ACTIVITIES

FINANCIAL SUPPORT

As of August 1988, the Genetics and Teratology Branch funded a total of 372 projects at a level of nearly $52 million (table 2). By far the largest support was provided for research in developmental biology. Because of new technology, especially recombinant DNA methodology, we anticipate an opportunity to foster even stronger programs of research for the future in the biomedical areas and in developmental biology and developmental genetics.

RESEARCH TRAINING

The Genetics and Teratology Branch provides training support through two funding mechanisms: (1) National Research Service Awards granted to institutions for support of both predoctoral and postdoctoral research trainees selected by those institutions (T32s and T35s); and (2) National Research Service Awards granted to individual postdoctoral fellows seeking full-time research training with a trainer of their choice (F32s). As of August 1988, the Branch was supporting 23 institutional training awards and approximately 23 individual training awards. The largest portion of the training support is in the area of developmental biology.

CONTRACT PROGRAM

A contract was awarded to the American Type Culture Collection (ATCC) in September 1985 to establish a repository of human DNA probes and chromosome-specific libraries as a centralized national and international resource. This resource is providing a reliable and efficient means for researchers to exchange cloned human DNA. The repository is a central storage and processing facility where well-characterized human DNA probes, collected from investigators in the scientific community, can be expanded, verified, and stored in multiple samples for distribution to other investigators working in the research fields of genetics and human genetic disease research. Quality control is maintained, and the probes deposited in this facility emphasize relevancy to human genetic disease. Representative chromosome-specific genes/probes are being acquired to span each individual chromosome, and the probes will represent important genes, polymorphisms, disease, and significant chromosomal locations for genetic linkage analysis. In addition to the DNA clones, the Division of Research Resources is providing funding to this contract for distribution of chromosome-specific libraries being constructed at the Lawrence Livermore and Los Alamos National Laboratories through funding by the U.S. Department of Energy. Over 550 probes have now been accessioned. There are over 1,400 registered users of the

Table 2

NICHD GRANTS AND CONTRACTS ACTIVE DURING AUGUST 1988

GENETICS AND TERATOLOGY BRANCH

Funds (in thousands)

Health Area	Total		Research Grants						National Research Service Awards		Research Contracts	
			Total Research		Research Projects (Incl.P01)		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	372	$51,950	315	$46,369	286	$44,674	29	$1,696	46	$3,295	11	$2,286
Clinical Genetics	26	4,159	24	3,817	22	3,687	2	130	-	-	2	342
Basic Developmental Genetics	91	10,430	75	9,826	64	9,229	11	597	15	499	1	104
Early Embryonic Development	85	11,659	71	10,045	65	9,743	6	303	14	1,614	-	-
Developmental Neurobiology	30	3,613	28	3,582	25	3,413	3	169	2	31	-	-
Limb Bud Development	13	3,103	12	2,835	12	2,835	-	-	-	-	1	268
Chondrogenesis	11	1,774	11	1,774	10	1,697	1	77	-	-	-	-
Myogenesis	12	1,315	10	1,270	9	1,216	1	55	2	45	-	-
Teratology	47	7,253	38	5,927	38	5,927	-	-	5	536	4	790
Ontogeny of Immunity	22	2,424	15	1,969	13	1,828	2	141	7	455	-	-
Neonatal Infection	18	3,915	14	3,020	13	2,943	1	77	1	115	3	781
Immunology of Breast Milk	4	452	4	452	4	452	-	-	-	-	-	-
Reproductive Immunology	13	1,853	13	1,853	11	1,706	2	148	-	-	-	-

Notes: 1) Excludes the Scientific Evaluation Grants.
2) The Minority Biomedical Support Grant (S06) is included in the research projects.
3) Excludes seven grants and four contracts funded from sources other than NICHD extramural funds.
4) Columns may not add to total due to rounding.

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repository, and over 3,500 clones have been distributed as well as 1,050 chromosome-specific libraries. Future clone acquisitions will concentrate on those most important to human genetic disease and well-spaced coverage of the entire genome.

Another service is provided to the scientific community through a contract with The Johns Hopkins University for the Developmental Studies Hybridoma Bank, which was awarded in February 1986. The bank now contains a collection of 45 selected, published, hybridomas and monoclonal antibodies available for limb development studies and other topics in developmental biology. Fusions for at least two new hybridomas each year are performed by the contractor, and solicitations for donations of additional published hybridomas from other investigators continue. Antibodies for limb studies, especially those markers that allow investigation of the early limb bud, are being emphasized. Antibodies for examination of nerve contributions to limb development, the vascular involvement in this process, and the mechanisms underlying limb regeneration are either considered for addition or have already been submitted to the bank. Furthermore, antibodies that can be used for cell lineage studies, not only of the early limb bud, but also of the beginning nervous system and the early mammalian embryo as a whole will be added to the repository in the future. The addition of the non-limb reagents is consistent with the broadening scope of the hybridoma bank envisioned by the NICHD. Therefore, the future emphasis will concentrate on hybridomas relevant to early embryonic primordia.

Two additional contracts are being funded to produce animal models for neural tube defects. One of these involves a dominant gene at the t-locus in the mouse, T^{Cu}, which produces homozygotes that provide a model of extreme failure of neural tube and axial development. T^{Cu}/T^{Cu} embryos are dramatically abnormal by 10 days of gestation, with the neural tube completely open from the cervical through the sacral region, and die by the next day. These embryos are morphologically identifiable by 8 days of gestation and occur at a frequency of 25 percent.

Mice that are heterozygous for T^{Cu} and the recessive t-complex "tail interaction factor," tct, are tailless and consistently (100 percent) show small sacral meningoceles or myelomeningoceles at birth. Such mice usually have some degree of hind-limb paralysis, urinary retention, and fecal impaction, and most die within 3 days to 2 weeks after birth. They can be identified morphologically as early as 12 days gestation, and their incidence in litters from $T^{Cu}/+$ X tct/tct parents is 50 percent. These models should be useful in studying the etiology of neural tube defects, and also (in the case of T^{Cu}/tct animals) in obtaining information on secondary effects. The detailed characterization of these models should enable better understanding of abnormalities in the process of neurulation, mechanisms of cell shape change, mechanisms of cell movement, roles of cell surface and extracellular matrix, contributing effects of other tissues, processes of cell proliferation and death, expression of cell phenotypes, and genetic and environmental factors. Breeding nuclei of $T^{Cu}/+$ and tct/tct animals were produced as part of this contract, and their availability to the scientific community has recently been advertised.

The other contractor will develop and characterize an animal model for neural tube defects using curly tail and the T (Brachyury) and culturing the mouse embryos <u>in vitro</u> to allow identification of future abnormal embryos, and the principal investigator will provide these animal models to other investigators wishing to carry out further studies of neural tube defects. The investigators will further look for extracellular matrix abnormalities in these developmental models. In

a model analogous to human diabetic pregnancy and to evaluate this model for enhancement of the neural tube defect. If extracellular matrix abnormalities are encountered, perturbation experiments will be used to reproduce the mutant phenotype in normal embryos or prevent the abnormality in future abnormal embryos. These studies are now under way, and the methods are being developed to identify early the affected mutant embryos.

RESEARCH HIGHLIGHTS

When reviewed in its entirety, the program of the Genetics and Teratology Branch clearly demonstrates the importance of basic biochemical and molecular research in providing solutions for developmental problems. A major priority of the Branch is research on structural defects, with special consideration for studies of normal and abnormal limb development as well as neural tube defects. In addition, normal and aberrant early embryo development, the bioeffects of ultrasound on the developing organism, developmental neurobiology, morphogenesis and malformations of the skin, immune system development, neonatal infections, immunodeficiency diseases of the fetus and newborn, developmental genetics, and gene transfer studies have all been identified as research emphasis areas. The following are research highlights and progress being made in our four program categories: (1) Developmental Genetics, (2) Developmental Biology, (3) Teratology, and (4) Developmental Immunology.

DEVELOPMENTAL GENETICS

Our understanding of human development and the causes of developmental defects and congenital malformations requires an understanding of the normal regulation of gene expression and the nature of regulatory controls that, when inoperative, can lead to abnormal development. Several strategies are currently being used to expand our understanding of developmental gene expression. The area that has had the greatest recent impact is the application of molecular genetic techniques to almost all aspects of developmental genetic studies. Clinical effects of mutations in single genes or gene families as well as gross chromosomal aberrations are being further described and the biochemical and molecular nature of these mutations are being uncovered. Family studies focusing on inheritance patterns and recurrence risks of specific developmental anomalies, and population genetic studies assessing both the frequency and distribution of specific developmentally regulated genes and their abnormal homologues are being pursued. Studies using twins and specific animal models are being conducted to define the extent of environmental influences on developmental gene expression.

Since development is a continuum, it is not surprising that maternal factors contained within the oocyte prior to fertilization are important in regulating the expression of the embryonic genome during the earliest phases of embryogenesis. The specific genes that are expressed and the molecular nature of the factors that regulate their developmental expression are critical issues to be addressed. In addition to the genes expressed early in embryogenesis, it is equally important to identify, structurally and functionally, the genes expressed during the cascade of events that ultimately leads to the development of a mature adult.

Clinical Genetics

Research teams are currently investigating the human genome at several different levels. Much recent success has been seen in using molecular genetic approaches to dissect the molecular nature of certain genetic diseases. Basic science studies have been applied to clinical investigations that address the genetic basis of identified inherited diseases.

Ribosomal DNA represents a unique type of DNA that has maintained a high degree of conservation over evolutionary time. In humans, ribosomal DNA represents a family of human genes currently being investigated in great detail. These genes are members of a repetitive gene family located on five nonhomologous chromosome pairs. Information generated about recombination of genes on nonhomologous chromosomes will be valuable in assessing the extent to which this phenomenon contributes to human birth defects. Even more intriguing is the evolutionary history of this interesting organizational arrangement.

Several other research groups are investigating the relationship between structural anomalies on human chromosomes, particularly the X and Y sex chromosomes, and specific functional aberrations. Of particular interest are several different families of repeated DNA fragments. One such family (DXZ1) has been implicated as the putative X chromosome inactivation center. A second family, termed b2-Aline, has been localized on both X and Y chromosomes. Both gene families could prove valuable in providing new information about the structural organization of the human genome.

Research to determine the particular portion of the Y chromosome that defines the male-determining region is being carried out by constructing deletion maps of the human Y chromosome. Such studies demonstrate that the male-determining functions of the Y chromosomes unambiguously map to the most distal interval of the short arm of the chromosome. Recent developments have included the actual identification, cloning, and sequencing of the testis-determining-factor gene. Analysis of the nucleotide sequence for this factor suggests that it is a zinc binding protein that can interact with nucleic acid in a manner that could either positively or negatively regulate gene transcription. In addition, a related gene on the X chromosome has also been identified, suggesting that there is fairly regular genetic interchange between the X and Y chromosomes and indicating a certain level of homology between these two genetic structures. Such homology has interesting evolutionary implications.

Studies are being pursued which investigate the molecular characterization of specific human gene loci associated with inherited human diseases. A cDNA clone for von Willebrand factor (vWF), the absence of which leads to a mild bleeding disorder, has been cloned. This cloned cDNA has been used to cytogenetically localize the gene for this factor on chromosome 12. A putative cDNA for the gene mutated in X-linked chronic granulomatous disease (X-CGD) has been cloned, and the gene has been localized to the short arm of the X chromosome. Analysis of the X chromosome encoded phosphoglycerate kinase (PGPK) gene has proven valuable. The entire gene has been cloned and sequenced. Analysis of the 5' and 3' flanking regions of the gene indicate interesting and opposite methylation patterns in the active and inactive X chromosome. This suggests that such methylation may represent a means of pattern fixation for gene expression. This is consistent with studies in nonhuman mammalian species (i.e., mice), which indicate that methylation is an important mechanism for genetic imprinting.

Basic Developmental Genetics

The universality of the genetic code implies that much about the expression of human genes during development can be understood through investigation of many different animal model systems. As a result, the NICHD supports studies in basic developmental genetics that incorporate observations from these many different experimental systems.

Recent studies have identified a small gene family encoding calcium-binding proteins expressed during sea urchin embryogenesis. Since a role for calcium is becoming increasingly apparent during human development, it will be valuable to investigate possible similarities between the sea urchin calcium-binding proteins and any human homologues. Attempts to interfere with the appropriate expression of this class of proteins using either antisense mRNA or specific antibodies will be particularly valuable in identifying cellular functions for these proteins.

The fruit fly has for years been the workhorse for studies in developmental biology and has consistently proven to be a versatile and important experimental system. Integrated studies looking at both oogenesis and early development have been valuable in identifying molecular components responsible for directing early embryological events. Studies specifically addressing the determination of germ line cells have shown that maternally derived genetic information is critically important in directing the determination and subsequent differentiation of embryonic germ cells. Monoclonal antibody studies, coupled with *in situ* hybridizations, have proven valuable in identifying the molecular nature of such maternal factors. The genes for these maternal factors have been identified and now can be analyzed for their developmental expression.

One of the most important breakthroughs in explaining fundamental mechanisms directing developmental processes involves the isolation and characterization of body segmentation genes. Several gene families that contribute to this developmental process have been identified. Consistent among all of them is a highly conserved 180 base-pair sequence of DNA termed a "homeobox." Of particular interest is the observation that homologous homeobox sequences have been identified in a wide spectrum of species, including frog, mouse, man, and even sea urchin (a nonsegmented animal). These observations further support the contention that important advances in our understanding of human development can be gained by investigating other experimental systems. Researchers are currently investigating homeobox-containing gene expression in several different experimental systems. Studies of the bithorax complex in *Drosophila* include identifying new morphogenetic functions for these homeobox genes and correlating those functions with genetic and molecular maps. Studies in the frog, *Xenopus*, focus on ascertaining the developmental function of homeobox-containing genes. Because large quantities of *Xenopus* embryos are readily available during early stages of embryogenesis, these studies will be particularly valuable in identifying a role for homeobox genes during early vertebrate development. Microinjection studies using both homeobox gene sequences and their equivalent gene products will be valuable in elucidating the specific function of these genes.

Similar studies are being conducted in mammalian species, particularly mouse embryos and embryonal carcinoma cells. In the mouse, there seems to be a number of different homeobox gene families, each of which has several members. Their relative developmental and tissue-specific expression should provide important insight into our understanding of mammalian development.

Recent developments in the production of transgenic mice have been particularly valuable in addressing the questions of temporal and spatial developmental gene expression. Several NICHD-supported investigators have played pioneering roles in the development of these technologies. One such technique involves the microinjection of specific pieces of cloned DNA into the pronucleus of mouse embryos. Such microinjected embryos can subsequently develop into term animals and be analyzed for the expression of the exogenous piece of DNA. A second valuable technique involves the use of specific retrovirus vectors. Such genetically engineered pieces of DNA can infect embryos and subsequently integrate into the mouse genome. Both techniques have been extensively exploited to introduce foreign DNA into an in vivo developmental system. In addition, both techniques can serve as a means of generating new mutations (insertional mutagenesis), caused by the incorporation of the foreign DNA into a developmentally sensitive integration site. Improvements in the ability to control homologous recombination should soon make it easier to direct the site of integration of the exogenous transgene. Transgenetics, as it has become known, has allowed the in vivo dissection of functionally important DNA sequences and enhanced our understanding of what structural features of the genome are important for accurate tissue-specific gene expression.

One specific example of transgenesis involves the generation of a unique mouse line that shows abnormal development of the foot. Since the exogenously introduced transgene has presumably integrated into a region of the genome that is necessary for normal foot morphogenesis, it will now be possible to recover that region of the genome and investigate its structure and regulative properties.

While attempts to generate new developmental mutants are valuable, such mutants already exist and are currently the focus of intense investigation. One class of such developmental mutants, called T/t mutations, are also being studied. Many different examples of this class of developmental mutation in the mouse exist, each of which expresses its developmental anomaly in a different temporal sequence. The T region of the mouse genome, which is on chromosome 17, has been cloned. Current studies are attempting to construct a detailed molecular map of genomic DNA sequences of t-haplotypes.

Limitations in the quantity of available experimental material have for years made direct experimentation on mammalian embryos difficult. While such limitations are becoming of less concern, valuable studies using murine embryonal carcinoma (EC) cells and embryonic stem (ES) cells as model systems for mammalian embryogenesis are currently being performed by several research groups. One such group is focusing on the expression of cellular proto-oncogenes during differentiation. Recent observations correlating proto-oncogene expression with growth factors and growth-related functions suggest that the regulation of such molecules is important during embryonic development. Previous studies have already demonstrated that there is variability in the levels of c-fos expression in embryonic tissues, and further investigation of how these differences reflect functional changes will be particularly valuable. Other investigators have identified several specific genes and are currently investigating how these genes are regulated during retinoic acid (RA) induced differentiation of these cells.

DEVELOPMENTAL BIOLOGY

Developmental genetic studies are complemented by studies in developmental biology, which put into perspective how individual cells divide and interact to produce a whole organism. These studies focus on cell-cell interactions, cell lineage relationships, the ability of cells to interact and form unique and biologically functional structures, and cell movement during development.

Early Embryo Development

The establishment o. morphological form is a question being addressed by several investigators. One group is using the unicellular ciliate Tetrahymena as an experimental model to explain intracellular positional information and to investigate how this positional information is expressed in both wild type and mutant cells. Monoclonal antibodies are being used to characterize specific cell surface antigens that reflect different positional values. A second group is looking at skeleton formation, using the sea urchin as a model developmental system. Studies currently under way are using both biochemical and immunological methods to investigate the interaction of mesenchymal cells to the substratum. Studies are currently under way to explore the role of calcium gradients in establishing pattern formation during early development, including attempts to measure actual calcium gradients in several different experimental systems. Additional studies using Xenopus oocytes as an experimental model system are addressing the specific issue of calcium channels, including attempts to clone the gene for the calcium channel protein. Evidence indicates that the protein itself may be a phosphoprotein, suggesting that phosphorylation states may have regulatory significance.

One of the major unanswered questions in developmental biology is the question of determination--how genetically identical cells become committed to different fates. This issue is being addressed in several different ways. One investigator is utilizing the interstitial cell population of the lower eukaryotic organism Hydra to investigate stem cell commitment during differentiation. A particularly novel aspect of these studies includes attempts to create a transgenic Hydra. Cells from such a transgenic organism could serve as excellent markers in chimeric aggregates and could be used to investigate the basis of the position-dependent pattern of differentiation in Hydra. Recently, fluorescent beads have been used to mark and trace individual cells during differentiation. Studies on how cell fates are determined in animal development are also being carried out in the nematode C. elegans. During early development in this organism, determination is directed by internal cues. Later in development, positional cues and cell interactions play an important role. Studies are currently under way to isolate and characterize mutant organisms that express different classes of pattern defects during early development.

Cell lineage studies are critically important in expanding our understanding of how different cell types are generated during development. Before one can investigate the expression of specific developmentally regulated genes in a particular cell phenotype, it is important to establish what the lineage history of that cell type is. One research team is using monoclonal antibodies to identify cellular subsets in a heterogeneous population of Hydra I-cells. Relatedness of these subpopulations will be determined by establishing overlap of paired antibodies. Studies in the sea urchin will use both cDNA clones and monoclonal antibodies to detail mesenchyme differentiation from micromeres. Mesenchyme cells

will differentiate in vitro, and the extent of autonomous versus interactive mechanisms that direct cell fate will be examined. Similar types of studies are being performed using the leech as a model experimental system.

A research area that is currently the focus of broad investigation involves the role of cytoplasmic determinants in development. Several diverse aspects of this issue are currently being supported. One study uses ascidian embryos as an experimental model. These studies are looking at both the localization and the temporal expression of muscle specific determinants, with the hope of functionally characterizing such a determinant. A second series of studies, using the same model system, suggests that mRNA molecules play a role in determining cell differentiation. Such studies are examining the distribution of specific mRNAs in the cytoplasm by in situ hybridization. In addition, the actual genetic regulation of these mRNAs is also being pursued. Studies using the nematode are aimed at determining the nature and function of P granules, already identified as putative cytoplasmic determinants, by using monoclonal antibodies and isolating cDNAs for P granule antigens. Attempts to influence the function of P granules by introducing antibodies to these molecules into embryos, isolating P granule-deficient embryos, and introducing P granule antisense sequences into embryos will be used to clarify the functional significance of these molecules during development. Additionally, a nematode transfection system is being developed in order to introduce exogenous genetic material into nematodes. Xenopus blastmeres are currently being used to characterize the nature of maternal factors, particularly RNAs, in directing differentiation. Such researchers intend to isolate and characterize oogenetically synthesized RNA and establish which RNAs are morphogenetically active. Interestingly, recent studies indicate that mammalian growth factor-like molecules play an important role during amphibian development. This observation suggests that growth factor-like molecules may play a more universal role in the development of many different organisms. Using mouse embryos as a model system, one group of investigators is addressing the role that cytoplasm plays in influencing the success of early mammalian development. Recent results suggest that mammalian development is obligate for both male and female genetic material since imprinting restricts the subsequent expression of certain transmitted DNA. The ability to specifically identify mammalian cytoplasmic determinants, either biochemically or genetically, is currently quite limited, but is clearly a long-term programmatic interest of the Genetics and Teratology Branch.

Limb Development

Studies of the developing limb bud examine the acquisition of positional information and the subsequent spatially organized expression of various connective tissue patterns. Maturation of cartilage and bone is examined in terms of chondrocytes and their surroundings as development proceeds. Muscle maturation studies examine genetic and nongenetic regulatory mechanisms that allow expression of different muscle fiber types. Studies of cell and tissue interactions underlying pattern formation have examined an ionic current that leaves the embryo where limbs are going to arise. Other studies have shown that isolated cells from the growing limb bud have a long-term positional memory which they retain when transplanted into a different region of a new limb. Sources of this positional information are being examined. One series of experiments has provided information on the involvement of cell surface and extracellular matrix constituents in mesodermal cell and epithelial-mesenchymal tissue interactions that confer upon mesodermal cells positional information necessary for proximo-distal outgrowth of the limb bud. Other experiments have pointed to a series of possible endogenous

signaling molecules from a "polarizing zone" of mesenchymal cells at the posterior margin of the limb bud. Molecules, such as retinoic acid (Vitamin A), are being tested for a role in the cellular interactions that provide positional information for antero-posterior limb growth.

Cartilage development studies have generated cell-specific markers (monoclonal antibodies) that recognize precartilage aggregates and that can serve as tools for the examination of chondrogenic precursor cells. Additional markers will be developed to examine other mesenchymal cells and cells of even earlier developmental stages in order to trace the origin of the chondrogenic and myogenic cell lineages. Other investigations are being conducted to determine various epigenetic influences essential for the transition of mesenchymal cells to cartilage, e.g., the round shape of mesenchymal cells, endogenous prostaglandins and cAMP, low extracellular hyaluronate levels, and synthesis and secretion of extracellular matrix molecules by developing chondrocytes. These examinations suggest that the specificity of proteoglycan aggregates from chondrocytes compared to precartilaginous cells is determined by the core protein of proteoglycan monomers. Investigation of collagen gene regulations provides insight into possible regulatory steps of collagen I and II genes and into the mechanism of the collagen I to II gene switch that may operate during the mesenchyme's transition to cartilage. These studies are also characterizing a new type X collagen molecule that is synthesized by the most mature chondrocytes and may have a role in the calcification of bone. Muscle development studies have identified a specific cell surface antigen and have examined the synthesis/degradation of cell surface components involved in the myoblast to myotube fusion regulating the expression of muscle-specific genes These investigators also examined the influence of the developing peripheral nervous system and muscle-specific genes. While current results of research on gene organization and regulation place muscle gene regulation primarily at the transcriptional level, it has been observed that in the case of heavy chain myosin, protein synthesis might be controlled at a post-transcriptional level by two small RNAs whose sequences show striking homologies with a small nuclear RNA, which is believed to be involved in RNA splicing.

Nervous System Development

Studies of the developing nervous system address a number of key developmental processes that are important in the maturation of this complex organ system. The mature nervous system is architecturally highly complex and contains an extremely large number of nerve cells, all of which make very specific connections. How the ordered structural arrangement of the neurons and their functional connections become established during embryonic development are two major questions being studied by NICHD-supported investigators in a number of animal models. One particular area of research examines how nerve pathways are set up between the retina and the visual centers in the brain analyzing what role cell surface recognition molecules play.

Other important investigations of the developing nervous system pursue the establishment of neuronal cell lineages from the primitive ectoderm. Using novel marking techniques, embryonic neuroblasts have already been identified and isolated at the gastrula stage and immunochemical probes have been prepared which can identify specific neuroblasts as they differentiate *in vitro* and *in vivo* into recognizable neural nets.

Formation of the neural tube, the rudiment of the central nervous system, occurs prior to the migratory events that are being investigated for some cell types of

the developing nervous system. A series of studies has established that neural tube morphogenesis occurs in two distinct phases, of which the primary one (primary neurulation) can be classified into three steps that establish the neural tube and much of the spinal cord. Closely related to this process is the initiation of the neural crest as thickening of the neural folds, which oppose each other for neural tube closure. After migrating from the neural tube, the neural crest then forms the peripheral nervous system in addition to other structures. Characterization of the process of secondary neurulation, which completes the spinal cord at its caudal portion, revealed contributing morphogenetic events which were different from those of the primary process.

To examine how neural crest cells differentiate into distinct cell types, two approaches are being used by NICHD-sponsored investigators. One group is transplanting neural crest cells from one region of the embryo into another to determine whether the environment plays a role in the final choice of transmitter phenotype. Transplantations of neural crest cells between avians and the mouse have shown that environmental cues are similar in both species and are capable of eliciting the appropriate final neuronal phenotype. Since the intact embryo is far too complex to allow much insight into the cellular and molecular mechanisms that regulate neural crest differentiation, an in vitro culture system has been developed. This method has shown that cloned, early crest cells, at the onset of their migration from the developing neural tube, are capable of giving rise to both neurons and pigment cells from presumptive neurons to melanocytes. Furthermore, melanocytes are able to undergo terminal differentiation early in embryonic development whereas future neuronal cells must undergo additional cycles of cell division before they can engage in differentiation.

Limb Malformations

Studies of developmentally caused limb malformations are important since these abnormalities constitute birth defects that occur in at least 1 out of 200 newborn infants and pose severe lifelong suffering and morbidity for many afflicted individuals. The etiology and underlying mechanisms through which many of these defects are produced are not known. Mechanisms which underlie abnormal limb formation are studied by investigators for a group of human-inherited connective tissue disorders, the chondrodystrophies. In these conditions, normal bone ossification and subsequent linear growth of skeletal structures is disturbed. To date, it has not been possible to effectively study formation and function of the growth plate involved in this bone ossification and elongation process. This is due to the fact that growth plate tissue does not lend itself easily to laboratory study because of its partially mineralized nature and its structural and biochemical heterogeneity. The difficulties have now been overcome with newly developed techniques, which have already shown the enormous complexity of the developing growth plate and have made investigation for linear bone growth and its abnormalities feasible. The studies under way will now include examinations to determine which events of normal endochondral ossification and growth plate formation are disturbed. The cartilage matrix that these chondrocytes produce and secrete will be examined at different stages of chondrocyte proliferation and differentiation in terms of major and minor cartilage collagens, proteoglycan substructures, and other matrix constituents. These investigations of normal growth plate development are already providing evidence which could potentially be exploited therapeutically in the chondrodysplasias.

Ultrasound

Although the benefits and advantages of diagnostic ultrasound are widely appreciated and ultrasound is accepted as an indispensable prenatal diagnostic tool, it is not clear from clinical, epidemiological, and animal as well as cellular studies of the past if the use of diagnostic ultrasound during pregnancy is free of risks to the developing fetus. There is little information available on long range and subtle effects. The Genetics and Teratology Branch funds work designed to provide better insight into potential effects on the developing fetus and to examine more precisely the employed ultrasound exposure conditions. These studies, to date, have determined relevant field parameters of ultrasound imaging devices, which will permit adjustment of equipment to meet certain output-intensity criteria. They have attempted to separate thermal from radiation effects and have developed anesthetic and chamber conditions for animal and cell suspension exposures. Studies of B-cell development have, to date, shown no significant effects of ultrasound radiation at the intensities and frequencies tested, nor have adverse ultrasound effects been observed in investigations of axonal outgrowth during gestation or on behavioral parameters in the postnatal period. Work is also under way on exposure of preimplantation embryos, followed by fetal morphological examinations during the midgestational period, as well as by determinations of the fetus' variability rates examined at birth. Morphological changes are further being determined after exposure at later gestational stages, and work in progress is designed to yield evidence of potential ultrasound damage to the developing reproductive system.

DEVELOPMENTAL IMMUNOLOGY

A breadth of application of immunology to the biological development of the fertilized human ovum, embryo, fetus, and newborn is demonstrated in five research categories. Studies of the ontogeny of immunity seek errors in maturation that lead to mild as well as severe immune deficiency states in man. Other investigations evaluate decreased immunologic competence associated with malnutrition in infants. Studies on the immunology of breast milk suggest that colostrum and milk may contribute protective anti-infectious components to the newborn. Studies of neonatal infections focus on immaturity and developmental deficiencies in body defenses against specific organisms. Researchers in reproductive immunology are looking for maternal-fetal immunologic mechanisms that protect the fetus from a potentially harmful maternal immunologic environment. Close attention is also given to aberrations in biological processes caused by specific immune reactions capable of contributing to birth defects.

Ontogeny of Immunity

Investigators have very successfully analyzed genetic mutations to arrive at an understanding of how genes function. It has been especially informative when a single gene for some polypeptide has a series of alleles in which many mutations have occurred. Mutant genes for structurally defective adenosine deaminase (ADA) or genes that change the amount of ADA per cell also represent an important genomic region to pursue. Cell lines from 19 different ADA-deficient children are available to one laboratory, and these lines are providing a readily available source of mutant DNA, RNA, and protein. The majority of these children are profoundly immunoincompetent and have a lethal disorder called severe combined immunodeficiency disease in which the metabolic abnormalities kill lymphoid stem cells.

Hereditary lack of ADA is the most common cause of severe combined immunodeficiency. The complete nucleotide sequence of the normal human ADA gene has been achieved, and the sites of mutation in four different alleles from patients have been described. Ongoing work will attempt to explain cellular ADA deficiency in molecular terms to link the changes in the structure and expression of the ADA gene to changes in the structure and/or amounts of ADA mRNA and protein. One team will clone and determine the nucleotide sequence of the regions of the genomic DNA that encode the mRNA containing a splicing defect in an ADA-deficient child. The team also plans to identify more precisely the location of the elements that are responsible for negative regulation of the human ADA gene.

Additional studies are designed to provide a molecular analysis of structure, organization, and polymorphisms of genes within the major histocompatibility complex (MHC) on the short arm of human chromosome 6. One such project is concentrating on the Class III MHC genes, specifically C4, C2, and Bf. Because of the extraordinary polymorphism of these genes and linkage with a large number of congenital and acquired human diseases, this region of the genome has been very informative. The Class III MHC complex contain genes that encode proteins of the complement system. Availability of recombinant DNA probes for the complement genes and their products, as well as methods for analysis of the expression of complement genes in tissue culture, has provided a description of the molecular defects in several of these disorders.

Neonatal Infections

Infectious diseases continue to be the most important cause of morbidity and mortality in infants and children. Investigators indicate that each year, in the United States alone, there are approximately 15,000 cases of bacterial meningitis and many more cases of nonmeningitic systemic bacterial diseases in children. Case fatality rates of 5 to 15 percent are reported for bacterial meningitis, and chronic neurological sequelae occur in 30 percent. Infections in newborn infants occur in 1 to 10 cases per 1,000 live births, the rate being highest in low-birth-weight premature infants.

The NICHD is funding studies of infections in infants using molecular immunobiological techniques to isolate and identify specific surface components or products of bacterial cells to better understand their role in infectious diseases of infants and children. Such studies include how microbial structures bind to receptors on host cells, cause an inflammatory response in animal models, or induce an immunologic response in congenitally infected fetuses, neonates, infants, children, and their mothers.

Cytomegalovirus (CMV) remains the most important cause of congenital viral infection in the United States. Greater than 90 percent of primary infections in mothers during gestation are asymptomatic. Prevention of CMV infections, especially in pregnant women, is a research goal with high priority. There is a need for the development of a safe and effective vaccine. Animal models are utilized for establishing routes and methods of horizontal and vertical transmission and for evaluating CMV infections at different stages of pregnancy to determine immune development of fetuses, neonates, and adult progeny. Other studies define the molecular and cellular mechanisms involved in immune recognition and immune response to human cytomegalovirus, and clinical trials with existing vaccine and vaccine challenge studies in human populations.

The guinea pig model has been used to provide insights into host defense mechanisms, placental transfer, and guinea pig CMV genome detection in cells. Results indicate that perinatal acquisition of CMV infection produces reductions of both T- and B-cell responses to mitogens, which differed in their timing during the course of acute infection in adult, nonpregnant, female guinea pigs. Perinatal acquisition of CMV infection in the neonatal animal results in thymic atrophy, splenomegaly, and nonspecific immune depression. With use of the inbred guinea pig as a model for study of congenital CMV infection, in contrast to previous studies with outbred strains, infectious virus was recovered from placentas and offspring of mothers with primary CMV infection during pregnancy, but not from placenta and offspring of mothers that were inoculated with CMV prior to pregnancy. Histological examination showed focal necrosis and inflammation in tissues obtained from offspring of both groups of mothers. Infectious virus was detectable in fetal tissues both at the time of maternal viremia, and later during the course of maternal infection, i.e., 4 weeks postinoculation.

Reproductive Immunology

The mammalian embryo (mouse) begins life as a free-living organism that differentiates into the inner cell mass, destined to become the embryo, and the trophectoderm, which will give rise to the placenta. Scientists continue to explore the mechanisms that initiate this differentiation, with some studies supporting the concept of cell position as an important factor. These mechanisms must be functional in cells of the cleavage stage embryo, the morula, and even the early blastocyst. One team is testing the hypothesis that molecules on the surface of the cell membrane may be "recognition" molecules that act as signals for differential development and resultant cell lineages. Specifically, they wished to better understand the role of histocompatibility antigens in development and reproduction. It was learned that expression can be detected on both inner cell mass and trophectoderm. These investigators were unable to produce monoclonal antibodies even though they were able to make successful fusions with myeloma cells. This difficulty in producing antibodies to minor histocompatibility antigens has been previously reported by other scientists. In examining maternal serological reactivity to paternal alloantigens, a significant response was noted, but only about two-thirds of the females responded, and the response occurred only after two prior pregnancies. This observation indicates that there is a maternal humoral immune response to the minor histocompatibility antigens of paternal origin in the absence of an MHC disparity. This finding provides evidence that target, minor paternal antigens can be detected on the surface of the early embryo, but, further, there is a noncytotoxic maternal recognition of these molecules.

Other current studies of the pregnant and nonpregnant uterus provide an understanding of how foreign materials may be processed and how they impact on the female's immune response system. Intrauterine infection is rare, and this is considered to be due to local macrophages, immunocytes, and a secretory immune system especially evident in the cervix. Investigators point out that successful mammalian pregnancies suggest that the uterus is immunologically discriminating toward the nonharmful but biologically important antigens of classes 2 and 3. In the rat model, they have shown that endometrial lymphatics are present in the virgin uterus, but they are small and require perfusion fixation and thin section light microscopy for visualization. Following mating, lymphatics in the endometrium were considerably larger and more easily visualized. With use of labeled cell injections, it was determined that cells placed in the uterine lumen did not readily gain access to the lymphatic system. The investigators are further characterizing the distribution of Ia+ cells in rat endometrial stroma, noting

their abundance in virgin tissue and that distribution is influenced by ovarian hormones. During pregnancy Ia+ cells become fewer, and the area around the conceptus is virtually devoid of such cells. Thus, the conceptus is isolated from maternal lymphatics and from potential accessory cells at the time of implantation.

STAFF ACTIVITIES

STAFF CHANGES

Dr. Danuta M. Krotoski joined the Branch in July 1988 to assume responsibility for the research program in limb development, nervous system development, limb malformations, and ultrasound. Previously, she was at the University of California in Irvine.

CONFERENCES AND WORKSHOPS

A workshop on The Role of Cytoplasmic Factors in Development was held on September 14-17, 1988. A group of experts discussed the varied molecular nature of cytoplasmic factors, their structures and modes of action, and how they interact to influence both the genetic and epigenetic signals that drive development. They also suggested how aberrations in cytoplasmic factors could lead to congenital malformations.

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Pregnancy and Perinatology Branch

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OVERVIEW

The program of the Pregnancy and Perinatology Branch shares, within the framework of the Center for Research for Mothers and Children, the overall research efforts on maternal and child health. The main focus, as implied in the name of the Branch, centers on events occurring during normal and abnormal pregnancies, their effect upon the developing fetus, the factors responsible for the initiation of labor, and the circumstances associated with disorders affecting newborn infants. Of particular interest is research to identify the causes, mechanisms, and characteristics of infants at risk for sudden infant death syndrome (SIDS). A new and rapidly expanding area addresses issues related to maternal and perinatal acquired immune deficiency syndrome (AIDS). Another major emphasis is directed towards the prevention of low birth weight (LBW), the most important problem in maternal and child health in the United States today.

Activities of the Branch are organized around six maternal-infant emphasis areas, which complement each other and must be viewed as a comprehensive approach to research during the pre-, peri-, and postnatal periods. These are: (1) High-Risk Pregnancies: Over the last 20 years, significant progress has occurred in maternal survival and well-being, which has surpassed improvements in fetal outcome. Research efforts on high-risk pregnancy are directed towards the closure of this gap and studies of both normal and abnormal pregnancies are being continued. (2) Fetal Pathophysiology: Studies of fetal pathophysiology are examining the factors influencing normal and abnormal embryonic development at the molecular, tissue, and organ levels. Emphasis is placed on studies facilitating the assessment of fetal status to provide meaningful antenatal diagnosis. Another area of special interest examines the mechanisms responsible for intrauterine growth retardation and its sequelae, increased morbidity and mortality. (3) Premature labor and birth: Premature labor and birth are a major cause of neonatal mortality and morbidity. Two-thirds of all infant mortality occurs among infants weighing 2,500 grams or less at birth. Furthermore, the Nation's high prematurity rate is responsible for our relatively high infant mortality compared to other countries. Consequently, this program supports studies of the normal onset of labor, the reasons for premature labor, and how premature labor might be stopped without detrimental effects. (4) Disorders of the Newborn: Disorders of the newborn are responsible for approximately three-fourths of the infant deaths in the United States and produce long-term disability for many individuals who are affected by them and survive. Research directed towards reducing the impact of these disorders includes studies of maternal health problems that affect the status of the infant, adaptation of the newborn infant to its environment, and problems in the early

weeks of life that influence subsequent development and behavior. (5) The Sudden Infant Death Syndrome (SIDS): Since 1974, the NICHD has made particular efforts to encourage research on SIDS. Research has demonstrated that the SIDS infant no longer can be viewed as having been perfectly healthy prior to death, but rather is believed to have had developmental abnormalities. Current research efforts are directed towards identifying the cause or causes of these abnormalities. (6) Acquired Immune Deficiency Syndrome (AIDS): The increasing number of women and infants infected by human immunodeficiency virus (HIV) calls for research to understand the magnitude of the problem and to determine the efficacy of potential therapeutic approaches. Recognition of the importance and rapid growth in this area led to the establishment of a new branch in FY 1989, the Pediatric, Adolescent, and Maternal AIDS Branch.

PROGRAM ACTIVITIES

FINANCIAL SUPPORT

The Pregnancy and Perinatology Branch supported 304 research grants and contracts during FY 1988 amounting to $57,936 million (table 3).

TRAINING

During FY 1988, the Branch supported research training through two types of grants: Individual Postdoctoral Fellowships (F32s, F33s, F34s) and Institutional Research Training Grants (T32s). Fourteen T32s supported trainees in 11 departments of pediatrics, two departments of obstetrics, and one nonclinical department. One was in the area of high-risk pregnancy, 2 in fetal pathophysiology, and 11 in the various aspects of perinatal medicine, which include disorders of the newborn. The Branch also supported 17 Research Career Development Awardees (K07, K08, and K11).

CONTRACT PROGRAM

The Branch contract program complements the grant and centers programs and meets special needs in perinatal research.

In 1969, the NICHD recognized a growing awareness of the potential for nonhuman primates in perinatal and developmental research. A special breeding colony was established in 1971 under contract to the University of California at Davis, which provides rhesus monkeys of known medical, reproductive, and genealogical history to NICHD-supported investigators in the United States.

The NICHD in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) awarded six contracts in FY 1984 (five clinical centers [NICHD] and one data management center [NIAID]) to conduct a clinical trial entitled "Prevention of Prematurity by Detection and Treatment of Gestational Genitourinary Infections: A Multicenter Randomized Controlled Clinical Trial." The objective of these ongoing contracts is to identify specific types of maternal genitourinary infections that are significantly associated with preterm labor and birth, and to

Table 3

NICHD GRANTS AND CONTRACTS ACTIVE DURING AUGUST 1988
PREGNANCY AND PERINATOLOGY BRANCH

Funds (in thousands)

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts	
			Total Research		Research Projects (Incl.P01)		Research Centers		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	304	$57,936	256	$46,862	233	$40,791	6	$4,577	17	$1,494	20	$1,856	28	$9,218
High-Risk Pregnancy	106	17,708	90	16,084	83	14,806	1	903	6	374	6	309	10	1,315
Fetal Pathophysiology	75	13,861	69	12,747	61	10,217	3	2,203	5	326	3	263	3	851
Premature Labor and Birth	44	8,976	36	6,953	32	6,039	1	720	3	193	2	97	6	1,926
Disorders of the Newborn	64	15,367	46	9,055	42	7,705	1	750	3	600	9	1,186	9	5,126
Sudden Infant Death Syndrome	15	2,024	15	2,024	15	2,024	-	-	-	-	-	-	-	-

Notes: 1) The Minority Biomedical Support Grants (S06) are included in the research projects.
2) Excludes four grants and four contracts funded from sources other than NICHD extramural funds.
3) Columns may not add to total due to rounding.

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test whether appropriate antimicrobial therapy for such infections can reduce the preterm birth rate in women at risk. The study has a double-blind design. Results will be available when the code can be broken and results analyzed.

Several contracts in perinatal AIDS are at various stages of data acquisition. Descriptions of these studies are provided under RESEARCH HIGHLIGHTS.

COOPERATIVE AGREEMENTS

Two requests for Cooperative Agreement Applications were issued during FY 1985 inviting proposals for multicenter cooperative clinical studies in Neonatal Intensive Care Units (NICUs) and in Maternal-Fetal Medicine Units (MFMUs). They were designed to investigate the safety and efficacy of new treatmert and management strategies for the care of sick infants in the NICUs, and problems in clinical obstetrics, particularly those problems related to prevention of low birth weight in the MFMUs. The two networks, each one consisting of seven centers, began their activities in FY 1986. After developing protocols, they began their implementation during the current fiscal year. The NICUs are accumulating data on the effect of the intravenous administration of immune globulins in the prevention of nosocomial infection in the low birth weight infant through a randomized trial. A survey of morbidity and mortality among low-birth-weight infants and study of outcome and resource predictors for very-low-birth-weight infants began in November 1987. Another protocol, ready to begin, will evaluate the use of tolazoline in the treatment of persistent pulmonary hypertension in newborns.

The MFMU network has started a clinical trial on the management of post-term pregnancies comparing elective induction versus expectant management with fetal surveillance. Through the support of the NIH Nursing Research Center, two nursing studies related to post-term pregnancies are being carried out. Another protocol will evaluate the use of low-dose aspirin to prevent preeclampsia.

A third cooperative agreement, involving two centers, addresses the question of routine ultrasound screening and risk assessment during pregnancy. Enrollment of patients is ongoing, but results will not become available before completion of the study.

An innovative cooperative agreement has been awarded to the Association of Medical School Pediatric Department Chairmen (AMSPDC) to support a Pediatric Physician Scientist Program. The program involves 5 years of training--2 years of basic research training funded by the NICHD and/or other private agencies, and 3 years funded by the sponsoring institution in a junior faculty position with guaranteed 75 percent free time for research under an identified preceptor. The program is administered by a task force representing the AMSPDC, NICHD, and participating private agencies. Several fellows have entered their second year of basic training. An independent evaluation committee has been established and will analyze the performance of this program and its contribution to the existing cadre of young academic faculty in pediatrics.

PERINATAL EMPHASIS RESEARCH CENTERS (PERCs)

As an adjunct to research projects in the above six areas, the NICHD established the Major Research Programs (MRPs) in 1977 to provide a multidisciplinary approach

to important unresolved problems in perinatal medicine. The official designation of the program was changed to Perinatal Emphasis Research Centers (PERCs) during FY 1984 in order to provide consistency in nomenclature for all research center programs at the NICHD.

RESEARCH HIGHLIGHTS

PERINATAL EMPHASIS RESEARCH CENTERS (PERCs)

Two PERCs are in the area of diabetic pregnancy, one is in hypoxia and maternal smoking, one addresses the issue of prematurity and initiation of labor, and two centers support studies on intrauterine growth retardation.

PERCs in the area of diabetic pregnancies have been very productive. Untreated diabetes mellitus is incompatible with successful outcome of pregnancy. As treatment of diabetes has improved so has pregnancy outcome, and presently maternal mortality for diabetic and nondiabetic women is similar. However, maternal morbidity continues to be higher in diabetics. Fetal mortality has been reduced, but not to the level in the general population. Increased incidences of congenital anomalies, macrosomia, late intrauterine death, and respiratory distress syndrome (RDS) remain a significant problem. Pregnancy per se has a diabetogenic effect, and some women develop abnormalities of carbohydrate metabolism known as gestational diabetes mellitus (GDM).

At one of the PERCs, investigators have measured total body water (TBW) in normal and diabetic pregnancies. It may be considered that total body water reflects body composition if it is assumed that water comprises a relatively constant proportion of lean body mass and that fatty tissue contains very little water. It was found that TBW and TBW/weight ratios in diabetic and normal pregnant subjects are similar. This finding indicates that similar increases in lean body mass and fatty tissue occur in normal and in insulin-dependent diabetic pregnancies. Studies of glycerol metabolism show similar turnover rates in healthy full-term appropriate-for-gestational-age infants in offsprings of diabetic mothers, and in small-for-gestational-age newborns indicating similar rates of lipolysis. Thus, glycerol metabolism in the newborn is not affected by maternal diabetes during pregnancy or by intrauterine growth retardation.

A second PERC center examines multiple aspects of diabetic pregnancies, fetal and neonatal metabolism and growth. Studies were carried out to test the effects of hypoglycemia on neonatal brain metabolism in rats with intrauterine growth retardation. These pups are more vulnerable to birth asphyxia, and in the early neonatal period they can maintain brain adenosine triphosphate (ATP) levels in the face of limited glucose supply, probably by utilizing alternative substrate. Other studies show that macrosomic female rat pups of hyperglycemic pregnancy maintain accelerated growth and exhibit hyperglycemia when they become pregnant with enhancement of fetal growth. Study of their offsprings showed similar abnormalities in the second generation.

It is known that offsprings of diabetic mothers have increased total body fat. In studies in chronically instrumented fetal sheep, it was found that prolonged fetal hyperinsulinemia decreased circulating total fatty acids and arachidonic acid in all major lipid classes. These reductions may represent insulin-mediated increased tissue deposition.

Under the title of "Fetal Activity/Responses to Hypoxia During Development," a team of PERC investigators are carrying out many interesting studies. One project examines the effect of hypoxia on the hypothalamo-hypophyseal system during development. Studies in the pregnant human and baboon demonstrate that the placenta secretes large amounts of biologically active corticotropin releasing hormone (CRH) into both maternal and fetal circulations during pregnancy. The CRH is secreted into maternal plasma in a reproducible pattern in the latter part of normal pregnancy. This suggests that sequential measurements of CRH may prove to be of value clinically.

This team of researchers established an excellent experimental model for the production of chronic fetal hypoxia in the sheep by means of relative maternal hypovolemia. When expansion of blood volume during pregnancy is inadequate, blood flow to the uterus is adversely affected leading to various degrees of chronic fetal hypoxemia and stimulation of vasoactive mediator systems (VMS) in both mother and fetus. The elevated levels of VMS in the chronically hypovolemic pregnant ewe are, in part, of uterine origin and may serve to maintain blood pressure and/or improve utero-placental profusion. These investigators have refined a chronically catheterized baboon model, with animals conditioned to a backpack and tether system. They demonstrated that long-term observations of behavioral, physiologic, and biochemical processes in the pregnant baboon and her fetus can be made without undue restraint or labor-suppressant drugs.

Initiation of labor is being studied at another PERC. Investigators are clarifying what brings about the onset of premature labor. There is evidence signaling that in 60 percent of the cases an "infection" is present. One step essential to parturition is the robust response of the decidua including the outpouring of prostaglandins, arachidonic acid, platelet-activating factor, and selected cytokines, among these interleukin-1. This type of answer is similar to that occurring in macrophages in response to certain stimuli, one of which is bacterial endotoxin lipopolysaccharide (LPS). Indeed if the decidua is macrophage-like, then a similar response to LPS is to be expected. The investigators showed that LPS was present in the amniotic fluid of some pregnancies complicated by preterm labor even though the amniotic fluid was sterile and the fetal membranes were intact. LPS was shown to stimulate prostaglandin production in endometrial stroma cells and to act on decidua. These findings lead one to conclude that decidual activation must be a step within the parturition process.

Two PERCs are directing their efforts to the problem of intrauterine growth retardation (IUGR). Their goals are to define the basic physiology and pathophysiology of this condition. The high incidence of perinatal mortality and morbidity in the IUGR infant may result from fetal nutrient deprivation, hormonal alterations and/or hypoxia, both chronic and acute. Basic and clinical studies are examining a variety of issues that are either directly or indirectly related to the main theme. Investigators are utilizing several animal models of IUGR. In rats, they produce small fetuses by the Wigglesworth procedure. A new model of growth retardation in the neonate is obtained by injecting epidermal growth factor (EGF) In the sheep they have developed and refined the uteroplacental blood flow restriction model. Studies looking at the ontogeny of EGF receptor binding in developing rat liver show that the ability of EGF to retard somatic growth is most marked in the period immediately following birth, but this ability may be present in fetal life. A new cutaneous syndrome described in the newborn rat is characterized by a marked change in skin viscoelastic behavior which has similar developmental stage sensitivity and dose response characteristics as the

ability of EGF to retard somatic growth. Therefore, there may be a possible mechanistic connection between the EGF-elicited cutaneous effects and the means by which the growth factor retards growth. Of great interest is the finding that one of the perinatal actions of EGF is its ability to lower systemic O_2 consumption in a dose-dependent manner, which may be explained by a direct action of EGF on brown fat or an indirect action by changing the "setpoint" of cutaneous sensory afferent pathways. In the very-low-birth-weight (VLBW) infant, the bone mineral content (BMC) is markedly decreased compared to term infants in the neonatal period. Studies show that bone mineralization catches up, and BMC appears to be appropriate after 2 years of age.

The second (IUGR) PERC has established an environmental chamber in which pregnant sheep can be subjected to heat stress. Preliminary observations show that normal fetal oxygenation in sheep with adult hemoglobin A result from a high level of uterine perfusion and a small PO_2 gradient between maternal and fetal blood. It is possible that exposure of adult hemoglobin A and B sheep to high environmental temperatures will produce different degrees of fetal hypoxia and fetal growth retardation.

Other investigators in this center demonstrated that sustained maternal hypoglycemia in the sheep results in a reduction of umbilical glucose uptake and thus of the supply of glucose tc the fetus. Uteroplacental glucose consumption is also reduced and is directly dependent upon fetal glucose concentration. Fetal endogenous glucose production could be documented and provides not only for glucose metabolism in the fetus, but also for placental consumption. Fetal growth is reduced resulting in a fetal weight for gestational age less than in normal control animals.

Another area of research aims to understand normal and abnormal fetal growth, by determining how the fetus allocates limited exogenous nutrients under conditions of spontaneous and experimentally induced IUGR, and by quantifying fetal synthetic pathways which may be regulated in response to IUGR. There are two possibilities: the "sequential hypothesis" (the allocation of macronutrients follow the same pattern as a normally grown fetus, but at a slower rate); and the "parallel hypothesis" (the allocation of nutrients proceeds at a different pattern than in normal growth). Preliminary data on guinea pig fetuses support the sequential hypothesis.

HIGH-RISK PREGNANCY

As more women become more physically active during pregnancy, both recreationally and occupationally, some scientific attention is being directed at the effect of exercise on pregnancy outcome. The relationship between exercise performance during pregnancy and outcome is quite complex. It is based on the interaction between several exercise variables (exercise type, intensity, duration, and frequency), which determine the magnitude of the physiological response to the exercise, and the gestation-specific physiological changes of pregnancy. Not surprisingly, the reported impact of exercise during pregnancy on birth weight has varied from a reduction, no effect, or an increase. In order to understand this difficult area of research, one investigator studied the spontaneous daily exercise performance of 20 well-conditioned female recreational runners before and during their entire pregnancies. Exercise intensity and duration were carefully measured and correlated with newborn weight and size, and with placental weight. The hypothesis is that recreational running, via effects on blood flow distribution

and energy utilization, limits fetal growth. Results show an inverse relationship between exercise performance in the last 20 weeks of pregnancy and birth weight. Women who voluntarily continued to run in the latter half of pregnancy at more than 35 percent of preconceptual level had infants whose mean birth weight was over 500 grams less than those of mothers exercising less than that level. The difference is due to a specific decrease in soft tissue rather than in length. The weight decrement, however, is not accompanied by greater newborn morbidity or mortality. Thus, it is uncertain whether the birth weight reduction should be considered a negative effect. Larger studies are needed to clarify this issue.

Are pregnant young teenagers physiologically mature? Although several studies have inferred maternal growth between successive adolescent pregnancies, linear growth during adolescent pregnancy itself has not been documented for two reasons. First, existing instruments are not precise enough to measure short-term growth, and second, stature in pregnancy may be influenced by postural changes. To overcome these problems, one investigator has used the knee height measuring device (KHMD) to monitor the growth of the lower leg during adolescent pregnancy. This device detects small amounts of linear growth over short periods, with a reliability of 0.5 mm. Primigravidae age 12-15 years and multigravidae age 15-18 years were compared to mature controls. During an average interval of 14 weeks during the second and third trimesters, the primigravidae gained an average 1.68 mm and multigravidae, an average 1.00 mm, compared with no growth for controls. These data support the hypothesis that linear growth continues during adolescent pregnancy and that some pregnant adolescents may not be completely mature physiologically. Continuing maternal growth has implications for the well being of the mother and fetus, and may be related to the higher risk of complications associated with adolescent pregnancy.

FETAL PATHOPHYSIOLOGY

A significant number of researchers are addressing various important issues to clarify our understanding of fetal physiology. Maternal anemia, which affects up to 5 percent of all pregnancies, places fetuses at increased risk for intrauterine growth retardation, stillbirth, and newborns at risk for hypoxia. The mechanisms for these poor outcomes are not well understood, although insufficient availability of oxygen to the fetus, resulting from anemia, may be an important factor. One investigator has begun a project with the sheep model to address the following hypotheses: (1) Chronic maternal anemia reduces oxygen delivery to the fetus; (2) Reduced fetal oxygenation triggers fetal circulatory compensations that maintain normal oxygen consumption and growth; (3) When compensatory limits are reached, fetal growth is reduced and the pattern of fetal growth is altered; and (4) Fetal growth reduction results from deficient fetal oxygen delivery, by affecting protein synthesis in certain tissues. Early results document that the chronically anemic, pregnant animal model can be maintained, with appropriate sampling catheters, over at least 1-month. It appears that maternal anemia results in a reduction in umbilical blood flow that is not compensated for by enhanced oxygen uptake by the placenta. The resulting fetal hypoxia does not apparently cause fetal growth retardation if the degree of hypoxia is only moderate. However, severe hypoxia appears to cause fetal growth retardation in which the growth of the brain is affected less than other body parts. The results of this research will begin to define the limits of maternal anemia within which the fetus can maintain normal metabolism and growth.

Endogenous opiates are believed to regulate catecholamine release but scant data are available regarding fetal dose response on the effect of the opiate antagonist

naloxone on such release. One investigator studied the effect of naloxone on plasma catecholamine responses to hypoxia in chronically catheterized fetal sheep. Hypoxia increased both norepinephrine and epinephrine, and this response was augmented by naloxone only at a low dose suggesting μ opiate receptor modulation. The same team is studying the cosecretion of opiate peptides (enkephalins) and catecholamines in response to physiological stimuli (birth) by measuring their concentrations in umbilical arterial and venous plasma. Results show that total enkephalin levels are one hundredfold greater than free enkephalins, but neither correlated with Apgar scores, cord pH, or route of delivery. The results suggest that enkephalins are cosecreted with catecholamines at birth and are important in neonatal adaptation.

Towards the end of gestation, and in preparation for extrauterine life, a number of changes occur in the fetal lung. A most important one is the increased production of surfactant material which lines the alveoli and prevents their collapse at low lung volumes. Several hormones have been reported to accelerate fetal lung maturation and stimulate surfactant production. A researcher has demonstrated that dexamethasone increases the activity of fatty-acid synthetase both in vivo and in cultured lung explants, and this stimulatory effect is due to new protein synthesis. Approximately 80 to 85 percent by weight of surfactant is glycerophospholipid, and 5 to 10 percent of surfactant is protein. These two components appear to be regulated independently. An investigator is examining the effect of fetal hyperinsulinemia on surfactant, and results suggest that it may cause the production of a surfactant deficient in the major human apoprotein (35K). This may provide an explanation for the increased incidence of respiratory distress syndrome (RDS) in infants of diabetic mothers.

PREMATURE LABOR AND BIRTH

The contribution of the hormone oxytocin to the physiology of parturition is still unclear, despite the fact that much is known about it and that it is used clinically for the induction of labor. On the one hand, studies have failed to show a consistent increase in maternal plasma oxytocin levels within increasing gestation, or immediately prior to labor. On the other hand, oxytocin receptors in myometrium have been shown to increase markedly before labor, suggesting an important biologic role for the hormone in parturition. A team of investigators has been working on synthesizing a chemical analogue of oxytocin that would antagonize its biologic activity. The analogues have actually been modifications of the hormone arginine vasopressin (AVP). Certain analogues were shown to inhibit uterine contractions in vivo and in vitro in the rat model. One analogue, 2-aminoindane, 2 carboxylic acid 2, was then tested on myometrial strips from pregnant baboons and humans. Oxytocin-stimulated uterine contractions in both tissues were totally inhibited by the analogue. Reversibility of the effect was demonstrated by displacing the analogue by washing. The availability of such oxytocin antagonists holds forth the promise of unravelling the biologic role for the hormone in various physiologic processes, including parturition. Clinically, the availability of a specific oxytocin antagonist may make the management of preterm labor and oxytocin overdosage more rational and effective. This is especially important because currently available methods of labor inhibition, such as beta-mimetics, are not highly effective and have undesirable side effects.

The increased synthesis of platelet-activating-factor (PAF) measured in fetal lung tissue may be related not only to the regulation of surfactant synthesis, but may also be released from lung tissue together with lamellar bodies through in utero

breathing movements. A team of researchers is investigating the possibility that the PAF in the amniotic fluid may react with the amnion tissue, initiating the signal for prostaglandin formation. An alternative hypothesis is that PAF may stimulate directly myometrial contractions. By this proposed mechanism the last major organ system to mature, the fetal lung, would transpose a signal from lung via the amniotic fluid to the amnion to initiate human parturition.

DISORDERS OF THE NEWBORN

In 1984, the NICHD launched a major special initiative--an expanded research program in the prevention of low birth weight. This important problem in maternal and child health may be the consequence of intrauterine growth retardation (IUGR), premature labor, or both. Most infants whose birth weights are between 1,500 and 2,500 grams survive and do fairly well, but the very-low-birth-weight (VLBW) babies (below 1,500 grams) require long periods of intensive care and have high mortality and morbidity. Postneonatal deaths (death between 28 days and 12 months of life) declined from 100/1,000 live births at the turn of the century to 50/1,000 by 1950, and 3.78/1,000 in 1984. In 1984, the infants below 2,500 grams, which constituted 6.7 percent of all births, contributed two-thirds of all deaths. The VLBW group, which constituted 1.15 percent of all births, accounted for one-half of neonatal deaths. The decline in infant mortality rate since 1960 is due mainly to a decrease in neonatal mortality (infants dying between 0 and 27 days of life).

Examination of the effect of maternal transport on the outcome of VLBW infants shows a significant increase in neonatal survival, but morbidities remain high. Follow-up of these infants to school age indicate that they displayed specific learning disabilities although they were of normal intelligence.

Brown adipose tissue (BAT) is critical to neonatal survival as it facilitates temperature adaptation after birth. A researcher studying the effect of maternal diabetes upon maturation of this tissue in fetuses found a significant reduction in BAT thermogenesis. This finding results from a specific reduction in BAT uncoupling protein without reduction of mitochondria. Both BAT uncoupling protein expression and stimulation of O_2 consumption are catecholamine dependent. Thus, impaired catecholamine response by fetuses from diabetic pregnancies may underlie the impaired energy metabolism seen in this group.

Hyperbilirubinemia of the neonate continues to attract the attention of investigators, and new methods for prevention are being developed. Tin protoporphyrin, a competitive inhibitor of heme oxygenase and thus bilirubin production, is effective for the treatment of this condition. Another compound, zinc protoporphyrin, was shown to inhibit in vivo total bilirubin formation in the adult rat, as well as inhibit in vitro heme oxygenase activity in adult and suckling rat tissues.

The patterns of use and adverse reactions to medications administered in neonatal intensive care nurseries are being uncovered and described by a team of researchers. They pointed out that, over a period of 10 years, morphine sulfate use increased from a low of 2 percent to a high of 24 percent in 1985. In addition, 11 percent of medicated newborns had adverse reactions such as respiratory depression, cardiovascular signs, and urinary retention. This study indicates the specific need for increased attention to morphine sulfate-associated morbidity and the importance of a good surveillance of drugs prescribed in the nurseries.

SUDDEN INFANT DEATH SYNDROME (SIDS)

Sudden Infant Death Syndrome (SIDS) is the most common cause of death among infants between 1 month and 1 year of age. Indeed, every week more than 100 American infants die of SIDS. Formerly called crib death, SIDS is defined as the sudden death of any infant that cannot be explained by prior medical history or postmortem examination.

Biomedical research includes studies involving both infants at risk and SIDS victims, as well as experimental animals whose early development is similar to that of humans.

Arousal is an important response to prevent severe hypoxemia and death during sleep, and an investigator carried out studies in lambs to determine the factors that may impair this response. He showed that repeated exposure to hypoxemia significantly increases the time of arousal and decreases the arterial hemoglobin oxygen saturation at arousal during both quiet and active sleep. To determine if endogenous opiates may play a role in this observation, an opiate antagonist, naloxone, was administered to the experimental animals. As it did not alter the arousal response decrement caused by hypoxemia, it is concluded that endogenous opiates are not involved as a causal or facilitating factor.

Studies in newborn lambs have shown that the respiratory response to hypoxia is mediated mainly by stimulation of the carotid body chemoreceptors. In the ovine model these receptors are active in the last trimester of fetal life. Being sensitive to PaO_2 changes, the normal rise occurring at birth silences the chemoreceptors. Postnatally, the PaO_2 sensitivity is reset from fetal to adult range during the first days after birth. A research team demonstrated that the normally occurring postnatal reset mechanism of the carotid body oxygen sensitivity can be reversed by long-term exposure to hypoxia. This finding might have implications for the ability to abort apnea at a time period when carotid body is an important factor in the control of breathing.

An investigator is carrying out a major quantitative anatomical study to detect subtle developmental aberrations in the brain stem of infants who have died of SIDS. Histological preparation of SIDS and non-SIDS brain stem specimens have been used in preliminary studies. These studies have solved some major statistical and procedural issues that are fundamental to the scope and goals of the proposed project. When the SIDS/non-SIDS code is broken, these studies will be of technical importance and will provide information about the growth and development of important areas of the central nervous system.

The SIDS Alliance, in cooperation with the NICHD, is supporting NICHD-approved but not funded SIDS research. In 1987 the SIDS Alliance recommended three programs for funding. These programs are studying heart rate variability under certain experimental conditions; the development of brain centers affecting vital functions in the cat, and hypoxic arousal responses and risk for SIDS.

ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

The AIDS epidemic continues to spread. The Public Health Service projection for the year 1990 forecasts 270,000 cases of AIDS in the United States, an eventuality of staggering cost in both human and economic terms. As noted by the Centers for

Disease Control in Atlanta, Georgia, the number of women infected by human immunodeficiency virus (HIV) is increasing. The primary route of HIV infection in children is from infected mother to child, and the NICHD has undertaken a number of research projects related to HIV infection in infants, children, and mothers.

The Pregnancy and Perinatology Branch has had primary responsibility within the NICHD and the NIH for the conduct of research in the area of pediatric and maternal HIV infection and disease (including AIDS). Research and development activities include: (1) The conduct (together with the National Cancer Institute) of a multicenter study of the vertical transmission of HIV infection from mother to child. Over 200 mothers and 150 children are enrolled in this longitudinal cohort study. Preliminary data suggest that the rate of vertical transmission may be as high as 40 percent. (2) A multicenter investigation of the neurodevelopmental outcome of HIV-infected children. Over 100 children have serial, standardized measures of cognitive function. Preliminary data indicate that cognitive dysfunction is a prominent and early sequela of perinatally acquired HIV infection. (3) The conduct (together with the Prevention Research Program) of a multicenter, randomized, placebo-controlled trial of the efficacy of intravenous gamma globulin in the treatment of HIV-infected symptomatic children. (4) The establishment of a multicenter study of the effect of HIV infection on hemophiliac children, to examine the natural history of the infection in these children. This study is being conducted in conjunction with the Office of Maternal and Child Health, the Centers for Disease Control and the Prevention Research Program. (5) The support of the development of a technique allowing testing for HIV antibody in samples of blood collected on filter paper. This technique, developed and validated by the Massachusetts State Laboratory Institute, permits the study for HIV antibody in samples of newborn blood routinely collected from virtually all live-born neonates in the United States. The Massachusetts group has tested over 30,000 samples of newborn blood and has estimated a statewide prevalence of 2.1 HIV seropositive women per 1,000 women bearing live children. Prevalence varied by geographic location of delivery service with a high of 8 positive per 1,000 women bearing live children in the urban inner-city population.

STAFF ACTIVITIES

COMMITTEES

Branch staff represent the NICHD on groups and committees in both the public and private sector as follows: ex-officio member of the Maternal and Child Health Research Grants Review Committee of the Health Resources and Services Administration; representative to the Committee on Fetus and Newborn of the American Academy of Pediatrics; representative to the Committee on Obstetrics; Maternal and Fetal Medicine of the American College of Obstetricians and Gynecologists; consultant to the Board for Correction of PHS Commissioned Corps Personnel Records; member of the Trans-NIH Sleep Research Committee; and member of the Grants Associates Board. Staff were also represented on the planning committee for the Surgeon General's Workshop on Pediatric AIDS.

CONFERENCES AND WORKSHOPS

At the Annual Meeting of the Directors of the Perinatal Emphasis Research Centers (PERCs), the Branch cosponsored with the Perinatal Biology Group a mini-symposium on neural development.

The Branch presented a research planning workshop entitled "The Onset of Labor: Cellular and Integrative Mechanisms," Bethesda, MD, November 1987.

The Branch organized an AIDS Research Planning Workshop, "A Research Agenda for Pediatric AIDS," Bethesda, MD, March 30-31, 1988. The summary of this workshop will be published in the February 1989 issue of Pediatrics.

OTHER

Staff made a presentation at the Annual Meeting of the Association of Medical School Pediatric Department Chairmen. Staff continue to serve as reviewers of manuscripts submitted to leading refereed journals in pediatrics, obstetrics, and pharmacology.

PUBLICATIONS

Shiono, P.H., Fielden, J.G., McNellis, D., Rhoads, G.G., and Pearse, W.H.: Recent Trends in Cesarean Birth and Trial of Labor Rates in the U.S. JAMA, 257(4):494-97, 1987.

Shiono, P.H., McNellis, D., and Rhoads, G.G.: Reasons for the Rising Cesarean Delivery Rates, 1978-1984. Obstet. Gynec. 69(5):696-700, 1987.

Hemminki, E., McNellis, D., and Hoffman, H.J.: A Review of Prenatal Care in the United States. J of Publ Health Pol. 8:330-350, 1987.

Maulik, D. and McNellis, D. (Eds).: Doppler Ultrasound Measurement of Maternal-Fetal Hemodynamics - Report of an NICHD Research Planning Workshop. Ithaca, New York: Perinatology Press, 1987.

McNellis, D.: "Specific Federal Programs for Improving Reproductive Health Care - What Have We Learned?" In Wallace, H., Ryan, G. and Oglesby, A. (Eds.) Maternal and Child Health Practices, 3rd edition. Oakland, CA: Third Party Publishing, 1988:401-407.

McNellis, D., Challis, J.R.G., MacDonald, P.C., Nathanielsz, P.W., and Roberts, J.M.: The Onset of Labor: Cellular and Integrative Mechanisms, Ithaca, New York: Perinatology Press, 1988.

Willoughby, A., Moss, H.A., Hubbard, V.S., Bercu, B.B., Graubard, B.I., Vietze, P.M., Chang, C.C., and Berendes, H.W.: Developmental Outcome in Children Exposed to Chloride-Deficient Formula. Pediatrics 79(6):851-857, 1987.

Yaffe, S.J. and Catz, C.S.: The Pharmacologic Management of the Neonatal Respiratory Distress Syndrome (Lo stress respiratorio neonatale intervento farmacologica). Federazione Medica XL-9.1987, 987-991.

STAFF CHANGES

Dr. Anne Willoughby left the Branch to become the Chief of the newly formed Pediatric, Adolescent, and Maternal AIDS Branch. Ms. Diane Sondheimer, who joined the Pregnancy and Perinatology Branch in May 1988, has been reassigned to the Pediatric, Adolescent, and Maternal AIDS Branch.

Endocrinology, Nutrition and Growth Branch

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OVERVIEW

The research program of the Endocrinology, Nutrition and Growth (ENG) Branch focuses on human development from conception through infancy, childhood, and adolescence. It emphasizes research on preventive and curative approaches to nutrition and endocrine-related disorders, and stresses health promotion as well as treatment. Much of the program is multidisciplinary and involves genetic, biochemical, developmental, and behavioral aspects of nutrition, endocrinology, growth factors, and physical growth.

The main research areas of the ENG Branch are closely intertwined. Linear and somatic growth are regulated by hormones, growth factors, and nutrient supply. Hormones, growth factors, nutrient substrates, and nutrient cofactors also work in concert to engender growth and development of the central nervous system and the gastrointestinal system. Hormones and nutrients interact as well in controlling the onset of puberty and lactation, maintaining menstrual cycles, and determining hunger, satiety, and food intake. The complex interactions of these factors with the genome and the cell cycle are only now beginning to be revealed. Obesity, diabetes, amenorrhea, and some kinds of short stature exemplify disorders that may be treated by both endocrine and nutrient interventions. Interdisciplinary research along these lines is encouraged and represents a sizable proportion of the Branch research program.

PROGRAM ACTIVITIES

FINANCIAL SUPPORT

As of August 31, 1988, the Branch supported 199 projects at a level of $30 million. These projects are analyzed according to programmatic mechanisms in table 4. Two-thirds of the total budget supports nutrition-related research, and one-third supports research on developmental endocrinology and physical growth. In FY 1988 the Branch received 409 research grant and research training grant applications. Of these 105 were funded by September 1988.

Table 4

NICHD GRANTS AND CONTRACTS ACTIVE DURING AUGUST 1988
ENDOCRINOLOGY, NUTRITION AND GROWTH BRANCH

Funds (in thousands)

Health Area	Total		Research Grants								National Research Service Awards		Research Contracts	
			Total Research		Research Projects (incl. POI)		Center Program Projects		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	199	$29,995	170	$27,536	154	$26,364	1	$323	15	$850	17	$921	12	$1,538
Developmental Gastroenterology	23	2,880	22	2,854	19	2,674	-	-	3	180	1	26	-	-
Childhood & Developmental Nutrition	12	1,724	10	1,201	10	1,201	-	-	-	-	1	195	1	328
Infant Nutrition	54	7,895	43	6,847	41	6,728	-	-	2	118	2	160	9	888
Obesity & Antecedents of Adult Disease	11	2,148	11	2,148	10	2,097	-	-	1	51	-	-	-	-
Behavioral & Cultural Aspects of Nutrition	8	950	7	939	7	939	-	-	-	-	-	-	1	10
Maternal-Fetal Nutrition	9	1,801	9	1,801	8	1,747	-	-	1	54	-	-	-	-
Adolescent Nutrition	4	1,325	4	1,325	3	1,295	-	-	1	31	-	-	-	-
Nutritional Status	1	323	1	323	-	-	1	323	-	-	-	-	-	-
Developmental Endocrinology	66	8,693	57	8,349	50	7,933	-	-	7	415	9	344	-	-
Developmental Physiology	6	856	2	378	2	378	-	-	-	-	3	167	1	311
Physical Growth	5	1,402	4	1,373	4	1,373	-	-	-	-	1	29	-	-

NICHD-OPE-PAS
August 9, 1988

Notes: 1) The Minority Biomedical Support grant (SU6) is included in the research projects.
2) Excludes four grants funded from sources other than NICHD extramural funds.
3) Columns may not add to total due to rounding.

RESEARCH TRAINING

The Branch currently funds eight Institutional (T32) National Research Service Awards (NRSAs). Three of these are devoted to developmental aspects of nutrition. The postdoctoral fellows supported by these awards are pursuing nutritional needs of prematurely born babies and babies born at term, especially amino acid, calcium, and essential fatty acid requirements. The other five Institutional NRSAs are concerned with developmental aspects of endocrinology, metabolism, and physiology. The postdoctoral fellows supported by these awards are working on projects that vary from studies of renal tubular function to calcium metabolism in childhood disorders of bone growth. The Branch also funds nine individual (F32) NRSAs in developmental endocrinology. In FY 1986, the Branch joined the Perinatal Emphasis Research Centers program of the CRMC by funding a Clinical Nutrition Research Unit (P50) at the University of California in Berkeley. A major aim of this CNRU is to further training of medical personnel in nutrition research.

CONTRACT PROGRAM

The ENG Branch is especially concerned with the composition, health values, and developmental importance of breast milk for the normal newborn as well as for the high-risk infant. To meet the special needs of this field, a coordinated research contract program was established to expand our knowledge of the composition and function of the many constituents of human milk. As a result of this contract effort, human milk is now recognized as a valuable source of growth factors and antimicrobial agents as well as nutrients for newborn infants, particularly those of low birth weight. In FY 1988, eight contracts were supported in this area of clinical research, at a total cost of $888,000. These contracts were initiated in response to a request for proposals on Development of Methods of Analysis of Human Colostrum and Milk.

RESEARCH HIGHLIGHTS

MATERNAL-FETAL NUTRITION

Vitamin B_6

An investigation of the dietary habits of a lower socioeconomic group of pregnant women in Washington, D.C. indicated that 68 percent of them have a suboptimal intake of vitamin B_6. This finding could have ominous significance, since studies of rats fed B_6-restricted diets during gestation and lactation revealed profound changes in metabolism in the brains of their progeny. Tryptophan concentrations became elevated in the frontal cortex at 14 days of age, with a resultant marked increase in the tryptophan metabolite 3-hydroxykynurenine. This metabolite is toxic to neuron-derived cell lines in culture, and produces convulsions when injected into the cerebral ventricles of rodents, providing a possible explanation for the convulsions seen in B_6-deficient neonatal animals. Suboptimal B_6 intakes also produce changes in neurotransmitters in other parts of the brain: gamma-amino butyric acid is reduced in the cerebellum in 4-week-old animals. Although concentrations normalize later, this interference with nervous system function during a critical period of brain development could have significant long-lasting functional results. There are also significant effects of B_6 restriction on the

dopaminergic system, as whole brain dopamine at birth and striatal dopamine postnatally are significantly reduced.

INFANT NUTRITION

Effects of Hormones in Milk

Whether or not hormones secreted into milk have physiologic functions is a question of great theoretical and practical significance. Prolactin is one such hormone, present in concentrations of 200-400 ng/ml in the milk of laboratory rats and other species, including humans. NICHD-supported investigators traced the accumulation of prolactin from the maternal circulation into milk within the mammary gland, and its passage from the gastrointestinal tract of the suckling offspring into their systemic circulations.

Recent results from this same laboratory suggest an important role for this milk-derived prolactin. Lactating rats were treated with the dopamine agonist bromocriptine, which markedly reduced the concentration of prolactin in the milk with little effect on lactation itself. When tested at 34 days of age, about 2 weeks after weaning, rats which had received prolactin-deficient milk at 2 to 5 days of age demonstrated neurochemical and hormonal changes: steady-state concentrations and turnover of dopamine in the median eminence were decreased, and consistent with these observations, serum prolactin concentrations were significantly elevated. The effects were prevented by concomitant administration of prolactin to bromocriptine-treated mothers, and they did not occur if the bromocriptine treatments were delayed until days 9-12 of lactation. In addition, pituitary cells obtained from 100-day-old offspring of bromocriptine-treated mothers are almost completely unresponsive in vitro to the effects of a dopamine agonist which decreases the release of prolactin and reduces the cytoplasmic levels of prolactin mRNA in cells obtained from controls. These results suggest that one function of milk-derived prolactin in the neonate is to influence the maturation of the inhibitory tuberoinfundibular dopaminergic controls over prolactin secretion, and that a deficiency in milk-derived prolactin during a critical period leads to a persistent reduction of dopaminergic inhibition of prolactin secretion.

Protein Turnover

A major problem in infant nutrition has been the search for ways of optimizing protein accretion in low-birth-weight babies. The method of protein turnover, or rate of production and degradation, has been used in the past to study nitrogen metabolism in these babies. Recently developed, more sensitive methods can be used to study the turnover of individual proteins, particularly those which are present in low concentrations. With these methods it has been learned that in small, parenterally nourished premature infants recovering from respiratory distress syndrome, the functional synthesis rate of albumin was greater than in a group of adults; the rate in enterally-fed, normally growing prematures was intermediate. The concentration of blood albumin, however, was lower in the infants, so their relative hypoalbuminemia resulted not from a diminished rate of synthesis but from a more intense catabolism and turnover of a smaller pool. In contrast, the relative hypofibrinectinemia of these babies was found to be due to a diminished rate of fibronectin synthesis.

Thus, these data indicate that a broad heterogeneity exists with respect to protein metabolism in premature babies. Certain protein pools appear to be turning over

with greater intensity than comparable pools in the older child or adult, while other protein pools turn over more slowly. The concept of an integral rate of whole body protein synthesis is an oversimplification of physiologic reality.

Fat Absorption

Formulas containing triglycerides with high levels of medium chain fatty acids (MCFA) are often fed to low-birth-weight infants because these triglycerides are easier to digest, the MCFA being rapidly absorbed from the intestine. Studies in eight such infants have confirmed previous work in a rat model which demonstrated that the MCFA are actually extensively hydrolyzed from triglycerides and absorbed directly from the stomach, providing a readily available energy source which can be absorbed much more rapidly than other energy sources available to the newborn.

Human Milk and Infections

Several studies have demonstrated that infants fed human milk have lower rates of gastrointestinal infection than infants fed formula. Evidence is strong that one mechanism for this is an interaction between specific constituents of milk and epithelial surfaces of the gastrointestinal tract. Another possible mechanism is the modulation by milk of factors in the infant's immune system so that increased amounts of protective substances are produced by the infant. An NICHD grantee has obtained preliminary evidence that very-low-birth-weight infants fed fortified human milk, excreted in their urine higher levels of almost all antimicrobial protective factors (e.g., lactoferrin, IgA, secretory IgA, and secretory components) than did those fed formula, although serum levels of these factors did not differ between the two groups. Other observations from fecal analyses suggest the presence of a human milk factor that induces the production of mucosal immune substances by these babies.

Breast Milk Jaundice

Although breast milk feeding is thought to provide the neonate with many benefits, there may be disadvantages to it as well. One troublesome problem is that of "breast milk jaundice," a prolonged hyperbilirubinemia which occurs in some nursing infants. An investigator supported by the ENG Branch has demonstrated that the stool of breast-fed babies contains on the average three times as much of the enzyme beta-glucosidase as does that of formula-fed babies. In babies with breast milk jaundice, other investigators have shown that this enzyme is not derived from the mother's milk, so it may be a product of the intestinal bacterial flora. In any case, it could contribute to hyperbilirubinemia by generating free bilirubin from bilirubin conjugates in the colon. This free bilirubin would then become available for reabsorption into the bloodstream. In formula-fed babies the intestinal flora convert the mono- and diglucuronides of bilirubin to reduction products which are not available for re-uptake.

CHILDHOOD AND DEVELOPMENTAL NUTRITION

Central Nervous System Development

The deleterious effects of ethanol on the developing nervous system are well known, but the mechanisms are not established. Previous investigations have generally utilized relatively high concentrations of ethanol. In a current study the effects of ethanol are being studied at concentrations in the range seen clinically in

humans, using primary cultures of cerebral glia (supporting cells) derived from newborn rat brain. Enzymes are used as markers for the different glial cells, glutamine synthetase serving for astroglial differentiation and 2,3'-cyclic nucleotide 3'-phosphohydrolase for oligodendroglial differentiation.

Results so far indicate that ethanol in clinically relevant concentrations does not affect DNA synthesis. No effect on the astrocytes is seen even with a prolonged duration of ethanol exposure (17 days). On the other hand, low concentrations of ethanol lead to a striking enhancement of the marker of oligodendroglial differentiation. These findings may provide a significant lead to an understanding of the effects of ethanol on the developing nervous system.

DEVELOPMENTAL GASTROENTEROLOGY

Bile Salt-Stimulated Lipase

Newborn mammals, including human infants, depend on fat as their main source of energy. Newborn infants, however, especially those born prematurely, have very low levels of pancreatic lipase and low bile salt concentrations. A bile-salt stimulated lipase (BSSL) present in human milk is thought to be an important factor in fat digestion by breast-fed infants, but experimental studies have been limited because BSSL had been identified only in the milk of higher primates. Recent findings indicate that BSSL is present in the milk of carnivores, and that ferret milk is particularly rich in this enzyme. The data indicate that BSSL is responsible for the high lipolytic activity of ferret milk, and that it is stable and active under conditions like those in the gastrointestinal tract. The ferret thus provides a model for studies of the regulation of synthesis and secretion of BSSL in the mammary gland as well as its role in rat digestion in the newborn. These findings illustrate the concept that no single animal model is appropriate for all studies of human milk.

Gastroesophageal Reflux

Gastroesophageal reflux is a common problem of infancy. Although it is most often a benign developmental nuisance, it can sometimes lead to inadequate growth, aspiration, esophagitis, and stricture. NICHD-supported investigators are working to learn the etiology and mechanisms of this condition, and to establish a treatment for it. During the course of their investigations, one group has discovered a new swallowing reflex. A puff of air directed onto the face provokes a reflex swallow in subjects younger than 12 months and in older subjects with neurologic damage. This reflex is not only of developmental significance, but it also has practical applications: facilitation of the insertion of nasogastric or orogastric tubes or pH probes, stimulation of swallowing during esophageal motility studies, and facilitation of the administration of medications. It could even be useful in the establishment of appropriate eating behaviors in neurologically damaged patients.

Chronic Diarrhea

Children in the first year of life often develop diarrhea after an episode of acute gastroenteritis. On the basis of clinical experience and experimental work suggesting that this condition is a result of sensitization to food proteins, a soybean protein-based infant formula is often substituted for other formulas in these patients. Often, however, the diarrhea continues or recurs despite this

change. Institute-supported investigators have demonstrated that 86 percent of a group of chronic diarrhea subjects improved within 7 days if lactose, the physiologic sugar of human milk, was substituted for sucrose of the soy formulas. Only 20 percent of the patients improved when the sugar was changed to dextrimaltose, or was not changed at all. The favorable response may be related to an interaction of this specific carbohydrate-protein combination that prevents soy protein from triggering the diarrhea.

DEVELOPMENTAL NEUROBIOLOGY

Neuron Proliferation

The regulation of neuronal mitosis, which is restricted to early development, remains to be defined. To approach this problem, NICHD-supported investigators have developed a culture system in which neurons grown on defined medium enter the mitotic cycle. The cultured cells express biochemical traits which indicate that they are sympathetic (catecholamine-secreting) neuroblasts. DNA synthesis in these cells is regulated by insulin and the insulin-like growth factors (IGF-I and II). IGF-I is 100 times as potent as insulin, while IGF-II is less effective, suggesting that IGF-I receptors mediate the mitogenic response. Nerve growth factor exhibits no mitogenic effect, indicating that the action of the insulin growth factors may be highly specific.

Support for the idea that this effect has physiologic significance comes from studies which show that IGFs are synthesized in the central nervous system during ontogeny and that IGF receptors appear in the brain at the onset of neurogenesis and decrease with cessation of neuronal proliferation.

Marginal Zinc Deficiency

Zinc deficiency in human beings is known to be associated with fetal deformity and growth retardation. It is particularly a problem in less developed countries, where diets are rich in cereals and other sources of phytate (which impairs zinc absorption) and low in meat and shellfish, but it also occurs in this country. In experimental animals, zinc is necessary for normal development of the central nervous system and for neural functions in the adult. In severe zinc deficiency, the *in vitro* polymerization by brain cells of the protein tubulin to form microtubules is impaired. *In vivo*, microtubules are involved in the maintenance of cell shape, the movement of subcellular organelles, and the formation of the spindle fibers of mitosis.

New results indicate that effects of zinc deficiency can be demonstrated with experimental animals even when the zinc deficiency is not so severe. A marginal zinc diet imposed at the beginning of pregnancy and continued through day 21 of lactation was associated with a reduction in the rate of brain tubulin assembly when compared to zinc-deficient controls. This was associated with a lower level of zinc in brain supernatants. These findings strongly indicate the participation of zinc in the regulation of tubulin assembly *in vivo*, and provide a clue as to the effects of zinc deficiency on development.

OBESITY AND ANTECEDENTS OF ADULT DISEASE

Long-Term Effects of Infant Diet

Investigators supported by the ENG Branch have tested several hypotheses relating to the effects of infant diet on serum lipoproteins, cholesterol metabolism, atherosclerosis, and obesity later in life. The first experiment examined the effects of the level of cholesterol in formula and compared the influence of breast milk and formula feeding on cholesterol metabolism and atherosclerosis. In baboons that had been breast-fed as infants, HDL cholesterol was lower and the VLDL+LDL/HDL cholesterol ratio was higher than in animals fed formula in infancy. A number of variables of cholesterol metabolism, including cholesterol production rate and bile cholesterol saturation, also differed significantly between breast-fed and formula-fed animals. A second baboon study was designed primarily to answer two questions: whether caloric intake influences lipoproteins and obesity in young adult baboons, and whether breast or formula feeding influence subsequent plasma lipoprotein concentrations or atherosclerosis. Overfeeding in infancy led to obesity in young adult female baboons, but not in males. As in the first experiment, the VLDL+LDL/HDL cholesterol ratio was significantly higher in young adult baboons breast-fed in infancy than in those fed formula. As adults, the breast-fed baboons had a higher prevalence and extent of arterial fatty streaks than did those fed formula. The lesion involvement of the arterial wall could be explained by the VLDL+LDL/HDL ratio. These results are evidence that manipulation of infant feeding of baboons causes permanent metabolic programming of lipid metabolism which influences the development of obesity and atherosclerosis. The animal model system may be useful for obtaining information impractical to derive from human studies.

BEHAVIORAL AND CULTURAL ASPECTS OF NUTRITION

Since its inception, the NICHD has been concerned about the impact of malnutrition during pregnancy, infancy, and childhood on cerebral development and consequent behavior. In order to study this question several large, prospective field studies involving nutritional interventions were organized and supported. These studies have been instrumental in changing worldwide understanding of the relative importance of calorie versus protein deficits for birth weight and development of young children.

To expand this area of investigation the ENG Branch has implemented a program on nutrition and behavior emphasizing the primary roles played by the cultural and social environments in determining diet and nutritional status. Research is aimed at understanding hunger and the development of food preferences and cravings, as well as the development of other dietary habits, such as anorexia, bulimia, food faddism, and rejection of all but a narrow range of foods.

In order to stimulate more research in the area of cultural and behavioral aspects of nutrition, the ENG Branch in conjunction with the Human Learning and Behavior Branch of the NICHD and the Nutritional Sciences Branch of the National Institute of Diabetes and Digestive and Kidney Diseases issued a request for grant applications entitled, "Behavioral Aspects of Nutrition," which appeared in the NIH Guide for Grants and Contracts on July 31, 1987. Fifty-eight applications were submitted in response to this request. The ENG Branch funded four of the most meritorious of these, which addressed problems of nutritional behavior in the following areas: behavioral and cognitive effects on children of diets high in sugar or aspartame; factors that affect infant feeding decisions and methods in

the Navajo community; studies of how diet influences the cerebral synthesis of serotonin and dopamine; and studies of dietary habits and choice of weight loss methods among adolescent girls.

PHYSICAL GROWTH

Tonsil and Adenoid Surgery

For many years tonsillectomy with adenoidectomy has been the most common elective surgical procedure performed on children. Several hundred thousands of these operations are performed each year. A longitudinal randomized study supported by NICHD over more than a decade has established the efficacy of this surgery in reducing the incidence of pharyngitis among severely affected children. Whether tonsillectomy alone or tonsillectomy with adenoidectomy is similarly efficacious among mildly affected children has been more difficult to determine. Preliminary data show no difference between the two types of surgery, but show a statistically significant better outcome with either surgical treatment than with medical treatment. Nevertheless, the difference in morbidity is small, and the benefit of surgery may fall short of justifying the risks and costs. If these trends continue when the study is extended, it may be possible to conclude that adenoidectomy need not be added to tonsillectomy routinely, and that generally accepted standards regarding indications for tonsillectomy are not sufficiently stringent. To achieve results robust enough to influence well-established standards of practice, follow-up of subjects for at least another year will be necessary.

DEVELOPMENTAL ENDOCRINOLOGY

Congenital Adrenal Hyperplasia

In previous work, NICHD-supported investigators have described the clinical characteristics and chromosomal localization of the gene encoding nonclassic congenital adrenal hyperplasia (NCAH). This is a relatively mild form of deficiency of the enzyme steroid 21-hydroxylase, but it is one of the most common autosomal recessive genetic disorders in the white population, and can cause short stature, severe acne, virilization of adolescent girls, and reduced fertility in both sexes.

NCAH is inherited as an allelic variant of the gene which encodes the 21-hydroxylase. This gene is located in the HLA histocompatibility complex, and the nonclassic variant is often associated with the HLA antigens B14,DR1. Recently, the investigators have cloned and analyzed the gene from a patient with NCAH. Five differences from the normal genetic sequence were found, but only one of these appears likely to have affected the functional integrity of the protein, i.e., codon 281, GTG, encoding valine, was changed to TTG, leucine. When DNA samples from eight unrelated NCAH patients with the haplotype HLA B14,DR1 were analyzed, all of them demonstrated the valine 281 mutation. In contrast, DNA from unaffected subjects and one patient with NCAH who did not have B14,DR1 did not show evidence of this mutation. Thus, the mutation in codon 281 seems to be a consistent marker of 21-hydroxylase deficiency associated with HLA B14,DR1.

Control of the Onset of Puberty

The inhibition of the onset of puberty by undernutrition or excessive exercise is of considerable clinical importance. Underlying this phenomenon is the question of how puberty is regulated by body growth. Most female mammals attain puberty when they have achieved a particular body size or composition, implying a linkage between nutrient processing and gonadotropin secretion.

Two new model systems developed to study this problem have succeeded in completely preventing reproductive development during nutrient restriction and compressing pubertal development when restriction ends into the shortest possible period. Puberty does not occur when the food intake of female rats is restricted so that their weight is maintained at 75 grams. If rats on this regimen are allowed ad libitum access to glucose water, they achieve puberty and ovulate, thus demonstrating that the suppression of puberty was due to caloric rather than protein restriction. If a normal caloric intake is maintained but the animals are forced to run rapidly, so that no weight gain occurs, the pulsatile secretion of luteinizing hormone is totally suppressed and puberty is prevented. When the running requirement is released, the rats gain weight rapidly and ovulation occurs within 2 or 3 days. The investigators involved are now testing whether the effect of running can be overridden by the pulsatile infusion of gonadotropin releasing hormone.

Fetal Macrosomia

Macrosomia in infants born to women with insulin-dependent diabetes mellitus (IDDM) is usually ascribed to fetal hyperinsulinemia, caused by excessive nutrient transfer across the placenta. However, macrosomia occurs in 20 percent of infants whose mothers had near normal glycemia and hemoglobin A1 levels during pregnancy. The possibility that antibody-bound insulin is transferred to the fetus to contribute to macrosomia has been considered but never proven. To examine this hypothesis, NICHD-supported investigators have adapted analytic procedures and validated them for separation and determination of bovine, porcine, and human insulin in 0.5 to 1.0 ml of umbilical cord serum of infants born to mothers with IDDM. Preliminary results indicate the presence of animal insulins in five of seven sera, thus demonstrating conclusively for the first time that maternal insulin can be transferred across the placenta. Whether or not the transferred insulin contributes to macrosomia can now be evaluated.

FUTURE RESEARCH

Nutritional Therapy of Inborn Errors of Metabolism

Although many nutritional therapies for genetic disorders are protective of cognitive functions, they often do not effectively eliminate disease complications, and they sometimes produce secondary nutrient deficiencies. The ENG Branch convened a planning meeting on March 8, 1988, involving consultants from the NIH and from the outside research community, to identify research needs in the area of nutritional therapy of genetic disorders. The workshop participants identified the following needs:

(1) Longitudinal studies of the adequacy of nutritional therapies in maintaining normal growth and development while maximizing therapeutic response. Currently, nutritional status is seldom reviewed even with patients on the most restrictive

therapies unless a manifest deficiency symptom occurs. (2) Studies of the effectiveness of nutritional therapies in producing normal protein turnover and accretion, and normal fat and carbohydrate utilization, using stable isotope techniques. (3) Studies of the development of secondary nutritional deficiencies in patients on therapeutic diets, due to interference with the availability of other nutrients. (4) Methods for improving palatability or acceptability of nutritional therapies. Current amino acid exclusion diets used in the management of inborn errors of metabolism are extremely unpalatable because they rely heavily on mixtures of free amino acids. Synthetic methods can now be used to produce specific amino acid-deficient peptides that are much more appealing. (5) Novel dietary approaches based on studies of the pathogenesis of disorders for which current therapies are only partially effective. (6) Development of animal models for the study of nutritional therapies of inborn errors, by searching for heterozygotes or by using recombinant DNA methods. (7) Investigations of how vitamins may affect active cofactor concentrations and activate specific enzymes in inherited disorders. (8) Development of a registry of patients with rare diseases, with a view towards pooling patients for nutritional studies.

The Branch plans to combine some of these research recommendations into a Request for Applications in collaboration with the Mental Retardation and Developmental Disabilities Branch of the NICHD and other units of the NIH.

Future Needs in Human Milk Research

Research on human milk and various aspects of lactation has made outstanding progress over the last 10 years, in part because of targeted research grant and contract support provided by the NICHD. To evaluate the state of the field and develop recommendations for its future directions, the NICHD sponsored a 1-day workshop on May 6, 1988. Areas examined included *in vitro* systems, animal models, clinical investigations, and epidemiological studies. At this meeting 13 individuals presented brief reviews of some of the scientifically active areas of the field and their probable future directions. These included systems for *in vitro* study of mammary cell function, nutritional requirements in lactation, and problems in the design of studies on the significance of nutritionally, hormonally, and immunologically active substances and milk. Many of these studies need to be done in animal models, but different models are appropriate for different substances of interest. Epidemiologic studies of the relationship between breast-feeding and later health are difficult, but possible, as indicated by recent studies on diabetes and lymphoma. Since breast milk is the optimal nutrient for most infants, studies of the physical and psychological determinants of breast-feeding are needed.

Development of Methods for Analysis of Human Colostrum and Milk

The ENG Branch is currently supporting eight contracts aimed at developing new methodology suitable for analysis of the components of human colostrum and milk. The methods being developed range from physical, chemical, and immunological techniques through novel microbiological assays. On July 27, 1988, the principal investigators of these contracts met to exchange information about their progress and future directions for their research. It was reported that for the first time reliable and relatively simple procedures have been devised for the determination of water-soluble vitamins in small amounts of milk, with the prospect that these can be applied to studies of the uptake and utilization of these vitamins by infants. Vitamin K secretion is being studied by new high-pressure liquid chromatography methods. Methods of great accuracy and specificity are becoming

available for the determination of lipids and nonprotein nitrogen-containing compounds. These should be useful in future studies of the nutritional significance of human milk components which are missing from artificial formulas based on animal milks or vegetable extracts. Monoclonal antibodies have been prepared against putatively immunoactive milk proteins and some of the low molecular weight, sulfur-containing substances which form mixed disulfides with them. Electron microscopic and chemical analyses are under way on cell surface components of breast epithelium, probably high molecular weight glycoproteins, as well as fragments of intracellular material which are secreted attached to milk fat globules. Finally, tests of new procedures for sterilizing human milk which result in minimal alteration of its biologically active constituents are proceeding.

STAFF ACTIVITIES

CONFERENCES AND WORKSHOPS

Pharmacology of Dichloroacetate

On September 2, 1988, seven consultants met and discussed the use of dichloroacetate in the treatment of lactic acidosis of a variety of origins. Dichloroacetate blocks gluconeogenesis and stimulates glycolysis, possibly by several different mechanisms, and markedly lowers the concentration of lactic acid in the blood by stimulating the activity of pyruvate dehydrogenase. Lactic acidosis occurs in a number of different hereditary and acquired disorders, and dichloroacetate is undergoing clinical trials in the treatment of this problem in adults. The purpose of the meeting was to explore the feasibility of a multicenter clinical trial of this compound in pediatric patients with various organic acidemias. The results of this meeting are currently being evaluated by the ENG Branch.

Initial Events in the Pathogenesis of Insulin-Dependent Diabetes Mellitus

A workshop on "Initial events in the Pathogenesis of Insulin-Dependent Diabetes Mellitus" was held on June 28-29, 1988. The aim of the conference was to evaluate progress toward resolving the natural history and pathogenesis of the disease, and to suggest new directions for future studies that might eventually lead to its prevention.

Central issues that dominated the discussions included how to ascertain when beta cell destruction actually begins and how to identify the earliest events in the pathological process. Participants emphasized that the autoimmune attack on the beta cell may begin at birth or even in utero. A major problem that must be solved is how to design studies capable of detecting the earliest evidence of the autoimmune attack. Another key issue is the determination of when and how environmental factors become involved in the process of beta cell damage. A major clinical dilemma is determining the best way, given current knowledge of detecting the prediabetic state with a high degree of sensitivity and specificity. The proceedings of this workshop have been summarized for publication in Diabetes (in press).

The Molecular Basis of Human Growth Disorders

A workshop entitled "The Molecular Basis of Human Growth Disorders" was held at the NIH on September 8-9, 1988. Drs. John Parks and Peter Rotwein cochaired the meeting. The purpose of the meeting was to discuss the causes for much of the growth hormone resistance seen in short stature. Precise etiological diagnosis of growth disorders is still in its infancy. The cause of the majority of cases of retarded growth is either unknown, undiagnosable or both. Many cases of growth retardation may have a mixture of biologically active and inactive GH, but appear by RIA to be GH sufficient. Topics included aberrant GH genes, aberrant carrier proteins, faulty GH receptors, altered somatomedin-C genes, and faulty somatomedin-C receptors. Postreceptor events were also discussed.

COMMITTEES

Dr. Gilman D. Grave represents the Institute on the NIH Diabetes Mellitus Coordinating Committee as well as on the Diabetes Mellitus Interagency Coordinating Committee and the Interagency Group on Physical Activity. He represents the Director, NICHD, on the National Diabetes Advisory Board and also serves on the Interagency Coordinating Committee on Human Growth Hormone and Creutzfeldt-Jakob Disease. In addition he represents the NICHD on the trans-NIH Work Group on Inherited Metabolic Disorders and serves on the Scientific Advisory Committee of the National Diabetes Research Interchange. Dr. Grave is also the custodian of the large serum collection of the Child Health and Development Studies. Since March of this year, he has chaired the NICHD Institute Clinical Research Subpanel and is a member of the NIH Human Research Review Panel.

Dr. Levin represents the NICHD on the NIH Digestive Diseases Coordinating Committee and is also liaison to the U.S.-Japan Cooperative Biomedical Sciences Panel on Malnutrition. He represents the NICHD on the NIH Nutrition Coordinating Committee and represents the Director, NICHD, on the National Digestive Diseases Advisory Board. Dr. Levin serves as NICHD liaison representative to the Committee on Nutrition of the Mother and Preschool Child of the Food and Nutrition Board of the National Academy of Sciences. He also serves as liaison representative to the Committee on Nutrition of the American Academy of Pediatrics. Dr. Levin represents the NICHD on the Board of Scientific Counselors of the USDA-Children's Nutrition Research Center in Houston, Texas.

Dr. Levin has been working to review and revise the chapters on maternal, infant and childhood nutrition of the forthcoming Surgeon General's Report on Nutrition, a DHHS initiative that began in 1983, and has made significant improvements and additions to this report.

Mental Retardation and Developmental Disabilities Branch

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OVERVIEW

A review of the current portfolio of grants supported by the MRDD Branch reveals an impressive diversity of projects directed towards a better understanding of the etiology and pathogenesis of diseases and syndromes responsible for mental retardation and other developmental disabilities. Currently there are more than 50 disorders under study. Down syndrome is the focus of more grants than any other condition but many inborn errors of metabolism, infection, hormonal and nutritional disorders, effects of toxic substances, and complications of low birth weight associated with and without prematurity are included.

Investigators are utilizing modern cytogenetic and molecular biologic techniques to identify the specific genes and gene products responsible for given diseases or syndromes; some 53 grants fall into this category. Fifty-nine grants are concerned primarily with behavioral or biobehavioral issues. The biobehavioral concept is important because in many studies behavior is the dependent or outcome variable with many complex interacting independent variables, both biologic and psychosocial.

PROGRAM ACTIVITIES

FINANCIAL SUPPORT

Because of the complexity and diversity of the conditions subsumed under the term mental retardation, research approaches must be multifaceted and diverse, basic and applied, biomedical, and behavioral. Therefore, the MRDD Branch supports a comprehensive program of research concerning the epidemiology, etiology, prevention, amelioration, treatment, pathophysiology, and diagnosis of mental retardation. In August 1988, the Branch's support of these activities totalled $45,715,000 for 169 research projects. In addition, the Branch supported 13 training grants ($1,706,000) and 14 ($3,509,000) research contracts.

RESEARCH HIGHLIGHTS

MENTAL RETARDATION RESEARCH CENTERS (MRRCs)

The Mental Retardation Research Centers provide facilities for support of research and research training relevant to mental retardation, developmental disabilities and related aspects of human development.

The original 12 centers, located throughout the Nation, were constructed primarily by Federal money authorized by Public Law 88-164, Title I, Part A. Under the provisions of the Act, the 12 Centers agreed, in principle, to use these facilities for their intended purpose for a minimum of 20 years. Over the past nearly two decades, the Centers have been reviewed periodically and provided annually with support for core facilities through the P30 center grant mechanism.

In FY 1987, these Centers received about $9 million to support central core facilities, thus enhancing program coordination and collaborative efforts. Support for all MRDD Branch research and training activities was approximately $29 million. Slightly less than half of these dollars were spent by investigators in the MRRCs. It should be noted that significant additional funds for research in the Centers derive from other Institutes at NIH as well as from other Federal, state, and private sources.

In 1987, a decision was made to open the competition and solicit applications from any institution possessing the requisite programs for a Center. A Request for Applications (RFA) was published, resulting in seven new applicants in addition to applications from the three extant Centers whose 20-year commitment had expired. A site visit was made to each applicant institution and, in June 1988, four Centers were recommended by the NICHD Advisory Council for funding. This year a second RFA was issued resulting in five applications. These potential Centers, including three from those currently extant, will be reviewed by the Mental Retardation Research Committee and recommendations presented at the June 1989 meeting of the NICHD Advisory Council. The same process is planned for 1990 and 1991. Assuming availability of funds, upon completion of this process there will be 12 MRRCs but their locations may be different than they were 20 years ago.

The range of research studies being conducted in the MRRCs encompasses every known major dimension in this area. This concentration of activity is supplemented by the work of investigators located in other universities, agencies, and research settings. The activities described in the sections which follow represent a few of the research accomplishments and highlights from the Branch portfolio during the past 3 fiscal years.

BIOMEDICAL RESEARCH IN MENTAL RETARDATION

Genetics and Genetic Disorders

Down Syndrome

Down syndrome is the most common genetic birth defect known to be associated with mental retardation and results from triplication of a portion of the long arm (q) of chromosome 21. Considerable progress has been made in identifying and localizing genes on human chromosome 21. Eighteen genes have been assigned to

chromosome 21, nine of which have been localized to the long arm, and eight which appear to be associated with the so-called "Down syndrome region." In addition, a large number of cloned DNA segments of unknown function have been isolated and regionally mapped. The majority of these segments detect restriction fragment length polymorphisms (RFLP) and, therefore, represent useful genetic markers. Five of the genes localized to human chromosome 21 have also been localized on mouse chromosome 16, considered to be homologous (in part) to the human 21 chromosome. These include the genes encoding the cytoplasmic form of superoxide dismutase (SOD-1), a proto-oncogene (ETS-2), the alpha and beta interferon cell surface receptor (IFNRC), the purine biosynthesis enzyme phosphoribosyl glycinamide synthetase (PRGS), and the amyloid precursor protein (APP). Because of the extensive genetic homology between human chromosome 21 and mouse chromosome 16, mice with trisomy 16 have been recognized increasingly as potential model systems for studies related to Down syndrome.

Mouse Model for Cytogenetic Disorders

A contract has been awarded to the Jackson Laboratory, Mount Desert, Maine, to provide the scientific community with a resource of mice that can be used to produce trisomy of whole mouse chromosomes in embryos or small segments of mouse chromosomes in viable adult mice. Whole chromosome trisomy is produced by mating mice heterozygous for two different Robertsonian chromosomes with one chromosome in common. Segmental trisomies can be produced from mice carrying a reciprocal translocation when one of the translocation products is a small marker chromosome. The objectives of the project are: to maintain and distribute Robertsonian chromosome-carrying strains of mice that can be used to produce embryos trisomic or monosomic for whole chromosomes; to produce reciprocal translocations that can, in turn, be used to produce live-born trisomic mice for small segments of the genome (segmental trisomies), with emphasis on chromosome 16; to determine the fertility of, and the frequency of aneuploid embryos produced by male and female carriers of (a) various double Robertsonian chromosome combinations, (b) reciprocal translocations, and (c) segmental trisomies; preserve frozen embryos from appropriate genetic backgrounds; and to attempt to improve the production of chimeric mice with a trisomy 16 component.

Fragile X Syndrome

The Fragile X Syndrome is a subgroup of X-linked genetic abnormalities. This syndrome is becoming increasingly recognized as one of the most frequent causes of mental retardation. With an estimated population prevalence of Fragile X ranging from 0.73 to 0.92 per 1,000 males, this syndrome ranks second only to Down syndrome as the most frequently identified cause of mental retardation associated with a chromosomal abnormality. Scientists have constructed somatic cell hybrids between human lymphoid cells obtained from either normal or fragile X males and Chinese hamster cells deficient in both HGPRT and G6PD. By maintaining genetic selection on HGPRT (locus proximal to the fragile X site) and staining the cells histochemically for G6PD (locus located about 7cM distal from the fragile site), they were able to determine if segregation of these syntenic markers occur. Using this method, they established that segregation of these markers is more frequent if the markers encompass the fragile X site in hybrids cultured under conditions known to induce the fragile site (i.e., thymidine stress). Hybrid cells that reveal marker segregation were found to contain rearranged X chromosomes involving the region at or near the fragile site, thus demonstrating true chromosomal breakage within this area. Two independent translocation chromosomes were identified involving a rodent chromosome joined to the human X at the location of

the fragile site. DNA analysis of closely linked, flanking loci was consistent with the position of the break point being at or very near the fragile X site. Fragility at the translocation junctions was observed in both hybrids, but at significantly lower frequencies than that seen in the intact X of the parental hybrid. This observation suggests that the human portion of the junctional DNA may contain a portion of a repeated fragility sequence. Since the translocation junctions join heterologous DNA, the molecular cloning of the fragile X sequence should now be possible.

Prader-Willi Syndrome

In infancy, the clinical features of Prader-Willi Syndrome (PWS) include marked hypotonia, hyporeflexia, poor feeding due to diminished swallowing and sucking reflexes, and cryptorchidism with hypoplastic penis and scrotum in boys or hypoplastic labiae in girls. In later childhood, the features are polyphagia, obesity, small hands and feet, short stature, hypogonadotropic hypogonadism, and mental retardation. It has been known since 1982 that, when high-resolution chromosome techniques are used, over 50 percent of PWS patients have a small, interstitial deletion on the proximal portion of the long arm of chromosome 15, that is region q11, q12, or q13. Less frequently, translocations involving chromosome 15 have been reported in association with PWS. These include Robertsonian translocations often between two chromosome 15s. Translocations of one chromosome 15 with another autosome have also been reported in PWS patients. They are frequently unbalanced, producing monosomy for the same region of 15q as do the interstitial deletions, but some translocations appear to be balanced. Pericentric inversions have also been associated with PWS. More recently, Institute-supported investigators reported an apparent duplication of the proximal region of 15q in two patients with Prader-Willi Syndrome. How both an excess number of gene copies and a deficiency of gene copies may produce the same clinical effect is not clear at the present time. Furthermore, several patients with deletion of proximal 15q without the clinical features of PWS have also been reported. Thus, discrepancies in karyotype-phenotype correlation are apparent. Isolation and characterization of DNA probes in the Prader-Willi Syndrome critical region on chromosome 15 will help in our understanding of how the multiplicity of chromosome aberrations can produce the PWS phenotype. Ultimately, molecular techniques will allow scientists to correlate the clinical features seen in PWS with specific gene products and functions.

Rett Syndrome

Rett Syndrome is a neurodevelopmental disorder that affects girls exclusively. Affected girls experience a plateau in their progress followed by a regression of psychomotor function following normal development until 6-12 months old. They exhibit autistic-like behavior, inability to use their hands, except for clapping-wringing-washing stereotypes. They have diminished expressive abilities, avoidance of eye contact, a lag in head growth, and seizures. The prevalence of Rett Syndrome has been estimated to be about one in 15,000 girls, and the condition has been reported in all races and in all parts of the world. The condition has not been described in males. This suggests an X-linked dominant pattern of inheritance which is lethal to males. The precise genetic transmission will be difficult to confirm because affected girls are unlikely to bear children. Neither the cause nor the pathogenesis of Rett Syndrome is known. Biochemical and cytogenetic evaluations have been reported as normal. Brain imaging and neuropathologic studies have demonstrated atrophy of both the cerebral and cerebellar cortices in some patients. Abnormalities in the electrical activity

of the brain have also been described. In the absence of a characteristic biochemical or genetic marker by which the diagnosis of Rett Syndrome can be confirmed, it is not now possible to delineate the range of clinical expression.

The Institute recently awarded two program projects on Rett Syndrome. The first project consists of four components: (1) clinical studies of 100 "classical" Rett Syndrome patients, including clinical delineation, epidemiology, neurophysiology, biochemical assays of body fluids and easily accessible tissues (not brain biopsy), magnetic resonance and/or CT imaging; (2) cytogenetic studies; (3) neuropathological and neurochemical studies; and (4) positron emission studies of neurotransmitters and their receptors. The second program project will study the clinical, epidemiologic, communicative, neurophysiologic, neurochemical, molecular genetic, and neuropathologic aspects of Rett Syndrome. Investigators involved in both program projects are collaborating to minimize duplication of efforts, to maximize utilization of biologic specimens, particularly brain tissues obtained at autopsy and to benefit from each other's expertise. They are also collaborating in the development of a registry with the assistance of the International Rett Syndrome Association.

INBORN ERRORS OF METABOLISM

Maternal Phenylketonuria

In 1984, the Institute initiated a collaborative study designed to evaluate the efficacy of a phenylalanine-restricted diet during or before pregnancy in reducing the morbidity associated with maternal phenylketonuria (PKU). About 130 clinics are participating in the United States and 18 clinics in all of the provinces in Canada. There are 179 women presently enrolled in this study (18 are currently pregnant). Of the 91 completed pregnancies, there were 60 live births, 16 spontaneous abortions, 14 induced abortions, and 1 stillbirth. Among the 72 women who received the phenylalanine-restricted diet, 17 started the diet prior to pregnancy, and the rest started the diet after pregnancy. In 8 women the diet was not medically indicated (i.e., the blood phenylalanine concentration was less than 6 mg/dl) and 11 women refused the diet. In addition to the enrolled subjects, there are about 1,700 PKU subjects older than 12 years whose whereabouts are known to the participating clinics.

Phenylketonuria

Using recombinant DNA technology, an Institute-supported investigator at Baylor College of Medicine in Texas has cloned the gene involved in PKU. In 1984, the same investigator assigned the phenylalanine hydroxylase (PAH) locus to 12q21->qter by restriction analysis of DNA from human-hamster somatic cell hybrids. By *in situ* hybridization, the assignment of the PAH locus was narrowed to 12q22-12q24.1. By using restriction fragment length polymorphisms related to the PAH gene, prenatal diagnosis of PKU homozygotes and PKU carriers has been achieved. The same group of researchers have succeeded in inserting a full-length complementary DNA clone of human PAH into a eukaryotic expression vector and in transferring it into NIH 3T3 mouse cells which do not normally express PAH. The transformed mouse cells expressed PAH messenger RNA, immunoreactive protein and enzymatic activity that are characteristic of the normal human liver products. This demonstrates that a single gene contains all of the necessary genetic information to code for functional PAH. Studies are currently under way to develop

a genetic animal model for PKU so that the demonstrated success in inserting the PAH gene *in vitro* can be tried *in vivo*.

PRENATAL DIAGNOSIS

Chorionic Villus Sampling (CVS)

In 1984, the Institute embarked on a multicenter, prospective clinical trial to determine the safety and accuracy of Chorionic Villus Sampling between the eighth and eleventh week of pregnancy for the prenatal diagnosis of genetic disorders earlier than is possible with amniocentesis. In this procedure a plastic catheter is inserted through the cervical opening using ultrasonographic guidance. A small sample of chorionic villi surrounding the embryo is obtained and cultured. The rapid growth of these villous cells enables the samples to be evaluated cytogenetically, assayed biochemically, and analyzed for DNA content within days after sampling. Using a common protocol, the national study includes seven contributing centers and one data coordinating center. Initial attempts failed to randomize subjects between the CVS group and a comparison group undergoing amniocentesis. The following criteria were established for enrolling subjects in the safety aspect of the study: (1) maternal age as primary indication for prenatal diagnosis, (2) baseline ultrasound done at the study center showing a normal singleton pregnancy with a size consistent with 49 to 90 days from the last menstrual period, (3) CVS procedure scheduled within 2 weeks of the baseline ultrasound and before 90 days menstrual age, (4) no active herpes or gonorrhea, (5) no intrauterine device in place, (6) no serious maternal illness which might compromise the pregnancy, (7) no known teratogenic exposure, (8) no anatomical obstruction to CVS, (9) no active vaginal bleeding, and (10) residence within a defined geographic area making close follow-up feasible.

Patients electing amniocentesis also were recruited in the first trimester for the safety study. These patients had chosen amniocentesis after first trimester genetic counseling, or had contacted the genetic center in their first trimester to schedule an amniocentesis. Eligibility criteria were identical to those for the CVS participants except for the scheduling of a CVS procedure. Patients having CVS at the seven participating centers, but not meeting the above criteria for the safety study, were asked to participate in the study of accuracy.

Because of the increasing interest in transabdominal CVS, a randomized clinical trial comparing the safety and accuracy of transcervical and transabdominal CVS was initiated in September 1987.

A preliminary analysis was made on 2,278 women undergoing CVS and the 671 controls seeking amniocentesis who were enrolled in the safety study and were due to deliver before April 30, 1987. Cytogenetic analyses were successfully performed in 97.7 percent of CVS and 99.1 percent of amniocentesis cases ($P<.05$) and revealed 1.7 percent and 1.4 percent aneuploidy, respectively. After adjusting for slight differences in gestational age and menstrual age at entry, the combined losses due to spontaneous and missed abortion, termination of abnormal pregnancies, stillbirths, and neonatal deaths were 0.7 percent higher in the CVS than in the amniocentesis group (80 percent confidence interval -0.7 percent to 2.0 percent). This difference is not statistically significant.

It is concluded that, based on preliminary analysis of available data. CVS is an effective approach to early prenatal diagnosis but probably confers a slightly higher risk than amniocentesis. Enrollment of women is in progress and a more

definitive analysis will be performed in the future. The results to this point have been encouraging.

Prenatal Diagnosis Using Fetal Cells in Maternal Blood

Genetic disorders contribute significantly to reproductive wastage and to infant mortality and morbidity. With the advent of prenatal diagnostic techniques, families have been provided accurate information upon which to make informed reproductive decisions. With the development of more sophisticated diagnostic and detection procedures, and the possibility of intrauterine treatment, either through fetal surgery or through therapeutic medical procedures, it becomes important to detect fetal abnormalities at earlier gestational ages.

A potential diagnostic procedure, certainly less invasive than either amniocentesis or chorionic villus sampling, relies on the presence of fetal cells in maternal peripheral circulation. This year, the Institute awarded two contracts for developing new methodologies or for refining existing procedures to separate fetal cells from maternal circulation. The fetal cells can then be processed either directly or after using appropriate tissue culture techniques for the prenatal diagnosis of genetic disorders using cytogenetic, biochemical, and molecular genetic techniques.

EXOGENOUS AND ENVIRONMENTAL FACTORS

Malnutrition

Protein

The Institute is currently supporting a program project to study the behavioral, neurophysiologic and neuroanatomic consequences of severe and mild nutritional deficits at different developmental stages in a rat model for protein malnutrition. The objective of the project is to document early, late, and intergenerational responses of brain function under conditions of severe or mild undernutrition with subsequent nutritional rehabilitation. Rats achieve maximal physical and brain growth on a 25 percent protein diet, yet show adequate physical growth but impaired brain function on an 8 percent protein diet. Poor physical growth and impaired brain function are found when a 6 percent protein diet is used. All diets are isocaloric and each contains 4 kCal/g.

Early in the developmental period, suckling rat pups learn to use cues in close proximity in order to localize hidden objects in their environment. Under normal conditions, pups develop the ability to use such proximal cues beginning on postnatal day 15, and the behavior is generally completely developed by postnatal day 17. When no proximal cues are present, rats can learn the location of the unseen object by using static spatial cues which are at some distance from the rat and from the object. The ability to use such distal cues can be observed in healthy rat pups by postnatal day 20. Animals subjected to postnatal malnutrition and who are malnourished at the time of testing show retarded development of distal cue localization abilities until postnatal day 28-30, although proximal cue mediated behavior is relatively unaffected.

Previous studies have indicated that rat pups show a predictable sequence of development of orientation to the nest region of the home cage. In the current program project, the development of home orientation has been tested in control

pups and in pups with histories of prenatal malnutrition who have been nutritionally rehabilitated from birth. Preliminary evidence demonstrates delayed development of the home-orienting behavior with activity levels comparable to or exceeding those of control animals. This finding eliminated concurrent undernutrition or reduced activity as a cause of nutrition-related changes and suggests a more direct role of prenatal undernutrition on brain centers associated with the control of such spatial activities. The time sequence of these studies has been planned to document changes during the major developmental period of hippocampal cell formation during the prenatal period and the first 30 days of life. These behavioral studies occur in parallel with neuroanatomic and neurophysiologic assessments permitting various correlations of biologic and behavioral development.

Effect of Prenatal and Postnatal Lead Exposure on Cognitive Development

The relation between prenatal and postnatal lead exposure and early cognitive development has been assessed in a prospective study of 249 children from birth to 5 years. On the basis of lead levels in umbilical-cord blood, children were assigned to one of three prenatal exposure groups: low ($\leq$3 μg/dl), medium (6 to 7μg/dl), or high ($\geq$10 μg/dl). Beginning at the age of 6 months, development was assessed semiannually through age 2, and at age 5. Capillary blood samples, obtained at the time of each developmental assessment, provided measures of postnatal lead exposure.

There was a significant association between infants' lead levels and their test scores. At all ages, infants in the high-prenatal-exposure group scored lower than infants in the other two groups. Scores were not related to infants' postnatal blood lead levels.

Infants of lower social class manifested lower infant test scores at lower lead levels than did the infants of upper social class but being in the upper social class was not sufficient to protect infants against adverse developmental effects associated with "high" cord blood lead levels (i.e., 10 to 25 μg/dl).

Scores at 2 years were significantly associated with lead exposure by age 5; a prenatal lead effect on development was no longer evident. At both ages 2 and 5, the higher lead levels were associated with difficulties in visual-motor coordination and visuospatial skills.

These findings raise the possibility that the fetus and young child are adversely affected at blood lead concentrations well below 25 μg/dl, the level defined by the Centers for Disease Control as the highest acceptable level for young children.

BEHAVIORAL AND BIOBEHAVIORAL RESEARCH IN MENTAL RETARDATION

Learning, Cognition, Perception, and Memory

Intelligence has been a "defining variable" for mental retardation. In recent years research has become increasingly focused on the processes and the components of intelligent behavior. There has been a growing interest in internal manipulations of information and problem-solving strategies. Similarly, the research emphasis has shifted from what the individual knows in a particular domain (that is, "facts") to the processing skills underlying the acquisition and use of that knowledge. One research team supported by the MRDD program is exploring such

an approach to the acquisition of mathematical ability by children. The research to date indicates that, if our interest is in predicting the learning trajectory of different students, the best predictor is not IQ, nor how much students know originally, nor even how readily they acquire new procedures, but how well they understand and make flexible use of those procedures in solving novel problems.

Researchers have analyzed problem solving in terms of the steps to solution, for example, representing the problem correctly, devising a solution plan, executing the plan, and deriving a general principle. Mentally retarded persons are especially deficient in applying what has been already learned to new problems. The Institute is supporting several research programs concerned with the processes involved in the formulation of problem-solving strategies and age-related changes in normal and retarded children. One grantee is investigating the conditions that aid or impede the transfer of previously achieved understanding from one situation to another. What changes with age is the complexity and organization of available knowledge. Knowledge which is organized and is consistent with a coherent theory is very likely to be transferred successfully.

Young children considered to be at risk for mental retardation have great difficulty detecting relationships such as similarity and dissimilarity among individual visual or auditory stimuli. One research project with children at risk for mental retardation has demonstrated low sensitivity to alterations in relational information from visual and auditory stimuli. Thus, for individuals with mental retardation to make many sorts of complex discriminations, the discrepancy between the target stimulus and the background in which the target is embedded must be of a greater magnitude than for normal children.

Another group of investigators at a Mental Retardation Research Center has been developing and refining a battery of tests for retarded adults in order to define the role of attentional factors in memory. It includes a delayed recognition test and tests of auditory and visual memory, picture and color recognition, spatial recognition, and spatial memory. The test battery appears to be sensitive to differential deficits associated with Down syndrome (DS).

LANGUAGE AND COMMUNICATION

Until recently, individuals who were classified as profoundly mentally retarded were very often thought to be unable to communicate. Two somewhat divergent fields of science have converged to clarify the capacity of these individuals to use elemental forms of communication. Studies from the fields of linguistics, psycholinguistics and child development have produced a complex theory and impressive series of empirical findings that map the development of communication abilities in individuals with normal intellectual abilities. Several investigators are using this structure to analyze the communicative abilities of individuals with mental retardation.

Numerous investigators have collected detailed information on mother-infant exchanges during the period when language normally develops. Transcripts of these exchanges have been used to describe and analyze delayed and deficient aspects of the development of language skills in disabled children. In a comparative study of language acquisition, one grantee has collected observations of communicative interactions in 46 families with normal children and is currently collecting similar data on 11 families with at least one child who has DS. Preliminary findings show that both sets of families respond appropriately in terms of the

developmental level displayed by the child. Children with DS showed large individual differences. Their developmental progress was significantly slower than that of normal children, especially in the motor domain. Individual differences in language skill acquisition within the sample of DS children appears to be associated with intelligibility of the child's speech and the degree to which the child can access sensorimotor experiences with understanding. These limitations influence the level of linguistic input and opportunities for cognitive and language learning.

The goal of one ongoing study is to determine whether in children with DS the gap between the cognitive level and expressive language widens with age. This study is examining the pattern of cumulative deficit and the relationship between language and nonlinguistic cognitive abilities in DS individuals from 6 to 21 years. The divergence of comprehension, production, and other cognitive skills in individuals with DS seems to begin approximately at age 6 and continues for some time. Normal developmental progression of expressive language skills depends on the interaction of cognition, comprehension, and prior talk. Understanding the cumulative deficits in language and cognition should lead to a better understanding of fundamental developmental differences in normal and DS children and provide useful information for early stimulation of disabled infants.

Environmental Interventions for At-Risk Infants and Children

Much of the behavioral work supported by the Institute has important implications for prevention of retardation or improvement in the status of infants and young children who are at risk for poor developmental outcomes. Some of these children are at risk for retardation because of medical conditions such as low birth weight and/or prenatal or perinatal damage. Although they are healthy at birth, others may be at risk because they are exposed to inadequate or inappropriate stimulation often associated with educational and economic disadvantage. Because prematurity and perinatal damage are more frequent in low income families, many children suffer from multiple sources of risk.

A major study at one of the MRRCs has been given long-term support by the Institute. The principal investigators have been conducting a prospective experimental longitudinal study of the effects of cognitively oriented intervention on children who come from extremely high-risk families. Children from extremely impoverished home backgrounds show consistent and highly significant benefits attributable to intensive delivery of cognitive stimulation within a university-based child care center. Further, they have found that the benefits of systematic preschool enrichment are best maintained when coupled with supplemental educational intervention during the first 3 years in school. Such children are more than three times as likely as control children (who received only careful medical and social service assistance during the first 8 years of life) to keep up with school grade expectations (i.e., they were not retained in grade). A group of children who received only the supplemental intervention from first through third grade also showed benefits, although not nearly of the same magnitude.

When children with mentally retarded mothers (IQs below 70) were studied, it was discovered that those children who received educational intervention throughout the first 5 years of life were nearly six times as likely as controls to function within the normal, rather than retarded range. In addition to the IQ and school achievement benefits, the intervention had broad effects on parent and family functioning and on aspects of adaptive behavior. For example, the experimental

group of teenage mothers was significantly more likely to complete high school and later continue with post-high school education, to receive less or no Aid to Families with Dependent Children or welfare, and to have smaller families than were comparable mothers in the control group.

TREATMENT OF BEHAVIOR DISORDERS

Self-Injurious Behavior

The study of self-injurious behavior (SIB) exhibited by persons with developmental disabilities is an important area of Institute-supported research. One grantee has worked for 8 years to develop a systematic methodology for describing, measuring, and treating SIB. A method for assessing SIB rates, intensity, and global impact in a variety of settings has been developed. Data obtained from functional assessment of SIB in a variety of cases suggest that the behavior is associated with several sources of motivation: escape from, or avoidance of, demands made by others; response to inappropriate attention from adults; or self-stimulation. A sizable portion of the 50 cases with SIB demonstrated multiple motivations for engaging n SIB.

In a study of six individuals who exhibited high rates of SIB, one investigator was able to demonstrate a significant reduction in SIB through the use of a multifaceted treatment program that included development of instruction-following behavior in demand situations, differential reinforcement and extinction in the attention condition, and provision of the opportunity to play with toys. Another study showed that, in combination with operant contingencies, it is possible to gradually eliminate physical restraints for individuals who have worn them for many years. Over the past 8 years, this research group developed a data base for studying prospectively a wide variety of treatments for the reduction or elimination of SIB. This systematic approach to examining the effects of various treatments will provide a basis for improving the treatment of these mutilating and life-threatening behaviors.

Recent research has shown that some individuals who exhibit SIB are responsive to pharmacological intervention. One hypothesized explanation for this is that the endorphin/enkephalin ligand system may be involved in maintaining SIB. That is, when individuals initially injure themselves, endogenous opioid ligands are released. Because these enkephalins are powerful reinforcers, these individuals may repeatedly hit themselves to release the endorphin/enkephalin substance(s). Discontinuing self-injurious hitting would have the same effect as withdrawal from an addicting drug, namely withdrawal distress. Thus, continued SIB may be maintained both by the reinforcing effect of the endogenous ligands and the avoidance of withdrawal distress. The Institute funds several research projects that focus on improving the understanding of SIB and the potential effectiveness of pharmacologic and other forms of treatment.

Behavioral interventions ranging from the use of positive reinforcement to punishment have been used to decelerate SIB and to probe the environmental components that contribute to SIB. Lesch-Nyhan Syndrome, which is characterized by serious self-injury, has been used as a model for improving the understanding of the biological nature of SIB.

ADAPTATION IN FAMILY, RESIDENTIAL, VOCATIONAL, AND EDUCATIONAL SETTINGS

Family Processes and Settings

The deinstitutionalization movement of the 1970s has led to increased interest in the families of the handicapped and retarded because more mentally retarded children are residing with their families for longer periods of time. In order to increase support for research on families, in 1987 the MRDD Branch issued a Request for Applications (RFA) for research grants to study the impact of retarded children and adults on their families, and the impact of family structure and processes on retarded children and adults. Five applications were funded. All of these projects have recently completed their first year of support. One is screening 600 pregnant women from an area that exceeds the national average for producing children at risk for developmental disabilities. The infants of these pregnancies will be followed with a standard protocol until they are 6 months of age. Beginning at 6 months, up to 30 infants who fit into one of seven risk groups will be followed until they are 2 years old. Another longitudinal study follows a cohort of 201 mentally retarded children and their families and a matched sample of families without a retarded member.

Still another project concerns the domestic life cycle of families with and without mentally retarded offspring, their formal and informal social supports, and their use of community services over time. The sample includes a group of individuals who have been studied by these investigators for over 25 years and a new cross-sectional sample of families with a retarded offspring and comparison group of families without a retarded offspring. A fourth project provides for a comprehensive evaluation of interactions of 250 mentally retarded children and their families, including mothers, fathers, and siblings. The aim is to identify predictors of poor child and family adjustment. The fifth project is investigating the impact of a retarded child on 300 families, using "time diary data," other measures of family function, and characteristics of family members, family units and their environments.

Residential Settings

There is much controversy about what comprises an "optimal" living arrangement for individuals with mental retardation. One research project has focused on longitudinal assessment of individuals with mental retardation, primarily those with severe and profound retardation, who previously had been institutionalized and were thought to be difficult to place in the community. Detailed quantitative observations of everyday behavior patterns in a variety of situations were made. It was found that environments that truly facilitate behavioral development for some individuals do not necessarily help others. The finding is directly relevant to placement decisions (i.e., for better "matching" of people and places in service settings). The observational methods which have been developed can be adopted for monitoring the residential adjustment of individuals.

A theory-based taxonomy of residential environments in mental retardation has been developed from this program of research. The results of controlled experimental and naturalistic studies of more than 600 individuals indicate that the structural features of residential environments (e.g., size, staffing patterns, location, categorical type, and funding source) are not closely linked to "quality of life" indicators or developmental advances. In contrast, the social milieu and the functional opportunities in the environment serve a far more important role in the well-being of severely and profoundly retarded persons.

Vocational Settings

During the past decade, individuals with severe developmental disabilities have been employed in a variety of settings from sheltered workshops to supported work sites. Placing individuals with severe developmental disabilities in work requiring simple repetitive responses has proven to be successful. Structuring the environment to maximize the individual's performance is an important factor in matching individual performance with job requirements. One investigator has used basic learning principles to study the development of skills requisite for assembling complex industrial products.. Training for each skill needed for a complex vocational task is time consuming and impractical. Using stimulus equivalence procedures to teach representational skills, this researcher is developing efficient and effective vocational training procedures. In a series of studies, component skills for completing a complex assembly task were analyzed. Four individuals with severe mental retardation were systematically trained in representational matching across assembly parts using a variety of stimuli ranging from black and white photographs to schematic drawings of the actual object. After training schematic-to-concrete, schematic-to-line, and schematic-to-cut-color relations to one pair of parts, equivalence of these stimuli occurred with the remaining pair of assembly parts without additional training. This training technique will allow individuals with severe developmental disabilities to learn complex assembly tasks required in-vocational settings.

Education

With the enactment of PL 94-142, an increasing number of children with mental retardation are being integrated into regular school classrooms. One anticipated result of bringing disabled individuals into the mainstream of public education was the fear that children with mental retardation would not be socially accepted. A six year study of the social acceptance of children with mental retardation found that the quality of the relationships between normal children and children with mental retardation is the result of a complex interaction of factors including: the appearance, academic ability, and social behavior of the child with mental retardation; the extent to which the normal children identify themselves with the disabled child and classroom placement; labels used to describe children with mental retardation; and teacher differences.

STAFF ACTIVITIES

CONFERENCES

A meeting entitled "Assessment of Behavior Problems in Persons with Mental Retardation Living in the Community" was held at the NIH on September 9, 1988, under the joint sponsorship of the National Institute of Mental Health and the MRDD Branch of NICHD. At this workshop a group of investigators presented the methods they use to assess persons with developmental disabilities who exhibit behavior problems in community, clinical, and research settings.

A conference on the "Effects of Inborn Errors of Metabolism on Pregnancy Outcome" was sponsored by the MRDD Branch on September 8-9, 1988. The conference convened a small group of investigators who discussed the effects of several maternal inborn errors of metabolism on reproductive capacity and on the fetus.

STAFF CHANGES

David B. Gray, Ph.D., formerly director of the National Institute on Disability and Rehabilitation Research, accepted a position in the MRDD Branch as Health Scientist Administrator for Behavioral Sciences.

PUBLICATIONS

Friedman, E.G., Koch, R., Azen, C., de la Cruz, F., Levy, H., Rouse, B., and Hanley, W.: Maternal phenylketonuria collaborative study (MPKUCS): USA and Canada. Abstract, The American Journal of Human Genetics, 41:3, Sept. 1987 supplement.

de la Cruz, F.: Epilogue. Understanding Mental Retardation--Research Accomplishments and New Frontiers. Kavanagh, J.F., Editor, Paul H. Brookes Publishing Company, Baltimore, Maryland, 1988.

de la Cruz, F. and Oster-Granite, M.L.: Neural Bases of Mental Retardation. In, Handbook of Human Growth and Developmental Biology, Vol. I: Part C, 1-20, 1988.

NICHD CVS Study Group: Diagnostic Accuracy in chorionic villus sampling (CVS). Abstract, The American Journal of Human Genetics, 43:3, Sept. 1988 supplement.

Human Learning and Behavior Branch

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OVERVIEW

The Human Learning and Behavior Branch (HLB) supports basic research and research training on behavioral development from the perinatal period to the beginning of adulthood. To attain the goals of this mission, the Branch employs a variety of support mechanisms. These include regular research grants, program projects, institutional training grants, individual postdoctoral fellowships, research career development awards, and contracts. The research knowledge gained provides a basis for understanding the ontogeny of normal development and criteria for assessing developmental delays and learning disabilities.

The primary focus of the Branch's program is to develop and support research that promotes children's health. This is accomplished by funding research grants that in the aggregate are designed to determine how the interaction of biological, psychological, and socioenvironmental factors result in normative behavioral development and to identify the factors that interfere with such development. Processes and behaviors specific to each stage of development are investigated. These include studies of behavioral development in children born at biological risk for a variety of behavioral disabilities: learning problems, delayed or impaired speech, and dyslexia. Biological, behavioral, and social science disciplines all play key roles in achieving the Branch mission; and interdisciplinary approaches are emphasized in this effort.

The program is divided into five major elements: (1) Behavioral Pediatrics, (2) Biological Bases of Behavioral Development, (3) Learning and Cognitive Development, (4) Communicative Abilities, and (5) Social and Emotional Development.

PROGRAM ACTIVITIES

During the time covered by this report, funds for the five program elements amounted to $28.2 million. Of the total 237 grants supported, approximately 85 percent are regular research grants. Such grants are provided to individual investigators who initiate the ideas and foci for their research projects. A full breakdown of financial support is provided in table 5.

Table 5

NICHD GRANTS AND CONTRACTS ACTIVE DURING AUGUST 1988
HUMAN LEARNING AND BEHAVIOR BRANCH

Funds (in thousands)

Health Area	Total		Research Grants						National Research Service Awards		Research Contracts	
			Total Research		Research Projects (Incl. P01)		RCP Awards					
	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds	No.	Funds
Total	237	$28,221	200	$24,924	189	$24,358	11	$566	32	$2,255	5	$1,043
Developmental Behavioral Biology	49	5,647	40	5,107	37	4,932	3	175	9	540	-	-
Human Learning, Cognition, Perception, Memory	67	5,315	54	3,961	50	3,787	4	174	12	1,068	1	286
Social and Affective Development	42	4,565	40	4,512	37	4,350	3	162	2	53	-	-
Human Communicative Processes	55	9,621	49	9,303	48	9,249	1	54	5	203	1	115
Behavioral Pediatrics	24	3,073	17	2,041	17	2,041	-	-	4	390	3	642

Note: Excludes seventeen grants and two contracts funded from sources other than NICHD extramural funds. Columns may not add to total due to rounding.

NICHD-OPE-PAS
August 9, 1988

RESEARCH TRAINING

Training of predoctoral and postdoctoral researchers is an integral part of the Branch mission. This is carried out through the award of institutional training grants and individual postdoctoral fellowships. During the time covered by this report, support of training amounted to $2.25 million. A major theme of the training program is that awards are interdisciplinary in nature and stress a biobehavioral approach to development.

RESEARCH HIGHLIGHTS

BEHAVIORAL PEDIATRICS

Research in this area is concerned with applying the principles of human learning and development to medical care and to health and illness behaviors of the pediatric population and their families.

Perinatal Behavior

The newborn infant was once considered incapable of learning and discriminating. Recent advances in the field of infant behavior and development have revealed that the capabilities of neonates have been seriously underestimated and have opened new avenues for understanding and examining the behavior and development of newborn infants. Included in this category is research related to the practice of pediatrics in the perinatal period, normative neonatal development, and behavioral effects of environmental exposure.

One researcher is investigating the assumption that neonates do not feel pain. Newborn infants undergoing invasive medical procedures in an intensive care unit are randomized to receive lidocaine or no anesthesia, as is currently common practice. Preliminary results indicate that physiologic, behavioral, and acoustic parameters exhibit significant alterations in response to routine medical procedures and that local anesthesia attenuates fluctuations in these responses. Further work is examining the relationship of vagal tone to activity of neurochemical mediators of pain, such as beta-endorphins.

A small business innovative research project (SBIR) is developing equipment for recording the pressure signal during nutritive sucking and the analysis methodology to relate these signals to clinical status of neonates. Results from phase I of the project revealed that the sucking variables alone or in combination could distinguish between full-term and pre-term infants to a very high level of significance. These results suggest that a neonatal sucking instrument, when combined with other measures, such as heart rate, can provide a sensitive measure of neonatal status. Phase II will focus on sucking organization of sick infants compared to healthy infants.

Behavioral Development in Relation to Medical Conditions

Practitioners and researchers have long been concerned about the impact of various illnesses and conditions on child development. Study of the effects of conditions such as prematurity and central nervous system infections on neurobehavioral development offer opportunity to provide guidance for medical practice as well as to improve understanding of the processes of behavioral development. One

investigator is analyzing cognitive functions and assessments specifically targeted at identifying infants at risk for developmental disability early in infancy. By systematically investigating information processing capacities, habituation, recognition memory, novelty preference, and cross-modal transfer, the foundations are being laid for early assessments and interventions of high risk infants. Another study is examining the effect of H. influenza meningitis on neuropsychological and behavioral functioning in a population of school aged children. Children with acute phase neurological complications during meningitis are at higher risk of developing neurological complications than those without such complications. Preliminary results also indicate that postmeningitis children do not perform as well on academic measures and on selected neurophysiological tasks as controls, suggesting that postmeningitis children will require more special assistance at schcol.

Otitis media affects approximately 80 percent of children in the first 3 years of life. Conflicting evidence and debate have failed to resolve whether children with frequent otitis media are more likely to experience cognitive or language delays. To investigate this, a recently initiated study will electronically monitor the middle ear status of 350 infants and children from birth to 5 years. Children with extended otitis media will be randomly assigned to receive either early ventilation tube replacement or conventional, more conservative management. Hearing, behavior, language development, and cognitive function will be evaluated through 5 years of age. Correlation of these outcome variables with early middle ear status will allow assessment of these interrelationships and the effect of early treatment for this condition.

Risk-Taking Behavior

The consequences of risk-taking behavior comprise the major source of mortality and morbidity in childhood and especially adolescence. Research directed to understanding and modifying risk-taking behavior is a major focus of the Branch.

Childhood Injury

As the major cause of mortality after the first year of life, prevention of childhood injuries is of high priority to the Institute and the Branch. Eight studies are under way to investigate mechanisms and antecedents of injury.

The first study seeks to determine the accuracy of parental information regarding home hazards in relation to perceived purposes of the observation, to assess the relationship between home hazards and children's injuries, and to analyze the interaction of parents and children in the presence of contrived hazards. Preliminary results revealed that, when their mothers were distracted, children with a history of injury had a higher level of hazard contact, disruptive behavior, and activity change than children without prior injury. The parents of injured children had significantly lower rates of play activities with their children. Furthermore, elevated scores on the standardized Child Behavior Check List identified children whose behavior placed them at risk of injury. Thus, this project has identified both behaviors to target for reducing the risk of injury and a convenient checklist to screen for children at risk.

Another study was recently initiated to investigate the relationship of childhood injuries to behavioral-developmental features of the child's temperament and attention, parental characteristics, and the family environment. This longitudinal study of twins also furnishes a unique opportunity to examine subtle conditions

associated with one twin being injured more than the other. Preliminary findings revealed that, when comparing medical records with paternal interviews concerning medically attended injuries 1 to 4 years earlier, parents were largely accurate concerning nature of injury and treatment involved. The study should ultimately specify the developmental aspects of the child and the child's behavioral and material environments that can be targeted for injury control.

Another study describes through longitudinal home visits with comprehensive video and audio recordings, the entire set of factors existing within a family which help maintain a child's safety within the home. Preliminary results reveal how children's spatial access to the home and their interaction with parents change with development. The study also suggests that there are a wide range of safety management systems as a result of different emphasis on physical, supervisory and training constraints and relates these differences to the parents' stated beliefs about child development and etiologies about child rearing.

BIOLOGICAL BASES OF BEHAVIORAL DEVELOPMENT

The Branch supports some 40 grants that focus on the biological basis of behavioral development. These comprise approximately 21 of the Branch portfolio. Included in this aspect of the program are studies of brain/behavior relationships; the biochemical, physiological, and hormonal basis of behavior; sensory motor processes; and comparative animal behavior. More specifically, the Branch solicits and funds research in five topical areas: (a) developmental behavioral neurobiology; (b) developmental behavioral genetics; (c) developmental behavioral endocrinology; (d) sensory and psychomotor development; and (e) developmental behavioral toxicology.

Hormonal Aspects of Behavioral Development

Research on brain/behavior relationships that are influenced by hormonal secretions has played a significant role in gaining an understanding of social, maternal, species specific, and sexually dimorphic behavior. A number of studies supported by the Branch are investigating how hormones help organize the central nervous system and thereby determine social behavior. This work is laying the groundwork for elucidating how androgens and estrogens interact to organize brain regions responsible for activating vertebrate social behavior. A number of studies supported employ birds as the subjects. The use of avian species is appropriate because their social interaction patterns are so well studied. Therefore, experimental manipulation of hormones can be related to social interaction patterns. For example, one of the Branch supported investigators is examining the hypothesis that estrogen secretion in the male Zebra-finch increases the number of androgen receptors in areas of the brain that are associated with sexual function and species-specific song. This work also includes studies of the role played by dihydrotestosterone (DHT) and estradiol on cholinergic and cholinoceptive neurones that have been implicated as being necessary for the control of steroid-sensitive vocalizations in this bird species. Avian models are also useful for elucidating the way that hormones help organize areas of the brain involved in other species-specific behavior. For example, research on mechanisms by which estrogen secretion increases androgen target cell numbers in areas of the brain thought necessary to mediate song in males and androgenized females is under study. The investigator is also attempting to identify areas of the finch's brain where estrogen acts during development to masculinize song control loci.

Results have shown that masculinization may occur by: (a) selective retention of certain cells during a time when others die off or (b) neuron proliferation. In this latter process, cells in certain song control nuclei of the brain are actually added during development.

Other studies supported by the Branch are aimed at understanding the relationships between hormones and the central nervous system in the initiation of maternal behavior. A recent conference was sponsored by the Branch, "Biological and Behavioral Aspects of Parenting in Mammals," to explore this and other relationships of hormones, brain and behavior. Several NICHD grantees have been investigating animal models that may help elucidate hormonal mechanisms which trigger maternal behavior upon parturition. One grantee, for example, is studying the role of prolactin in the initiation of maternal behavior. His studies focus upon the sites that prolactin acts in the brain during various reproductive states. Another one of his studies focuses upon the behavioral effects of prolactin when it is directly administered in to the lateral ventricles and other brain loci of female rats which have not given birth. Included in his research program are experiments to examine the specificity of prolactin's probable site of action by treating rats centrally with hormones in the prolactin-like family of hormones such as growth hormone and placental lactogen. Recent findings from his laboratory indicate that progesterone and ectopic graft secretions stimulated a rapid onset of maternal behavior in both hypophysectomized and nonhypophysectomized female rats. Exposures to progesterone or the grafts alone were without effects. Such work exemplifies another aspect of behavior in which hormones exert a controlling influence. This research is part of a genre which will significantly enhance our understanding of how brain interactions with hormones mediate parenting behaviors in mammals.

Developmental Behavior Genetics

An important approach for asking questions concerning behavioral development involves the use of behavior genetics methodology. This aspect of our Branch program focuses upon questions designed to determine the relative contributions of heredity and environment in development of intellectual capacity. One Branch supported investigator is using a "full" twin design to ascertain the proportion of variance in IQ that can be attributed to inheritance. The study employs twins reared apart and compares their scores on intellectual development through adulthood. The IQs of their adoptive parents and their biological parents are also measured to help sort out the questions of nature and nurture. The investigators found that at 1 year of age, 8 percent of the variance in IQ could be attributed to genetic factors. At 7 years of age, 35 percent of the observed variance could be ascribed to heredity. At the time of adulthood, they estimate that some 40-50 percent of the variance in IQ is related to genetic factors.

LEARNING AND COGNITIVE DEVELOPMENT

Learning

The Branch portfolio contains a number of grants which are designed to gain a basic understanding of learning mechanisms within both comparative and developmental frameworks.

A new and exciting trend in biological science is the analysis of mechanisms of interest at the molecular level. Work at this level of analysis for learning is

in its formative stages of development. The Branch supports examples of this type of research. One such project involves the exploration of mechanisms that may subsume associative conditioning in the marine mollusk, Hermissenda. Results from the work to date have demonstrated that a conditioned stimulus (CS) paired with the direct application of serotonin (5-HT) can mimic conditioning in this animal. The studies have also shown that long-term memory associated with conditioning is dependent upon protein synthesis. The work is important because it demonstrates a way to employ biological tools for analyzing the basis for simple forms of learning and memory.

Another trend that is apparent in our grant portfolio is research on learning as early in life as behavior can be measured. By studying learning processes as they emerge, Branch supported researchers have a better chance of characterizing the necessary mechanisms that underpin these behavioral processes. Thus, a number of our researchers are studying learning during the perinatal period of development.

One of our grantees, for example, has continued his research on behavioral development in the rat fetus. His work has demonstrated that learning (classical conditioning) can be achieved in this organism as early as day 17 of gestation and is consolidated by day 19 of gestation. Such learning by the rat fetus is retained beyond the second week of postnatal life. The research has also shown that there is a systematic ontogeny to the organization of rat fetal movement. This research has opened the way for the systematic study of learning in the rat fetus and portends new methodological breakthroughs for conducting behavioral toxicology research.

Closely allied to the aforementioned studies is work being conducted on early learning in the neonatal rat pup. One of our researchers continues his studies of motivation in 1-day-old rat pups. His work to date has demonstrated that behavioral activation is not essential for reward or learning. His recent studies have shown that lateral preference learning is lost when the commissural projection systems of the rat pup's brain are developed and in place (2nd postnatal week). This investigator has also elucidated specific brain loci that are correlated with motivation. He has employed 2-deoxyglucose autoradiographic methods to map areas of the brain that are necessary for the type of early learning that he is investigating.

One exciting new trend that has been emerging during the past 4 years is that researchers who have studied learning using animal models have begun to analyze functional learning in the human neonate. One such scientist has focused his work on the learning mechanisms employed by 1-day-old human babies to recognize their care giver. His work supports the conclusion that classical conditioning is a likely mechanism by which babies learn about their mother. Results have revealed that click sounds similar in spectographic properties to those made by nursing mothers can serve as effective conditioned stimuli for head turning and lip puckering in neonates. Such associative learning may help promote mother-infant attachment.

In summary, the Branch has continued to support learning research at the cutting edge of this field. The work is making contact with molecular biology and is studying mechanisms of learning as they emerge during perinatal development. Most importantly, those who have long worked in animal research are applying their analytic skills to the human neonate to help elucidate the functional significance of learning mechanisms as they occur in natural ecological settings.

Cognitive Development

The HLB Branch supports research on the mental processes of infants and children and on changes in these processes as a result of development, practice, and exposure to different favorable or unfavorable experiences. Examples of mental processes include attention, perception, learning, and memory.

Infant Cognition

The Branch supports a substantive number of grants in the area of infant cognition. Most of these deal with the attributes of objects that lead infants to behave toward them as entities and as belonging to a class of other objects. The mechanisms by which infants collect and store information about objects, i.e. attention, sensitivity to similarity and to contrast among displays, the cross modal enrichment that occurs when information arrives through multiple sensory modalities, and knowledge representation are also being investigated by researchers supported by the Branch. The growing interest among cognitive development researchers in the predictions that can be made from cognitive status in infancy to later cognitive status and in social influences on the acquisition of cognitive skills is also reflected in the grant portfolio of the Branch.

One of the investigators in the area of infant cognition completed two studies of the development of infants' ability to use gestalt relations as information for the unity of partly occluded objects. The principle of gestalt that was investigated in relation to infants' perception is that of good continuation (that we see contours as following natural lines). The findings revealed that 4-month-old infants, who fail to use gestalt relations, are nevertheless able to detect those relations in the visual mode and in the haptic mode. Infants were habituated to a center-occluded object whose ends were united by the relations of good continuation and similarity, then they were with a center-occluded object whose ends were not united by these relations. Significant dishabituation occurred in each modality. The experiments provide evidence that 4-month-old infants do detect gestalt relationship in partly occluded objects. Their failure to use those relationships to specify the unity of such objects does not stem from a failure of perceptual discrimination.

Cognitive Development in Childhood

In addition to the investigations described above, the HLB supports research exploring the development of cognition beyond infancy. One research question that ties the infant cognition research and the childhood cognition research is that of concept formation. The term concept formation refers to the abstraction of categories on the basis of exposure to specific exemplars from a given category.

One HLB supported investigator studies the acquisition of relational concepts; another studies concept learning under conditions that allow the discovery of predictive relations among characteristics. Concept formation can be subsumed under the more general topic of knowledge acquisition, that is a focus of other investigations. For example, one of the HLB grantees asks: When someone points to an object and labels it, how does the child determine that the term refers to the object category as opposed to all the other possible things it could mean? Other HLB supported investigators are asking questions and providing answers about knowledge representation in memory and about knowledge use. One investigator found that the child's representation of narrative discourse is depicted in terms of a network of events and their causal relations. Another investigator is studying

the strategies children use when solving math problems. It should be noted that the last two examples relate to children's everyday cognition, the cognition that children use on a daily basis when they attend school. This interest in children's acquisition, representation and use of information or cognitive skills that serve them well in their daily lives is reflected in many of the investigations that are supported by HLB.

A specific example of research in the area of knowledge representation comes from an HLB-supported study aimed at understanding, (a) the development of early knowledge about experienced events and (b) young children's retrieval and use of event knowledge. Three- and 4-year-olds practiced in a "science experiment" event over 8 weeks. Children in one group were questioned later on the same day of each event occurrence; children in the second group were questioned one week after each event, before the next event occurrence; children in the third group were questioned after the eighth event occurrence. Preliminary data show that 4-year-old children report more and need less prompting than 3-year-olds. Children in both age groups report more as a function of experience. Approximately a month after the eighth event session, eight children in each age group were presented with six line drawings depicting actions which occurred in the "science experiment" procedure. With the exception of one 3-year-old child, all children were totally accurate in judging the temporal order of the occurrence. Two other groups of children were asked to judge the importance or centrality of the events. The task proved significantly more difficult than the temporal ordering task. Agreement within age groups and with adult judgments increased with age. A study of judgments of typicality of events showed that children's judgment were more variable than those of adults and that children's judgments correlated more highly with those of adults as a function of age.

COMMUNICATIONS

This part of the Branch supports studies of genetic, neurological, maturational, and environmental factors influencing the development of speaking, listening, writing, and reading. The study of symbol acquisition by great apes, modality specific aspects of language development, the development of speech reception and production, and the complex processes involved in the acquisition of reading have been the focus of the HLB Branch funded research. Discovering biological bases of human communication, early identification of abnormal language development, defining and categorizing poor readers, and linking the results of basic research to improving intervention programs are other areas funded by the Branch.

Reading

Over the past 20 years, studies funded by the Branch have provided experimental evidence of the importance of how children make the transition from written language. Poor readers have great difficulty segmenting printed and spoken words into individual components (Phonemes). Students were tested to measure their proficiency in reading nonsense words and unfamiliar words. Less skilled readers performed poorly, suggesting that poor readers have great difficulty decoding the written word into a sound code for processing. Preschool children who are good at rhyming words or parts of words are found to be good readers when examined in grade school. Comparisons of second grade poor readers with sixth grade poor readers showed that the inability to use sound decoding skills to read new words remained deficient even when other reading-related skills improved. These findings illustrate that phonological skills are important in acquiring proficiency in

reading the written language and that some phonological skills may be improved through practice.

Reading--Processing Abilities

In addition to phonological abilities, reading requires rapid storing of information in short-term memory and retrieving information from long-term memory. Studies comparing dyslexic and normal reader skills in recalling words immediately after hearing them presented find that dyslexics are less able to use short-term memory for recalling the verbally presented words. Tests of retrieval of words, objects, colors, and numbers were all performed slower and with more errors by dyslexics. This implies that the retrieval processes of dyslexics are impaired. In an association and rule-learning comparative study of storage and retrieval of visual-verbal and visual visual associations, the dyslexic children performed poorly when compared to normal readers only on the association task requiring storage and retrieval processes of visual-verbal associations. These findings indicate that dyslexics have a specific deficit in coding, storing, and retrieving symbols with sound equivalents.

Eye movement measures are being studied not to investigate a causal role in the acquisition of basic skills, but as a way of characterizing changes in reading comprehension and to study cognitive processes involved in reading. As people read, they make a series of discrete eye movements (saccades) and pauses (fixations) which can be tracked using sophisticated computerized equipment. One Branch supported investigator compared the eye movements of young children who were learning to read with the eye movements of adult proficient readers. His studies show that by first grade the attentional and oculomotor systems are fully developed in their ability to target a word in the text for attention and to move the eyes to that word. In contrast to children, adult good readers tend to spend less time, have fewer fixations, and have a reduced number of gazes on words with which they are familiar. Adults spend proportionally more time when they encounter new or unfamiliar words as measured by increasing fixations and gazer frequency. As children advance in their reading skills, the fixation times and the probability of refixating on a word show similar curves but with different parameters. These findings may indicate that, as basic oral to written transition skills are mastered, more time is devoted to the comprehension of the words being read.

Reading--Test of Comprehension Levels

The development of reliable and valid assessment tools to normalize and/or criterion reference reading comprehension abilities has been a difficult task. A Branch supported Small Business Innovative Research grant has developed a computer-based reading scale that assigns comprehension difficulties to a wide variety of commonly read texts. The scale was constructed by using a two-component model using measures of syntactic and semantic difficulty. Textual materials were then subjected to an analysis for difficulty level. Reading materials were classified on a scale ranging from 100 to 1100, primer texts at the low end and encyclopedia text at the high end. By reanalyzing the results of nationally standardized reading comprehension tests with his scaling technique, the investigator was able to construct a "universal norm" based on a population of over 200,000 students. The scale may provide teachers a pragmatic test for assessing which reading materials students are most likely to comprehend at different stages of their development.

Reading--Genetics

A study sponsored by the NICHD for 11 years has used a variety of genetic methods to examine the hypothesis that at least some part of the explanation for reading deficiency is explained by genetic differences. Using a family studies approach, the investigators conclusively demonstrated a familial component to reading disability. However, since family members share both genetic and environmental influences, these findings alone do not provide sufficient evidence of a genetic component to reading disability.

In order to demonstrate a genetic component to this finding of familial influence, these investigators have examined reading skills through the use of twin and chromosomal linkage methods. By examining the concordance rates for reading disability in identical (MZ) and fraternal (DZ) twins, an estimate of the genetic (heritable) component of reading deficits was estimated to be approximately 30 percent. Another study used probability studies to examine the possibility of genetic linkage of a specific type of reading disability with a cytological marker on chromosome 15. This apparently autosomal dominant transmitted type of reading disability was found to be linked to chromosome 15. Independent studies are currently being supported to confirm or refute this finding.

Given variability in the development of reading abilities, measuring reading skills at different ages could produce different genetic loadings for component skills. If phonological processing skills are important in the development of language and the capacity for categorizing acoustical signals into elementary units is present very early in development, then one might ask to what degree this specific reading characteristic is heritable. One study recently showed that, while visual coding skills are not heritable, some precursors of the phonological coding skill may have a significant genetic component. Studies of reading performance, symbol processing speed, and spatial/reasoning have found that disabled readers perform worse than normal readers on all measures. While the rate of improvement of disabled and nondisabled readers was similar for reading performance and spatial/reasoning measures, the disabled readers did not improve at a comparable rate to nondisabled readers in their ability to rapidly process symbols. This finding argues for differential developmental capacities for the component skills that are necessary for proficient reading. The results support the hypothesis that there may be different genetic influences for these component processes and probably differential effectiveness in remediation of the various deficiencies.

SOCIAL AND EMOTIONAL DEVELOPMENT

Social Development

The Branch is supporting research on the social development of children. It is interested in investigations of social development from early infancy through the toddler age, early childhood, and adolescence. From birth on, the child is a part of a social environment. He or she communicates with "social others," entering into close interpersonal relationships with some of them, learning how to behave in a socially acceptable, desirable, and captivating way. At present, investigators are studying social information processing; the relationship between information processing and the development of social competence and peer relations; the role of family variables (e.g., family interactions processes) in the development of the child's peer relations; qualitative features of sibling relationships; and the role of ethnicity in friendship and in social adjustment.

One investigation focuses on the development of companionship and intimacy during preadolescence and adolescence. The research participants, who were in second, fifth, and eighth grades, rated the importance and extent of companionship and intimate disclosure they experienced. Companionship was perceived as a desired social provision at all three grade levels. Family members were found to be important providers of companionship for children in the second and fifth grades, but they became significantly less so in the eighth grade. Same sex peers were important as children grew older. Opposite sex peers did not become important as companions until the eighth grade. There were no age differences in the global desire for intimacy. Parents were important providers of intimate disclosure for the youngest children, but they were less important among the younger adolescents.

Emotional Development

The Branch is supporting investigators who study emotions and emotional development in infancy, in childhood and in adolescence. Emotions like joy, hope, affection, interest, anger, disgust, surprise, fear, sadness, guilt, and shame exist in the repertoire of children and many of these emotions are known to also be present in the repertoire of infants. The study of emotion has recently entered a period of great resurgence of interest. This interest is mediated by important advances in the measurement of emotions in the face, voice, autonomic nervous system, and the brain. In addition, important conceptual advances are taking place in understanding both the nature of emotion elicitation, and the functions of emotional reactions.

The grants reflect some of the lines of research that are now being pursued by researchers of emotional development. One investigator studies the relation between facial signs of discrete infants' emotions and the concomitant central nervous system processes. Another investigator concentrates on one of the complex emotions--empathy. She plans to differentiate various modes of empathy, to study the convergent validity of various measures of empathy, and to examine age-related changes in it. Another researcher studies what might be considered a cognitive prerequisite of empathy--that is, children's ability to use personal information to infer emotional reactions of other people. Another complex emotion that is being studied by an HLB Branch supported investigator is that of helplessness in reaction to failure experiences.

One HLB supported investigator studies children's ability to use person-specific information to predict and explain the emotional reactions of other people. Children in kindergarten, second grade and fifth grade heard six stories of either the protagonist's behavior or experience in one situation, followed by a second, similar situation. They were asked to infer the protagonist's emotional reaction in the second situation. Other children only heard about the protagonist's behavior or experience in the first situation and were asked to infer what the protagonist thought of the situation. The findings indicate an increase with age in children's ability to infer other people's appraisals from their prior behaviors or experiences, and an increase with age in children's ability to make emotional inferences that reflect their appraisal. Children are better at inferring other people's appraisals of situations than at using that understanding to infer these people's emotional reactions to later, similar events. When inferring other people's appraisals and probable emotional reactions, children are marginally more influenced by information about the people's prior behaviors than by information about their prior experiences.

SOCIAL INFLUENCES ON COGNITIVE, SOCIAL AND EMOTIONAL DEVELOPMENT

The Branch supports a number of grants that investigate the processes by which the culture and the family contribute to the cognitive, the social, and the emotional development of children.

Social Influences on Cognitive Development

There is ample information in the research literature showing that the family and the culture in which the child grows have an effect on his or her cognitive development. These data, as well as the interest in developing intervention programs, have led scientists to ask questions as to how members of the family and how institutions such as schools affect psychological processes involved in knowledge acquisition, its representation in memory, and its use in problem-solving situations.

One recently funded project will investigate the different types of parental interaction strategies and the factors that contribute to variation in those strategies. The same project will assess the contingency of mother and child behaviors and the extent to which parental behaviors help 4 and 1/2-year-old children become competent.

Social Influences on Social and on Emotional Development

Evidence from anthropological research indicates that social and emotional behaviors of individuals have both universal and culture-specific aspects. The many culture-specific aspects suggest that social and emotional development are shaped by social factors. Developmental psychologists conducting research in the areas of social and emotional development are asking questions such as who are the socializers, what are the circumstances under which socialization occurs and what are the specific interactional processes by which it occurs. The Branch supports investigations pertaining to the social influences that shape social and emotional development. These studies focus on family variables (e.g., working parents, step-families, quality of marriage) and maternal variables (responsiveness of the mother) as possible shapers of social and emotional adjustment.

One HLB-supported study revealed a correlation between dysfunctional family processes, more life stress, less cohesion, less adaptability, and increased behavior problems in children.

STAFF ACTIVITIES

REQUESTS FOR APPLICATIONS

Requests for Applications were issued as follows:

Behavioral Aspects of AIDS Prevention in Children and Adolescents (88-HD/MH-01)
Behavioral Mechanisms and Causes of Childhood Injury (88-HD-03)
Development and Change in Planning Skills Throughout the Life-Span (88-HD/AG-11)
Effects of Non-Parental Infant Day-Care on Child Development (88-HD-08)
and Learning Disabilities: Multidisciplinary Research Centers (88-HD/NS-11)

WORKSHOPS AND CONFERENCES

Biobehavioral Foundation of Language Development. June 12-15, 1988. Branch Organizer: Norman A. Krasnegor

Exchange of Information on Research Program Projects on Dyslexia. May 2, 1988. Branch Organizer: David B. Gray

Generalizing from Experience: An Issue of Development. September 23, 1988. Branch Organizer: Sarah L. Friedman

Issues of Reliability and Validity of Measurements of Risk Behaviors Associated with HIV Infection Among Adolescents. September 15-16, 1988. Branch Organizer: Norman A. Krasnegor

Socialization of Emotion. May 15-17, 1988. Branch Organizer: Sarah L. Friedman

Social Influences on the Development of Children's Practical Intelligence. June 26-28, 1988. Branch Organizer: Sarah L. Friedman

PUBLICATIONS

Breznitz, Z. and Friedman, S.L.: Toddlers' ability to concentrate: The influence of maternal depression. Journal of Child Psychology and Psychiatry, 29:267-279, 1988.

Friedman, S.L. and Malloy, M.H.: A review of Ensher, G.L. and Clark, D.A. "Newborns at risk." Child Development Abstracts and Bibliography, 62:109, 1988.

Friedman, S.L., Scholnick, E.K., and Cocking, R.R. (Eds.): Blueprints for thinking: The role of planning in cognitive development. New York: Cambridge University Press, 1987.

Friedman, S.L., Scholnick, E.K., and Cocking, R.R.: Reflections on reflections: What planning is and how it develops. In S.L. Friedman, E.K. Scholnick and R.R. Cocking (Eds.). Blueprints for thinking: The role of planning in cognitive development. New York: Cambridge University Press, 1987.

Kochanska, G., Radke-Yarrow, M., Kuczynski, L., and Friedman, S.L.: Normal and affectively ill mothers' beliefs about their children. American Journal of Orthopsychiatry, 57:345-350, 1987.

Krasnegor, N.A.: Adolescent Drug Use: Suggestions for Future Research. In Adolescent Drug Abuse: Analyses of Treatment Research, NIDA Monograph Series 77.

Krasnegor, N.A.: On Fetal Behavior. In Smotherman and Robinson (Eds.) Fetal Behavioral Development. Telford Press, 1988.

Krasnegor, N.A., Grave, G.D., and Kretchmer, N. (Eds.): Childhood Obesity: A Biobehavioral Perspective. Telford Press, 1988.

Scheidt, Peter C. and Participants: Behavioral Research Toward Prevention of Childhood Injury: Report of a Workshop Sponsored by the National Institute of Child Health and Human Development, September 3-5, 1986. AJDC 142:612-617, 1987.

Scholnick, E.K. and Friedman, S.L.: A plan for plans: An analysis of theories and research. In Friedman, S.L., Scholnick, E.K., and Cocking, R.R. (Eds.) Blueprints for thinking: The role of planning in cognitive development. New York: Cambridge University Press, 1987.