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EXECUTIVE SUMMARY

The Endocrinology Nutrition and Growth Branch (ENGB) is pleased to present a summary of its activities to the National Advisory Child Health and Human Development (NACHHD) Council. ENGB supports research on endocrinological and nutritional influences on growth, body composition, puberty, skeletal accretion, and brain development. The Branch also emphasizes research on the prevention during childhood of chronic disease later in life, such as obesity, atherosclerosis, diabetes, and osteoporosis. Another major emphasis lies in pediatric pharmacology and the development of better and safer drug therapies for newborns, children, and adolescents.

The Branch supports a network of 13 Pediatric Pharmacology Research Units (PPRUs) that perform clinical trials of drugs in children. The Branch also supports 20 Child Health Research Centers (CHRCs), the mission of which is to speed the translation of basic research advances to the patients' bedside and train the next generation of academic pediatric investigators. This report covers ENGB activities that occurred since its last presentation to the NACHHD Council, in September 1996.

The ENGB is one of the six branches of the Center for Research for Mothers and Children (CRMC). The Branch provides a focus at the NICHD for research and research training in nutritional science, antecedents in childhood of disease later in life, developmental endocrinology, and physical growth. Within these areas, ENGB pursues the following topics.

PREVENTION OF CHRONIC DISEASE

The burdens of obesity, cardiovascular disease, diabetes, and osteoporosis continue to increase in this country and abroad. These chronic diseases have their roots in childhood. Because these conditions are difficult or impossible to reverse in adulthood, ENGB encourages research on preventing their onset during childhood as the focus of its recent programmatic initiatives.

- **Obesity:** The Branch has provided long-term guidance, support, and encouragement to biological and behavioral scientists tackling the genetic, environmental, and behavioral origins of obesity. In addition to research directed at the causes of obesity, the Branch funds research on behavioral modification and other innovative approaches to reverse obesity in childhood.
- **Atherosclerosis:** The Branch supported a large initiative to study several thousand children in families that are susceptible to premature coronary artery disease. This effort revealed several robust predictive markers for the disorder in childhood, including elevated serum triglycerides, high plasma fibrinogen, and obesity. The Branch will hold a conference in September 2000, to build on these findings.

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- **Diabetes:** In an effort to prevent this disease, the Branch has pioneered methods in ascertaining risk factors and stratifying levels of risk for type 1 (juvenile) diabetes mellitus according to genetic and immunologic markers. This work forms the basis of the Diabetes Prevention Trial, a major, multi-institute study aimed at preventing or delaying the onset of type 1 diabetes in relatives of index cases. ENGB is also working with the Juvenile Diabetes Foundation International to co-fund a large prospective study of infants who have relatives with type 1 diabetes, to ascertain the earliest changes in gene expression in those infants who become diabetic. In addition to its efforts in preventing type 1 diabetes, the Branch also supports large research programs aimed at preventing type 2 diabetes mellitus and gestational diabetes mellitus.
- **Bone Health:** The Branch has greatly augmented its support for osteoporosis prevention research since its last report to the NACHHD Council. Currently, Branch researchers are studying the bone mineral densities (BMDs) of several thousand children prospectively to assess the effect of dietary and behavioral interventions.

NUTRITION

Some advances made by ENGB in the field of nutrition include:

- **Infant Nutrition:** In a series of major research initiatives, Branch-supported researchers have established the nutrient requirements for term infants, as well as the nutrient, antimicrobial, and hormonal contents of human milk and colostrum. The Branch is now encouraging research on the nutrient requirements and optimal feeding regimens for infants born prematurely. As the survival rate of these delicate infants increases, the question of their nutrient needs becomes more pressing. ENGB staff is also involved in understanding the effects of essential fatty acids on infant visual acuity and brain development, to determine whether docosahexaenoic and arachidonic acids should be added to infant formula. Investigators funded by the Branch continue to make important contributions in this field of research.
- **Maternal-Fetal Nutrition:** The placental origin of intrauterine growth retardation is an important, but understudied, area of research. The Branch encourages research on the role of placental nutrient transporters in the net transfer of nutrients to the fetus. Mutations in the genes that code for nutrient transporters are also of interest, and will be the target of future initiatives. In addition, epidemiologic observations on the fetal origins of adult cardiovascular disease have energized the field of maternal-fetal nutrition. The Branch is funding several large studies of maternal nutrient intake in relation to infant outcome. The Branch also recently issued a Request for Application (RFA) calling for more research on molecular mechanisms to explain how perturbed intrauterine environments may predispose fetuses to cardiovascular and other chronic disease later in life.

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- **Developmental Gastroenterology:** Because premature babies are at high risk for necrotizing enterocolitis, the Branch targets this inflammatory intestinal disorder of newborns as a high research priority. As a result of programmatic initiatives, the Branch is funding research on the permeability of the fragile intestine of premature infants; the Branch is also investigating blood flow regulation of the intestine in animal models of prematurity.

ENDOCRINOLOGY

In the field of endocrinology, some of the Branch's activities include:

- **Pediatric Endocrinology:** Through a series of conferences, the Branch has been at the forefront of efforts to curb the inappropriate use of growth hormone (GH) to treat children who, although of short stature, are not deficient in GH. ENGB staff worked closely with the NICHD Intramural Developmental Endocrinology Branch to design a randomized, placebo-controlled clinical trial to test the efficacy of GH in these children. Results of this trial will determine indications for pediatric use of GH in this country. The Branch has also had a longstanding interest in the treatment of disorders of pubertal onset, as well as congenital adrenal disorders. Branch-funded research on these topics has determined current diagnostic methods and treatment of such disorders.
- **Growth Factors:** The Branch also funds seminal research on epidermal growth factor, basic fibroblast growth factor (bFGF), and their receptors, which has led to a better understanding of post-receptor intracellular events and potential clinical applications of these growth factors. In animal models, bFGF, when given peripherally, crosses the blood-brain barrier and stimulates neurogenesis. bFGF also stimulates stromal cells of human bone marrow to differentiate into neurons, thus providing an accessible, renewable reservoir for neuron creation for treating degenerative neurologic diseases of the brain and spinal cord.

GROWTH

The Branch-supported Fels Longitudinal Study of Growth and Development has provided growth data for the North American Standard Tables of Height and Weight, as well as for a comprehensive atlas of bone age and skeletal development. These standards are in widespread use in the United States and abroad. This unique study now includes three-generation pedigrees, providing a basis for genetic studies of skeletal growth and body composition.

PEDIATRIC PHARMACOLOGY

Less than one-quarter of the medications currently on the market are approved by the Food and Drug Administration (FDA) for use in children. To rectify this imbalance, ENGB initiated a cooperative Network of PPRUs as a national resource to facilitate clinical studies of drug action and disposition in infants and children. The goal of studies conducted by the PPRU Network is to provide a scientific basis for improving the efficacy, dosages, and safety of using drugs to treat infants and children. An important practical result of these studies will be an increase in the number of drugs labeled for use in children. The success of the Network in its first three years of operation spurred Congressional legislation that mandates pediatric drug studies for all new drug applications submitted to the FDA for approval.

CHILD HEALTH RESEARCH CENTERS

One major development in pediatric science in the United States is the creation of a Congressionally mandated program of CHRCs. The 20 centers currently supported by this program are devoted to accelerating the application of discoveries in basic research to the care of sick children. The Centers are also instrumental in increasing the number of research-oriented pediatricians.

INTERRELATIONSHIPS AMONG RESEARCH AREAS

The fields of nutrition, endocrinology, and growth are intimately intertwined. The central nervous system, as well as hormones, GFs, and nutrient supply regulate linear and somatic growth. Hormones, GFs, and nutrients, in turn, work in concert to engender growth and development of the central nervous system. Hormones and nutrients also interact in: regulating energy metabolism; determining blood pressure; controlling the onset of puberty, ovulation, and lactation; maintaining menstrual cycles; and determining hunger, food intake, and satiety. Both endocrine and nutritional interventions may successfully treat obesity, diabetes, amenorrhea, osteoporosis, and some kinds of short stature. To this end, ENGB encourages interdisciplinary research on these topics.

OVERVIEW OF FUNDING: 1996-1999

In the fiscal year ending September 30, 1999, the Branch supported a total of 192 research projects, at a level of \$47.8 million. Figure 1 analyzes these projects by subject matter. Approximately one-half of the 1999 budget addresses research and training in nutrition and the antecedents of adult disease, while one-quarter target research and training in developmental endocrinology. The remaining one-quarter supports research on physiology and physical growth, as well as 20 CHRCs and 13 PPRUs.

Since its last report to NACHHD Council in September 1996, the ENGB research budget has grown by \$10.4 million. Beginning in FY 1999, \$2 million of the overall increase accounts for support of six new PPRUs. Furthermore, support for the research program on osteoporosis prevention accounts for another \$3.5 million of the increase, while an additional \$2 million represents support for the CHRCs. The average cost of a research grant increased by \$15,000, which accounts for the rest of the increase.

The remainder of this report describes specific aspects of the Branch's activities from 1996 to 1999, in the research areas mentioned previously.

PREVENTION OF CHRONIC DISEASE

The burdens of chronic diseases in the United States are enormous; further, such diseases, especially obesity, type 2 diabetes, asthma, and osteoporosis continue to increase. In general, these diseases are the products of gene-environmental interactions, where the genetic component reflects the interactions of multiple genes. Many of the genes involved are polymorphic at specific loci, which determine the degree of disease susceptibility. Advances in genomics and informatics make a concerted attack on these problems possible, leading to the development of genetic markers that predict disease susceptibility and advances in specific interventions for use during infancy and childhood. Microarray chip technology now permits the analysis of the simultaneous expression of tens of thousands of genes. Such differential gene expression allows researchers to understand the earliest divergent pathways that lead to chronic diseases later in life, which may help to design rational interventions. The Branch plans to issue an RFA on genetics of disease susceptibility for funding in FY 2002.

ATHEROSCLEROSIS

Coronary arterial atherosclerosis remains the primary cause of death in the United States today, often causing death during the most productive years of life. An estimated 1.5 million people in the US have heart attacks each year; that's one every 20 seconds. Of those afflicted, nearly one-third die, including 75,000 people between the ages of 35 and 65. Approximately one out of every five men will have a coronary attack before age 60.

The pathological process of coronary atherosclerosis often begins in adolescence or earlier. Dr. Robert Cooke, a pediatrician formerly on the staff of Johns Hopkins who worked with the Kennedy family to found the NICHD, pointed out that atherosclerosis should be considered a pediatric disease. Therefore, pediatricians should search aggressively for children at high risk for atherosclerosis.

Part of the Branch's prevention mission is to identify predictive markers of premature atherosclerosis. To begin this initiative, in 1994, the Branch funded four studies of several thousand children from coronary-prone parents, matched against children from families unaffected by coronary atherosclerosis. These studies showed that children of parents with premature coronary disease often have

elevated levels of serum triglycerides. This discovery may soon prove useful in identifying future coronary-prone individuals in pediatric practices.

The studies also revealed that Hispanic children have lower levels of high-density lipoproteins cholesterol than age-matched white or African American children. Additionally, childhood obesity is a robust discriminator between children of coronary disease-prone families and children of families not prone to coronary heart disease. The studies also identified a significant difference in plasma homocysteine levels among those with parental history of coronary artery disease (Greenlund et al., 1999).

The atherosclerosis studies also showed that multiple risk factors interact to cause atheromatous plaques in the coronary arteries of children and young adults. The extent of these early atherosclerotic lesions correlates with body mass index (BMI), systolic blood pressure, serum total cholesterol, serum low-density lipoprotein cholesterol, and serum triglyceride concentrations. In a study of children and adolescents who died accidentally, autopsy revealed that the amount of arterial surface covered by plaques varied with the number of risk factors, increasing by 8.5-fold in those with three risk factors, and by 12-fold in those with four, when compared to those with no risk factors (Berenson et al., 1998).

The Branch also conducted an observational cohort study of children and parents in 276 families, classified according the presence or absence of ischemic heart disease at or before age 55. Results from this study show that early onset ischemic heart disease is associated with higher plasma fibrinogen levels in the parents. The main finding among the children was that levels of plasma fibrinogen are strongly associated with measures of obesity (Shea et al., 1999).

In September 2000, ENGB will hold a workshop to identify new directions for its research program on the antecedents of atherosclerosis. Topics under consideration include stress and central serotonergic tone, clustering and tracking of risk factors, and the use of information technology to mine databases in an effort to identify risk factors. The Branch plans to issue an RFA in 2001 to stimulate research projects in these areas for funding in FY 2002.

DIABETES

Type 1 Diabetes Mellitus

Research and research conferences supported by the Branch in the past helped to establish type 1 (juvenile) diabetes as an autoimmune disorder with a strong genetic component. In this disease, activated T-cells of the immune system initiate a cytotoxic attack on the β -cells of pancreatic islets during infancy, childhood, or adolescence. Branch-supported research showed that first-degree relatives of index cases of type 1 diabetes are at high risk of becoming diabetic. Glutamic acid decarboxylase and tyrosine phosphatase, key enzymes involved in β -cell activity, are antigens that are targeted by this autoimmune

response in genetically susceptible individuals. Physicians are currently using such immunological markers to establish the risk of type 1 diabetes in relatives of index cases.

The Diabetes Prevention Trial

Type 1 diabetes affects one-in-300 people in the US, accounting for much of the costly retinal, renal, neurologic, and cardiac diseases treated in this country every year. Successful identification of the prediabetic state presents the prospect of treating high-risk children with immunomodulators prior to the onset of clinical disease. To exploit the ability to stratify future risk of type 1 diabetes, the NICHD joined the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of Allergy and Infectious Diseases (NIAID) in conducting the Diabetes Prevention Trial, an antigen-based intervention designed to delay or prevent the onset of type 1 diabetes in first-degree relatives who are found to be at high-risk by immunological testing. As of July 2000, 80,000 subjects had been screened and 325 subjects enrolled and randomized into this ambitious trial of low daily doses of subcutaneous insulin. A parallel trial of oral doses of insulin is ongoing in a group of relatives who were found to be at moderate risk of type 1 diabetes. The results of these trials are not yet available. However, if this primary prevention technique works, the cost savings could be counted in the billions of dollars.

Prospective Assessment in Newborns for Diabetes Autoimmunity (The PANDA Study)

The Branch partnered with the Juvenile Diabetes Foundation International in 1999 to co-fund the PANDA Study, a major project designed to detect the earliest changes in gene expression in the pathogenesis of type 1 diabetes among 12,000 infants who are at various levels of genetic risk for diabetes. After analyzing 11,520 genes in diabetic mice at different time points in development, the researchers identified more than 100 genes associated with diabetes progression. They can now apply the technique to samples of blood collected from infants enrolled in the PANDA Study to study the same predictive genes identified in the mouse model.

Islet Cell Regeneration

Type 1 diabetes is caused by a paucity of insulin-producing β -cells in the islets of Langerhans. The islets' β -cells have no regenerative capacity after birth; that is, once the cells are destroyed they cannot be recovered. Branch-supported investigators are searching for mechanisms by which β -cells may be regenerated. These studies utilize a strain of transgenic mice that have demonstrated a high level of pancreatic duct cell proliferation and neogenesis of islet cells during adult life. These mice bear the interferon γ (IFN- γ) gene expressed in the pancreatic islets. The IFN- γ -induced islet neogenesis is similar to embryonic islet morphogenesis, which provides a model system for studying β -cell development.

Tissue Specific Insulin Gene Expression

Branch-supported investigators are also trying to understand why insulin is produced only in β -cells of the pancreatic islets, even though every nucleated cell of the body carries two copies of the insulin gene. Currently, researchers are studying the rat insulin II gene, which is homologous to the single human insulin gene. These studies showed that the β -cell-specific activation of this gene depends on a 43-nucleotide enhancer region. Four different transcription factors must bind to this enhancer for the gene to be fully expressed; mutation in any one of their binding sites drastically reduces insulin production (Huang et al., 2000) This research should enable scientists to determine what controls insulin gene expression, to eventually stimulate other cells of the body to produce insulin.

Type 2 Diabetes Mellitus

In addition to its type 1 diabetes prevention efforts, the Branch also supports research designed to prevent type 2 diabetes. ENGB, in conjunction with the Office of Research on Minority Health and the NIDDK, issued an RFA to implement a multicenter, randomized clinical trial evaluating the efficacy of interventions designed to delay or prevent the onset of type 2 diabetes mellitus in 4,000 individuals at increased risk. National health surveys during the past 35 years show that the percentage of the African American population that has been diagnosed with diabetes is increasing dramatically. Insulin resistance is the earliest abnormality detected in individuals who will develop type 2 diabetes. Weight reduction and exercise can successfully reduce insulin resistance; however, it is not known whether reversing insulin resistance will prevent or delay the onset of type 2 diabetes. One arm of this trial is evaluating the efficacy of lifestyle changes in delaying the onset of type 2 diabetes. Because pharmacologic agents may also prevent or delay the onset of type 2 diabetes, testing the efficacy of one of these agents constitutes the second arm of the trial. Metformin can reduce hepatic glucose production and improve glycemia and hyperinsulinemia, but data in the literature are inconclusive with respect to the ability of this agent to prevent or delay the onset of type 2 diabetes. The study's third arm is a control group.

Gestational Diabetes Mellitus

Currently, gestational diabetes mellitus (GDM) occurs in two-to-three percent of pregnancies. Even though the diabetic condition usually disappears after delivery, about half of the affected women will develop type 2 diabetes within five-to-15 years, depending on their ethnicity. During their pregnancies, these women have high levels of blood sugar, which reach the fetus through the placenta. The islet cells of the fetal pancreas respond by producing high levels of insulin, leading to larger babies with birthweights of nine-to-14 pounds. Such large babies are usually delivered by caesarian section. Because of their higher levels of insulin, these babies are at risk for neonatal hypoglycemia.

To ascertain the worldwide incidence of GDM and assess the need for caesarian section objectively (without regard to glycemic status), ENGB is funding a prospective international study of 25,000 pregnant women at 16 sites, five of which are in North America. The study design stems from a GDM workshop that was jointly sponsored by ENGB and the Pregnancy and Perinatology Branch (PPB) of the NICHD in December

1992. Participating physicians will be blinded to levels of plasma glucose below 105 mg/dL, to remove the subjects' glycemic status from decisions about operative delivery. This study could lead to improved care of women affected by GDM, reducing both overtreatment and undertreatment of the condition on a worldwide basis. The NIDDK has agreed to co-fund this major undertaking.

Future Directions in Diabetes Research

The NIH portions of Senate Report 106-293 for FY 2001 Appropriations state, "The Committee believes that the [NIH] should support more juvenile diabetes grants. The NICHD is encouraged to work with NIAID and NIDDK on efforts to develop a vaccine to prevent juvenile, or type 1 diabetes." In carrying out this directive, the NICHD will join the NIAID and the NIDDK in issuing three RFAs to stimulate more research on the immunopathogenesis and possible immunomodulation of the autoimmune process that leads to β -cell destruction. ENGB staff will continue to participate in the Congressionally established Diabetes Research Working Group to oversee the dispersal of a \$150 million special appropriation for diabetes research in FY 1998-2002.

The Branch is now seeking grant applications to study continuous subcutaneous monitoring of children with type 1 diabetes for episodes of hypoglycemia which attend intensive insulin therapy. The need for such a study is urgent. The incidence of hypoglycemia in adolescents who are assigned to intensive insulin therapy is three times that of adults. Investigators need to address the important issues of compliance, control, hypoglycemia, and prevention of complications in a pediatric population. A novel feature of this initiative is the study of the efficacy of subcutaneous glucose monitors in detecting hypoglycemic episodes. Preliminary data indicate the occurrence of prolonged periods of profound nocturnal hypoglycemia in diabetic children on insulin therapy.

The PPRU Network is currently completing a clinical research study entitled, *Tolerability and Pharmacokinetics of Inhaled Insulin in Children 6-11 Years of Age with Type 1 Diabetes*. If the trial shows that inhaled insulin is as efficacious as insulin injected subcutaneously, investigators will have attained a milestone in the history of the treatment of diabetes. The issues of fear of injections and children's compliance with intensive insulin therapy could recede into the past.

The NICHD joined the NIDDK in co-sponsoring an RFA entitled, *Type 2 Diabetes in the Pediatric Population*. This important initiative is designed to ascertain the causes for the startling increase in prevalence of type 2 diabetes among children and adolescents. Preliminary data indicate a four-fold increase of this disorder within the past decade. Studies are needed urgently to ascertain the true prevalence of the problem and to develop predictive markers for the disorder in children prior to its onset.

OBESITY

Obesity in children is a dangerous condition that threatens their future health. Obesity contributes significantly to five lethal diseases: stroke, hypertension, heart attack, diabetes, and some cancers. Unfortunately, this country has experienced an epidemic of childhood obesity in the past 20 years. The prevalence of obesity in boys and girls age 6-11 and in boys age 12-17 has doubled from 5 percent to 10 percent or more. The situation is particularly alarming among African American females 6-11 years old, whose obesity prevalence has more than tripled from 4.6 percent to 16.6 percent. As a result of this obesity epidemic, a dangerous second epidemic of type 2 diabetes is now occurring in adolescents, the prevalence of which has quadrupled within the last 20 years.

To examine associations among timing and duration of overweight, Branch-supported researchers are relying on lifetime serial data from participants in the Fels Longitudinal Study, who are now adults. The analysis indicates that the earlier the onset of overweight, the more serious the consequences, in terms of increased levels of risk factors for adult obesity and cardiovascular disease (Wisemandle et al., in press). Investigators also related the changes in BMI in kg/m^2 during childhood to adult overweight status and levels of adiposity in the same individuals at 35 to 45 years of age. The Fels Study is the first to relate changes in timing and intensity of childhood adiposity to the effects on levels of fatness in the same persons decades later. Investigators analyzed the patterns of change in BMI into three critical periods for the development of overweight: BMI rebound at ages 4-8; pubescence; and post-pubescence. The researchers also found that, in girls, an adiposity rebound that occurs one year earlier doubles their risk for developing subsequent adiposity in adulthood. The earlier the BMI rebound, the more likely a child is to develop adiposity in pubescence. The pubescent period is important for both sexes in that an increase in BMI by 1kg/m^2 per year trebles the risk of becoming an obese adult (Guo et al., in press).

The pattern of BMI changes during childhood had stronger effects on adult overweight in the same individuals than their birth weight and adult lifestyle variables. Regardless of whether these children became obese because of environmental or genetic factors, there is a strong tendency for their overweight to persist into adulthood. These findings indicate that pediatricians may prevent obesity in adults by focusing on the timing and intensity of obesity development in childhood.

Metabolic Programming and the Fetal Origins of Obesity

Epidemiologic analysis shows that the disease burden of obesity falls disproportionately on African American, Hispanic, and Native American minorities, especially among those in impoverished circumstances. Some evidence also suggests that obesity and its inimical companions, insulin resistance, hypertension, and dyslipidemia, may be transmitted from mother to daughter. This important concept was a topic of an international research conference held by ENGB in September 1999, on the Fetal Origins of Adult Disease. One Branch-supported investigator presented an interesting example of this kind of metabolic programming that involved the children of diabetic mothers. Specifically, the higher the mother's plasma glucose, the greater the insulin in fetal amniotic fluid. Higher amniotic

fluid insulin levels, in turn, correlate with obesity and insulin resistance in the offspring during childhood and adolescence.

The Genetic Epidemiology of Childhood Obesity

NICHD-supported investigators are conducting a multipoint linkage analysis and a candidate gene association study to search the genomes of 250 markedly obese individuals to screen for a polymorphism of the human glucocorticoid receptor gene that is associated with increased sensitivity to glucocorticoids. The results indicate that 12 percent of the subjects are carriers.

Investigators also screened the entire coding region of the human melanocortin receptor 4 (MC4R) gene in these individuals to identify mutations that are associated with autosomal dominant obesity. The ligand for MC4R in the brain is α -melanocyte stimulating hormone, which inhibits feeding. A novel mutation in one subject contained a heterozygous 15-base deletion.

An association analysis of quantitative BMI in a population-based study in Muscatine, Iowa, demonstrated that a variant of the gene coding for the leptin receptor explains a significant proportion of the BMI variability in Muscatine families. A transmission disequilibrium test analysis of this same polymorphism showed that the variant was transmitted more frequently than expected to children with high BMI, but not to children with low BMI.

Future Research Directions in Obesity

Molecular capabilities now far outstrip the precision of clinical methods for defining the obese phenotype. Investigators can now identify single base-pair variations in genes and their regulatory regions with great efficiency. Further, molecular techniques permit a base-by-base digital analysis of genes responsible for obesity. Rodent models will also continue to generate candidate genes for such analysis. The likelihood of success with genetic strategies depends on isolating a predisposing factor, such as primary insulin hypersecretion, within a set of individuals and linking the phenotype to variants of candidate genes. Research needs to focus on both the refinement of relevant phenotypes and on families with extreme outliers. Emphasis on very obese children and their lean siblings should narrow the number of genetic factors involved.

Research should also address the development of rapid screening tests for relevant gene products, such as the MCR4 receptor, so that genetically affected individuals can be selected for subsequent study. One key goal is to identify individuals at genetic risk before they become obese, so that researchers can characterize their phenotypes related to storage of body fat as precisely as possible. In this way, research can demonstrate the mechanism by which these genes lead to obesity and may lead to preventing or reversing their effects.

In addition, research in behavioral science should emphasize development of new interventions. Relevant topics include: the origins of food craving and satiety; the value of food in reinforcing overeating; environmental variables influencing physical activity;

and the role of neurotransmitters regulating food intake. One important question is whether behavioral and pharmacological interventions can be combined in synergistic ways to improve weight loss and maintenance. While it is important to treat obesity, it is even more important to prevent it.

One further area of study is sedentary behavior, which is a risk factor for obesity, type 2 diabetes, hypertension, atherosclerosis, and osteoporosis. Physical activity ameliorates these conditions and may prevent their onset. The molecular mechanisms that link activity to disease prevention are unknown. However, techniques to study differential gene expression and proteomics in sedentary versus active conditions will provide molecular clues for possible nutrient or pharmacologic interventions to prevent such chronic diseases. The NICHD is planning a program announcement (PA) with other Institutes to address this issue in 2001.

BONE HEALTH

Osteoporosis

Osteoporosis is a major public health threat for 28 million Americans, 80 percent of whom are women. Once acknowledged as a natural part of aging, osteoporosis is now regarded as a preventable disorder as a result of new understanding of its cause, diagnosis, and treatment. Osteoporosis may have its origins during childhood due to impaired peak bone mass acquisition. Peak bone mass is attained early in the third decade of life, but is determined by bone mineral acquisition during puberty. Many children enter their adulthood with compromised skeletal systems from poor nutrition and exercise habits. The average teenage girl consumes only about 800 mg of calcium per day, which is 500 mg less than the current recommended daily allowance and 700 mg less than the amount recommended by the NIH Consensus Conference on Calcium Intake. Small changes in bone mineral content and size early in life may have profound effects on fracture risk later in life.

Researchers are now focusing on how inherited, environmental and nutritional factors influence bone mineral acquisition. These and other factors determine bone size, mass, and integrity. The failure to achieve genetically determined maximal peak bone mass correlates with the development of osteoporosis later in life. The ENGB is currently funding 13 grants in the area of osteoporosis prevention, which were initiated in response to two RFAs issued by the Branch since 1996.

Initiatives resulting from the first RFA focus on the effects of exercise and nutrition on calcium metabolism and bone mineral acquisition. The amount of dietary calcium needed to optimize peak bone mass remains uncertain. Since sodium reabsorption and calcium retention are regulated by the same mechanism in the renal tubules, an excess of sodium may affect bone density through increased calcium excretion. Investigators have established the effect of dietary sodium on the relationship between calcium intake and calcium retention in children. Other studies are designed to examine the adaptation effects of low calcium intake on calcium absorption, excretion and bone turnover.

Additionally, investigators will evaluate the relationship between hormonal and pubertal status and the adaptation to low calcium in healthy girls.

New initiatives resulting from the second RFA include six randomized controlled trials designed to test behavioral change interventions in children. Two studies are school-based with large study populations of 1800 children each. All studies that address this RFA share the same intervention: increased calcium intake and increased exercise. In addition, the studies incorporate a variety of behavior modification techniques and social learning theory approaches. The overall assumption is that lifestyle changes made during childhood will also continue into adulthood and will lead to an increase in peak bone mass and a decreased incidence of osteoporosis.

The Young Women's Health Study

Despite the established benefits of regular exercise, the prevalence of sedentary lifestyles is increasing. NICHD studies show that weight-bearing physical activity, such as gymnastics, is associated with increased adolescent bone gain in girls. In the Young Women's Health Study, investigators followed 84 girls from age 12 to age 18, to focus on the contribution of teenage calcium intake and physical activity to the total body bone mineral gain and peak hip BMD. They observed that the teenage sports-exercise scores correlated with increased hip BMD at age 18 years. Interestingly, they also observed no relationship between calcium intake during ages 12-to-16, with either total body bone mineral gain or hip BMD at age 18 years. These investigators project that an increase of $.05 \text{ g/cm}^2$ of hip bone density represents a 50 percent reduction in osteoporotic fracture risk. In this study, a difference of $.05 \text{ g/cm}^2$ in hip bone density was associated with the amount of physical activity that distinguishes a sedentary teenager from one who engages in some form of exercise on nearly a daily basis.

The Milk Matters Campaign

By age 17, approximately 90 percent of the adult bone mass is established. Although it is known that calcium in milk and other dairy products builds strong bones, figure-conscious teenage girls are reluctant to consume dairy products for fear of gaining weight. To help ameliorate this problem, the NICHD started the Milk Matters campaign in 1997. This public health education campaign aims to increase the consumption of milk among children and adolescents. As a result of interaction between the staff of ENGB and the advertising company that created the Milk Moustache campaign, the Milk Moustache campaign now features children and adolescents, in addition to postmenopausal women, the original target audience. In this way, the NICHD gets its prevention message to millions of children and adolescents every day.

Future Directions: Bone Mineral Density Database for Children

The understanding of risk factors and pathophysiology of impaired mineralization in childhood has surpassed the ability to measure and identify children with osteopenia. These measurement tools are vital for implementing the proper actions needed to prevent the progression of osteopenia. Disorders such as malabsorption, renal dysfunction, anorexia, asthma, joint disease, and hypogonadism, are associated with low peak bone mass and osteoporosis. Close monitoring of the bone density in such children is essential.

The ability to diagnose and treat children with chronic diseases that affect bone remains limited because the current age-specific norms for BMD are often inaccurate. Standard reference data are needed that consider skeletal maturation, volume, body size, and pubertal factors which appear to be more important determinants of BMD than chronological age. In addition, a universal approach to the interpretation of pediatric bone mineral measurement is urgently needed for this field of research to move forward. Such data would be valuable resources for both clinicians and investigators, enhancing their ability to diagnose and treat children with osteopenia. The proposed initiative planned for FY 2001 will provide important normative reference data.

NUTRITION

MATERNAL NUTRITION AND INFANT BIRTHWEIGHT

Low birthweight babies are at risk for increased rates of morbidity or mortality. In this country, about two-thirds of the birthweight babies who are born prematurely are appropriate in size for their gestational age; however, the remaining one-third, or about 100,000 per year, are small for their gestational age because of intrauterine growth retardation (IUGR). On a worldwide basis, undernutrition of the mother before and during pregnancy accounts for nearly all of the IUGR, especially in developing countries. The causes of IUGR in the US, on the other hand, are poorly understood. Some causes of the condition in the US include fetal infection, fetal genetic syndromes, and placental impairment of nutrient supply. Such impairment results primarily from circulatory disruption caused by maternal diabetes, hypertension, thrombotic disorders, and resulting placental infarction. Presumably, genetic causes also account for impaired placental transport of nutrients; however, little is known about genetic variation in placental nutrient transporters which represents an important area for future study.

Currently, the only clearly demonstrated nutritional factors that impact birthweight are maternal pre-pregnancy weight for height and weight gain during pregnancy. Because these two factors account for only a portion of the variance in birthweight, investigators are searching for other contributions to the variance, including specific nutrients such as vitamins, polyunsaturated fatty acids, and trans fatty acids. Additionally, the epidemiologic associations of IUGR with cardiovascular disease later in life imbue this field of research with a new sense of urgency.

In view of these outstanding questions and their importance to future generations, ENGB is now funding three prospective studies of maternal diet in a total of 22,000 women in relation to pregnancy outcome. The studies are also designed to assess the role of specific nutrients in infant growth, cognitive development, and infant blood pressure. The significance of these studies is to identify optimal diets for fetal growth and infant development, and perhaps to ensure a healthy life well beyond infancy.

In September 1999, the Branch held an international conference on the fetal origins of adult disease, in conjunction with the National Institute of Heart, Lung, and Blood Institute, the NIDDK, the National Institute on Aging, and the Office of Research on Women's Health. The participants at this meeting, including Professor Barker, presented their findings and suggested new areas of investigation, including the effect of nutrient deprivation during pregnancy on the endocrine pancreas and kidney. An RFA based on this conference was issued in August 2000; successful grant applications will be funded in FY 2001.

INFANT FORMULA

Two long-chain fatty acids, arachidonic acid (AA) and docosahexaenoic acid (DHA) are primary structural constituents of neurological membranes in the brain and retina. Breastfed babies receive AA and DHA in their mothers' milk. The importance of these components in the diet of newborns, especially premature infants, is subject of intensive research activity by ENGB-funded investigators. These investigators showed that newborn infants do not have the capacity to synthesize all of the AA and DHA they need for optimal development. This is especially true for pre-term babies because they are denied the transfer of DHA and AA from mother to fetus, which normally occurs during the period of rapid brain growth in the third trimester of pregnancy.

Recently, ENGB-supported investigators evaluated the effects of dietary DHA supplied during infancy on later cognitive development of healthy term infants in a randomized clinical trial. The study compared infant formula supplemented with DHA only or with DHA and AA versus control formula, which provided no DHA or AA. Children in the DHA-supplemented groups performed significantly better in tests of visual acuity.

At 18 months, the children were tested using the Bayley Scales of Infant Development. Supplementing infant formula with AA and DHA was associated with a mean increase of seven points on the Mental Development Index (MDI). Furthermore, both the cognitive and motor subscales of the MDI showed a significant developmental age advantage for DHA-and DHA/AA-supplemented groups when compared with the control group. Significant correlations between plasma DHA at four months of age and MDI at 18 months of age suggest that early dietary supply of DHA is a major determinant of improved performance on the MDI (Birch et al., 2000).

Although DHA and AA are not added to infant formulas in the US, both substances are routinely added to infant formula throughout Europe and Asia. "Whether DHA and AA should be added to infant formula in this country is a pressing public health concern," said Duane Alexander, M.D., Director of the NICHD, in a press release announcing these findings on March 7, 2000. "This study is an important step in the comprehensive array of studies needs to determine whether both substances should be added to infant formula." The urgency of this problem prompted the FDA to engage a panel of experts under the auspices of the Life Sciences Research Organization to review the extant data and make a set of recommendations to the FDA. NICHD staff are closely involved in this activity.

LACTOFERRIN

Infants who are fed breast milk are better protected against enteric diseases than formula-fed babies. Bacillary dysentery due to *Shigella spp* is among the most communicable and severe forms of bacterial gastroenteritis. Epidemiologic studies have shown that breastfeeding decreases the severity of *Shigella spp* infection in infants who become colonized early in life. Immune and non-immune components of milk are involved in this protection. Among the non-antibody factors is lactoferrin, an iron-binding glycoprotein that decreases the availability of iron required for bacterial growth. However, the anti-bacterial activity of lactoferrin is not solely due to its iron-binding capacity; a pepsin-derived fragment of lactoferrin has iron-independent bactericidal activity. ENGB-sponsored investigators found that lactoferrin also impairs the virulence of *S. flexneri* by degrading certain bacterial proteins, called invasion plasmid antigens. These results may lead to the use of lactoferrin as an antibiotic or a prophylaxis against intestinal pathogens (Newburg et al., 1998).

LACTADHERIN

Rotavirus is the most common cause of diarrhea in infants and young children worldwide. In the US, rotaviruses cause up to 50 percent of cases of gastroenteritis in infants. However, human milk contains a glycoprotein, lactadherin, which binds to rotavirus and inhibits its replication. In a study of rotavirus infection in infants, higher-than-average lactadherin values in human milk were associated with a 12-fold increase in protection against enteric symptoms. Milk glycoproteins, such as lactadherin, can inhibit microbial binding to host-cell receptors. Such molecules might be developed as prophylactic or therapeutic agents suitable for oral use (Newburg, Peterson, et al., 1998).

STUDIES OF THE EFFECTS OF PHYTOESTROGENS IN INFANT FORMULA

Researchers have considerable concern about possible effects of phytoestrogens such as isoflavones in food, especially in food derived from soybeans. Isoflavones have only about 0.1 percent of the activity of mammalian estrogens, but they occur in some foods in high concentrations. Attention has focused on the postulated endocrine disrupter activities of isoflavones in soy-derived infant formula. Scientific opinion is divided as to whether this constitutes a hazard. Thus far, researchers have found no clinical effects in humans, but observations on rodents are disturbing. Symptoms noted in rodents include growth suppression in the fetus, an anovulatory syndrome in females, and altered sexual behavior in males. More than 20 percent of infants in the US are being fed soy-based formulas, usually because of poor feeding, reflux, or cow-milk protein sensitivity. The sale of soy-based formula is now prohibited in New Zealand.

Because of these concerns, the NICHD and the Center for Food Safety and Nutrition of the FDA held a workshop to address the issue in May 1997. The workshop resulted in a research project, co-funded by the NICHD and the Formula Manufacturers' Council. The

study involved subjects who participated in growth studies as infants and who are now 21 to 35 years old. Feeding histories include 300 individuals fed soy formula, 600 fed cow-milk formulas, and 300 fed human breast milk. The follow-up examined variables such as breast development, menarche, sexual orientation, fertility, menstrual characteristics, and reproductive system malignancies.

No significant differences were found between the two groups, in either females or males, with regard to adult height, weight, and indices of sexual precocity, cancer, or infertility. However, the soy-formula subjects reported menstrual periods 0.4 days longer than women in either the cow-milk or human-milk group, although without heavier bleeding. These women also reported greater discomfort with menstruation, even though they sought medical care for their symptoms less frequently. The findings of this study are reassuring about the safety of soy-based infant formula.

PROTEIN NUTRITION IN VERY LOW BIRTHWEIGHT INFANTS

Neonatologists are faced with ever-increasing survival rates of 22-to-28-week infants, who are under high degrees of stress, sepsis, and polypharmacy. Despite the needs of these vulnerable infants for feeding strategies that mimic *in utero* growth rates for both brain and body, researchers have yet to develop optimal feeding strategies. To ameliorate the situation, the NICHD's ENGB and PPB, in connection with the Children's Nutrition Research Center of the United States Department of Agriculture and the Robert Schwartz Center for Metabolism and Nutrition at Case Western Reserve University, co-sponsored a conference entitled, *Protein/Nitrogen Metabolism and Accretion in Very Low Birthweight Infants*, in November 1999. Dr. Satish Kalhan and Dr. Dennis Bier co-chaired the conference, which was designed to emphasize how basic biochemical research could be applied in clinical practice. Participants addressed the need for non-invasive studies and new technologies that can reveal intracellular events in order to monitor minute-to-minute cellular nutrient needs of brain and body. They pointed out the need to learn more about the systems and signals that target nitrogen accretion and to define key endpoints. Better intervention strategies are needed that might include hormones, GFs, and essential fatty acids in addition to nitrogenous substrates. Participants also addressed the relationship between diet quality and body composition and the need for specific amino acids and dipeptides, such as glutamine, taurine, and glycytyrosine. Conferees also raised the question of whether very low birthweight infants have sufficient protein synthetic machinery and whether they can respond appropriately to signal proteins such as insulin and IGF-1. All of these problems are compounded in infants who are immunologically compromised, septic and on multiple drugs. Dr. Kalhan has prepared a summary of the conference for publication in *Pediatric Research*.

In December 2000, a companion conference will be held on carbohydrate metabolism in very low birthweight infants, supported by the same sponsors and co-chaired by Drs. Kalhan and Bier.

ZINC, DIARRHEA, AND PNEUMONIA

Zinc is a micronutrient that is involved in fundamental processes of cellular growth and differentiation. Cells with a rapid rate of turnover, such as lymphocytes, are most affected by inadequate levels of zinc. Diarrhea and pneumonia are the two leading causes of death in children in developing countries; therefore zinc supplementation would be an important means to improve child survival. A meta-analysis of ten trials of zinc supplementation in children in developing countries revealed a large effect on diarrhea, reducing its incidence by 25 percent, and an even greater effect on pneumonia, reducing its incidence by 41 percent. This latter effect is greater than that estimated for any other intervention to prevent pneumonia. The effectiveness of zinc as a single nutrient supplement is striking; its effect on pneumonia prevention accounts for the entire estimated contribution of malnutrition as a risk factor. In normalizing the function of cells in multiple tissues, zinc supplementation enhances the child's ability to combat entire disease states, not just single infectious organisms. Thus, zinc supplementation may prove more economical than vaccines in some cases, when directed against specific organisms (Butta et al., 1999).

DEVELOPMENTAL GASTROENTEROLOGY

Intestinal Permeability

The gastrointestinal tract of the pre-term infant is more permeable than that of the term infant. This increased permeability is associated with gastrointestinal mucosal damage, such as that seen in necrotizing enterocolitis. To determine the effects of early versus late feeding in pre-term infants investigators studied 132 pre-term infants whose mean gestational age was 27 weeks. The results indicate that feeding human milk or pre-term formula, beginning on the fourth day, is associated with lower permeability when compared to withholding milk or formula until the fourteenth day. Antenatal steroid use was also associated with a decrease in permeability. In addition, permeability was lower at 28 days in infants who are exclusively fed human milk, compared with those fed formula. The relationships between permeability and early feeding, antenatal steroid use, and human-milk feeding have important clinical implications regarding prevention of sepsis and necrotizing enterocolitis in pre-term infants (Shulman et al., 1998).

Intestinal Defenses against Microorganisms

The gastrointestinal tract is a major site of entry for bacteria, viruses, and parasites. The epithelial barrier normally prevents penetration of these pathogens into internal tissues and fluids. Sampling of pathogens in the lumen of the intestine is done by specialized epithelial cells, the microfold or M-cells, which transport microorganisms across the epithelium toward the underlying gut-associated lymphoid tissues that activate the mucosal immune system. However, pathogens sometimes use the M-cell transport mechanism to penetrate the intestinal barrier and invade the underlying tissues. NICHD-supported investigators are working to identify M-cell surface components that can serve as receptors for attachment and transport of microorganisms. By studying the interactions of viral and bacterial pathogens with M-cells, investigators seek to elucidate the role of these cells in the pathogenesis of infectious diseases that begin at the mucosal surface of

the intestinal tract. Defining the M-cell features that allow microorganisms to exploit M-cell transport will enhance our understanding of microbial pathogenesis, and could promote the development of intestinal-specific vaccines and oral immunization against retrovirus and bacterial toxins (Neutra et al., 1999).

ENDOCRINOLOGY

GH DEFICIENCY

ENGB staff continue their work with leading pediatric endocrinologists to curb the use of GH in children who cannot benefit from it. The industrial production of human GH by recombinant techniques has greatly increased the supply of this potent, 191-amino acid polypeptide. With the increased availability of GH, therapy for non-deficient (non-GHD) children who are two or more standard deviations below mean height for age has become a controversial issue, with far-reaching medical and social ramifications. NICHD-supported investigators have shown that when exogenous GH hormone is injected into non-GHD short children, 25 percent do not respond, while 50 percent show only modest increments in growth. Even among those who respond initially, the long-term effect on final height is not known for years. In the US, the number of children eligible for GH treatment ranges from 11,000, if strict criteria for GHD are applied, to 1.3 million, if all those with heights below the third percentile are candidates. The respective cost of GH therapy would jump from \$155 million to \$20 billion per year if the less stringent criterion became the standard of care (Cuttler et al., 1996). So far, pediatricians in the US have shown gratifying restraint in prescribing GH for non-approved indications, since only 20,000 children are receiving GH therapy (Finkelstein et al., 1998).

Pediatric endocrinologists are awaiting the results of a randomized, double-blind, placebo-controlled NICHD study designed to ascertain the effect on final height of exogenous human GH in non-GHD children who are two or more standard deviations below the mean height for their age. The study has generated considerable controversy and has received much attention in the lay press. This trial, which will determine the indications for future GH prescription, was designed at an ENGB-conference on disorders of human growth and represents an example of cooperation between extramural and intramural scientists at the NICHD.

CONGENITAL ADRENAL HYPERPLASIA (CAH) AND OTHER DEFECTS OF ANDROGEN METABOLISM IN CHILDREN

NICHD-supported investigators have made numerous discoveries in the fields of steroidogenesis and adrenal development, including the clinical variant of CAH known as late-onset or non-classical CAH. This disorder is a significant cause of infertility in women who are also afflicted with hyperandrogenism, insulin resistance, and hirsutism.

Investigators have discovered eight functionally important mutations of the gene encoding 21-hydroxylase. These mutant alleles engender three different levels of 21-hydroxylase activity in affected individuals and are present in various combinations in affected children. The many possible combinations of faulty alleles, and the variety of enzyme activities associated with these mutations, explain the spectrum of clinical phenotypes. Affected individuals may suffer life-threatening degrees of salt loss, virilization with severe hypertension, or obesity, hirsutism, and infertility (New & Wilson, 1999).

Non-classical CAH due to steroid 21-hydroxylase deficiency is one of the most common autosomal recessive genetic disorders currently known occurring in about one out of every 30 Ashkenazi Jews, and in one to 100 individuals in a mixed Caucasian population. Thus, the non-classical allele of the 21-hydroxylase gene may be the most common autosomal recessive genetic disorder. Scientists are treating affected female fetuses *in utero* with dexamethasone during the first trimester of development. This novel fetal therapy prevents the overproduction of masculinizing steroid precursors by the adrenal gland and results in the birth of girl babies with normal female genitalia, despite their defective alleles of the genes encoding 21-hydroxylase or 11 β -hydroxylase (Cerame et al., 1999).

NEUROENDOCRINOLOGY

Pubertal Onset

One of the most common problems seen by pediatric endocrinologists is the delayed onset of puberty. Less common, but more vexing to the families involved, are disorders of precocious puberty, in which menstruation may begin as early as infancy. Puberty represents the final stage in the maturation process of the hypothalamic-pituitary-gonadal axis. This process evolves from the neonatal period, when there is no hypothalamic regulatory control of the pituitary gonadotropins, to early childhood, when there is significant restraint of the hypothalamic pulse generator and suppressed secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, thus rendering the pituitary-gonadal axis quiescent. The mechanism underlying the intrinsic central nervous system (CNS) inhibitory mechanism during early childhood as well as the disinhibition of the CNS-pulse generator that allows for the onset of puberty remains speculative. However, NICHD-supported scientists have shown that chronic, intermittent administration of excitatory neurotransmitters induces precocious puberty in non-human primates. These studies also showed that the prepubertal restraint on GnRH release may be mediated by neuropeptide-Y.

Despite extensive investigation of the hormonal changes underlying the pubertal transition, many questions remain regarding gender differences in gonadotropin secretion and sex-steroid feedback. Additional peptides, such as inhibins, activins, and follistatins, initially thought to be localized to the gonads, but now known to be produced in other tissues as well, also appear to participate in the regulation of pituitary follicle stimulating hormone (FSH) secretion. The recent advent of specific, two-site immunoassays for

inhibins A and B and activin A has made it possible to measure these regulatory peptides in the peripheral circulation of prepubertal girls. Whether gonadal peptides regulate FSH secretion during pubertal maturation is still unclear. The study of the neuroendocrine control of the reproductive hormones and the exploration of the intricate role of gonadal peptides in this process may lead ENGB investigators to better understand the pathophysiology of disorders of pubertal onset, reproductive abnormalities, and infertility.

Peripheral bFGF Stimulates Brain Neurogenesis

Until recently, scientists considered the number of cerebral neurons to be fixed at birth and unresponsive to environmental signals later in life. To disprove this tenet, NICHD-supported scientists examined *in vivo* effects of bFGF, because it stimulates proliferation of multiple precursors *in vitro*. They found that bFGF stimulates neurogenesis in the brains of newborn rats, apparently crossing the blood-brain barrier to stimulate mitosis. Hippocampal DNA synthesis was stimulated by bFGF in older animals, indicating the persistence of bFGF-responsive cells. Peripheral bFGF increased the number of mitotic nuclei three-fold in the forebrain subventricular zone and olfactory tract. These observations suggest that bFGF regulates ongoing neurogenesis via a unique, endocrine-like pathway, which could potentially provide new approaches for treating damaged brains during development and into adulthood.

Peripheral injection of small doses of bFGF also stimulated neurogenesis in the neonatal cerebellum, increasing the proportion of mitotic granule cell precursors. Moreover, intact bFGF entered brain parenchyma to stimulate mitosis, suggesting ongoing communication between somatic tissue and neurogenetic regions. The existence of a physiological pathway transporting peripheral growth factors to neurogenetic regions also has therapeutic implications (Wagner, Black & DiCicco-Bloom, 1999).

Bone Marrow Stem Cell Differentiation

Marrow stromal cells (MSCs) are a subclass of bone marrow stem cells, capable of differentiating into bone, cartilage, and fat cells. NICHD-supported investigators found that rodent- and human-cultured MSCs can be induced to differentiate into neurons. The finding raises the possibility of using differentiated MSCs to treat neurological diseases caused by neuronal cell loss or destruction.

Researchers expanded adult rat MSCs as undifferentiated cells in culture for more than 20 passages. A simple treatment protocol including antioxidants and basic fibroblast growth factor induced the stromal cells to exhibit a neuronal phenotype and express neuron-specific proteins. This is the first report that peripheral mesenchymal cells can differentiate into neurons *in vitro*.

Human MSCs subjected to this protocol also differentiated into neurons. Consequently, adult MSCs can be induced to overcome their mesenchymal commitment and may constitute an abundant and accessible reservoir for the treatment of some neurologic diseases. MSCs offer significant advantages over other stem cells because they are readily accessible and provide a renewable population. Autologous transplantation

overcomes the ethical and immunologic concerns associated with use of fetal tissue transplants, another treatment for these conditions (Woodbury et al., in press).

GROWTH

THE FELS LONGITUDINAL STUDY OF PHYSICAL GROWTH AND DEVELOPMENT OF THE SAMUEL S. FELLS RESEARCH INSTITUTE FOR THE STUDY OF PRENATAL AND POSTNATAL ENVIRONMENT

The Fels Longitudinal Study is the oldest and largest growth study in the world. The study started in 1929 under the auspices Dr. Authur Morgan, and includes valuable serial growth data on 1,260 individuals whose height, weight, body composition, bone density, plasma lipids and lipoproteins have been carefully measured at regular intervals from birth through adulthood. The NICHD has supported this study since 1976. Data generated by this study form the basis for the North American Standard Tables of Height and Weight, which are used to record and monitor children's physical growth, as well as to predict adult height. With more than 100 million charts already distributed, physicians can make judgments about the normality of an individual's body size and proportions compared to those of the Fels population. Additional tables were developed so that the measured height of a child could be adjusted for parental stature.

The Fels study has also generated charts of normal bone age based on four million observations of knee, hand, and wrist radiographs. These charts are in widespread use by pediatric endocrinologists and radiologists, who deal with problems of growth retardation or inappropriate bone age acceleration, as in cases of precocious puberty. These data also allow researchers to estimate the heritability of skeletal age at annual increments, from age three to 15, and to determine the genetic and environmental correlations between skeletal age estimates across this age range. Initial results show that the degree of skeletal maturity is highly heritable during childhood through the adolescent growth spurt; and that different sets of genes define the heritable component of skeletal maturity over the course of childhood. More than half of the presently known single-gene defects that impact child health and development cause skeletal malformations. Elucidating the process of normal skeletal maturation during childhood will advance the understanding of abnormal skeletal development.

A great strength of serial growth data lies in its statistical power, especially in regard to exact curve-fitting by complex mathematical equations. This approach to analyzing vast quantities of biologic data permits the discovery of new descriptors of normal growth and may shed light on underlying genetic mechanisms. Data from the Fels Longitudinal Study indicate that obesity in childhood tracks from age three onward, into adulthood. In addition, the timing and rate of adiposity rebound, from four-to-eight years old, strongly predicts the degree of obesity in adulthood (Guo et al., in press).

The information in this document is no longer current. It is intended for reference only.

Researchers are now exploiting this rich data set, which contains three-generation pedigrees, to ascertain the segregation of growth patterns for detecting linkage of candidate genes to various phenotypes of growth. The application of quantitative biological and molecular techniques to the Fels data has imparted new excitement to the use of this carefully maintained, 71-year-old, random-sample, longitudinal data set. For example, the influence of candidate genes on phenotypes of BMI growth curves was analyzed by a quantitative sibling transmission disequilibrium test, revealing that the gene coding for Insulin-like Growth Factor-1 was strongly linked to one of the BMI phenotypes. This result indicates that random sampling of a population can uncover genetic loci for quantitative traits, in addition to the more commonly used extreme sampling methodology, in which morbidly obese individuals are oversampled.

Recently, serial data on the BMI and blood pressure of the Fels Study participants were analyzed from birth to adulthood, to test the “Barker hypothesis” about fetal origins of adult disease prospectively, instead of retrospectively. The data confirmed Barker’s observation of higher systolic blood pressure in males of low birthweight. However, investigators could account for the blood pressure increment by a five-kilogram greater increase in BMI in the low birthweight group after age 18 that did not occur in the normal birthweight group. This important observation underscores the statistical power inherent in the Fels Study data set.

PEDIATRIC PHARMACOLOGY

HISTORY OF THE PPRU NETWORK

In 1964, Dr. Harry Shirkey first called attention to a major public health problem in children: drugs used to treat diseases in children were not being first tested in children for safety and efficacy. He coined the term “therapeutic orphans” to describe these children. Because drug companies have little economic incentive to study drugs in children, only one-in-five drugs prescribed for use in children has ever been tested in that population. Untested drugs carry no label with an approved indication for use in children. When such drugs are prescribed for children, their use is called “off label.” This off-label usage puts the child at risk for adverse drug reactions; the physician is also at risk for a medical malpractice suit, if something dire and unexpected ensues.

The FDA’s regulatory position was first articulated in 1970, when the agency stated that “drugs *used for children must be tested in children.*” The Committee on Drugs (COD) of the American Academy of Pediatrics supported this view in a position paper that appeared in *Pediatrics* in 1995. The COD pointed out that every time a physician prescribes an unlabelled drug for a child he or she is performing an uncontrolled experiment with an enrollment of one with no protocol or outside overview. The COD stated that it is unethical *not* to study drugs in children.

In 1990, the Institute of Medicine (IOM) sponsored a workshop to address the lack of pediatric labeling. A major concern was the feasibility of performing drug-labeling studies in children. To address this concern, the IOM recommended that the NICHD provide the infrastructure for collaborative efforts of pediatric pharmacologists to conduct clinical trials of drugs in children. As a result, the NICHD created a Network of seven academic institutions in 1994 to demonstrate that such studies could be ethically and efficiently performed in children. In the ensuing five years, the Network successfully performed over 100 studies in newborns, children, and adolescents. The Joint Conference Committee of the House and Senate (Bill S. 830) recognized the Network's efforts, as did the FDA (Final Rule of 1997) and the Pharmaceutical Research and Manufacturers Association. The IOM workshop also recommended that economic incentives be provided in the form of extended marketing exclusivity. This recommendation forms the basis of the pediatric provisions of the FDA Modernization Act of 1997 (FDAMA).

THE PEDIATRIC PROVISIONS OF FDAMA

The success of the PPRU Network in its first three years of operation influenced the enactment of the pediatric provisions of FDAMA. To encourage pharmaceutical companies to study drugs in children, the FDAMA provides for a six-month extension of exclusivity for marketed drugs with remaining patent or any other kind of exclusivity. The economic reward of such an incentive is often substantial. For a blockbuster drug, the extension of exclusivity may garner an extra \$50 million for a pharmaceutical company. This incentive has already proven to be a powerful engine for driving new pediatric drug trials.

THE EFFECT OF FDAMA ON THE PPRU NETWORK

During the past five years, the PPRU Network has become a national resource for the conduct of pediatric clinical trials. The impact of FDAMA on Network-sponsored pediatric drug trials is reflected in the number of studies conducted within the Network since 1998. In 1999, 54 industry-sponsored protocols were active, a 300 percent increase over pre-FDAMA activity. During the first six months of 2000, 55 protocols were active (Figure 2). The increase in the number of protocols is associated with a marked increase in the annual number of patients enrolled in all protocols. For instance, enrollment for this year will exceed 1,000 patients. Additionally, the number of pharmaceutical sponsors has increased in the last two years by 41 percent. Table 1 lists the pharmaceutical companies and the number of studies supported from January 1998, through June 2000.

Not only has the number of pediatric studies increased, but the range of therapeutic categories studied has also expanded. The distribution of therapeutic categories reflects the major therapeutic categories on the FDAMA list of drugs that are designated as having therapeutic benefit for children (Figure 3A and 3 B). PPRU studies have included pediatric subjects who are well-distributed by age to provide information across the entire developmental spectrum (Figure 4).

In contrast to pre-FDAMA studies, more of today's protocols target early phase studies in children with an emphasis on pharmacokinetic/pharmacodynamic studies, which are important to understand developmental differences in drug response. Pharmacokinetics (PK) involves the kinetics of drug absorption, distribution, and elimination with a major aim of designing a dosing strategy that produces the desired pharmacologic effect, while keeping adverse effects to a minimum. Pharmacodynamics (PD) deals with the relationship between drug concentration at the receptor site and pharmacologic response.

The PPRU Network has played a major role in the implementation of FDAMA. To date, FDAMA has granted exclusivity to 22 marketed drug products that successfully completed pediatric studies. The PPRU Network performed all or part of the clinical trials to support the exclusivity determination for eight of these drugs. During 1998-99, pediatric labeling was added to six marketed products based on studies conducted under FDAMA provisions. The PPRU Network conducted the studies to support the labeling changes of four of these six products. Because of the lag time from completing studies to effecting labeling changes, more pediatric labeling changes are anticipated from completed studies and studies now underway. During the two years that FDAMA has been in effect, the market has shown an impressive surge in the number of sponsored pediatric trials. To accommodate for the increased demand the NICHD expanded the PPRU Network in January 1999, from seven to 13 units (Figure 5). The expanded Network has access to an all-inclusive pediatric population with approximately 160,000 pediatric inpatient admissions and 2,300,000 outpatient visits annually.

FUTURE RESEARCH DIRECTIONS FOR THE PPRU NETWORK

The RFA for the five-year continuation of the PPRU Network (1999-2003) added new research requirements to the performance of labeling studies. The new aims include: studies on the developmental characteristics and genetic polymorphisms of drug metabolizing enzymes; research on new pediatric therapeutic modalities, including molecular approaches to the treatment of diseases; application of new technology to PD studies and drug delivery systems; and development of new pediatric formulations.

The initiation of PPRU Network drug trials in young children uncovered the need to expand current knowledge of pediatric clinical trial methodology and pediatric pharmacology. The explosion in the number of drug studies that occurred after implementation of the pediatric provisions of FDAMA brings to light the problem of finding enough children to conduct efficacy trials. Another problem involved in these studies is the difficulty in establishing efficacy and safety for drugs used in chronic childhood conditions, due mostly to the lack of adequate biomarkers to establish disease severity, recurrence, and response to therapy. The NIH Ad Hoc Committee on Surrogate Markers has identified a biomarker as "a characteristic that is measured and an indicator of normal biologic processes, pathogenic or response to a therapeutic intervention," (e.g., use of clonal markers to quantify leukemic cells, CD4 counts, or measurement of viral load to estimate response to therapy in AIDS). To address these research issues, the

NICHD is planning to publish a PA in 2001 to stimulate research on clinical outcome, biomarkers, and surrogate endpoint development and validation.

The most challenging area of performing drug clinical trials in children is conducting efficacy studies in pre-term and sick newborn infants. Only a few of the 140 drugs currently used in newborn intensive care nurseries were demonstrated to be effective. This population is the most vulnerable to adverse drug effects because of the immaturity of their drug metabolizing enzymes and the pathophysiologic changes that affect drug disposition in this group of patients. A group within the Network was established to perform clinical trials in newborns of drugs with a narrow therapeutic index or toxic potential.

Another major challenge for the future will be to close the gap in the current understanding of the ontogeny of drug metabolizing enzymes, drug transporters, and drug receptors and ion channels. The current change in the central pharmacologic paradigm from symptomatic relief to targeting the cause or mediators of disease processes makes acquisition of this knowledge a priority. To address this issue, NICHD sponsored a workshop on the subject with the participation of world experts to identify research needs and investigative priorities. The NICHD recommendations generated at the workshop in were used to develop an RFA on developmental pharmacology, which was released in January 2000. This RFA was co-sponsored by the National Institute of Neurological Disorders and Stroke and the National Institute on Drug Abuse (NIDA). The NICHD also joined the National Institute of Mental Health (NIMH) and NIDA to issue a PA on developmental psychopharmacology, which was published in June 2000.

Adverse drug reactions (ADRs) are among the most common causes of death in hospitalized patients. Despite a large body of literature implicating the immune system and genetic factors in producing ADRs, the incidence of ADRs in the pediatric population is particularly understudied. New biomedical technology, including using pharmacogenomics, proteomics, and *in vitro* cell lines to test for toxic effects, now permit the study of mechanisms that lead to ADRs. Pharmacogenomics combines informatics and pharmacogenetics to determine the ways by which genes and gene variations affect the discovery, development, toxicology, and use of drugs. Scientists expect that pharmacogenomic screening of patients will lead to the identification of genetic variations that cause toxic reactions. In fact, investigators coined the term “proteome” to describe all the proteins that are expressed or modified following expression by the genome in the lifespan of a cell. Proteomics entails the analysis and characterization of proteins in the proteome. Researchers are now developing proteomic techniques to identify specific proteins that may act as molecular markers of toxicity.

Additionally, ENGB-supported researchers are applying new statistical techniques, such as data mining, to the non-biased assessment of the incidence of ADRs. Data mining is ideal for finding drug-ADR combinations in conjunction with other variables, such as age, which are highly associated when compared to the generality of the data stored in a very large databases of adverse reactions.

The NICHD is planning a workshop in April 2001 to bring together epidemiologists, pharmaco-epidemiologists, molecular pharmacologists, and toxicologists to identify research needs, including development of toxicity biomarkers, identification of patients likely to develop ADRs, and determination of an appropriate model to monitor ADRs.

TRAINING OPPORTUNITIES RESULTING FROM THE PPRU NETWORK

Resources for conducting pediatric drug studies, now and in the future, is a concern that the PPRU Network is addressing. Each PPRU offers a teaching environment in which pediatricians, pharmacists, and others gain supervised experience and training in pediatric clinical trials methodology. Most PPRUs have teaching arrangements with schools of pharmacy to offer training to both undergraduate and graduate pharmacy students in pediatric clinical pharmacy, PK, drug analysis, and drug trial methodology.

The involvement of children in clinical trials requires study designs that minimize discomfort in patients, do not disturb family life, and are conducted in child-friendly environments. Implementation of pediatric drug trials also requires adequately trained study coordinators and research nurses. Currently, nurses learn special skills by trial and error, because this type of training is not available in either basic or advanced nursing degree programs. The PPRU Network has established formal training plans to teach the need skills.

Pediatric Clinical Pharmacologists

There is also a dearth of pediatric clinical pharmacologists and training opportunities for these professionals. To remedy this situation, ENGB announced the *Mentored Specialized Clinical Investigator Development Award*, in January 1999. The objective of this fellowship is to provide skills in the design, execution, and interpretation of pediatric drug clinical trials, with emphasis in PK modeling, PK-PD correlations, and drug metabolism. The Branch funded its first fellow on December 1, 1999, and is in the process of evaluating candidates for support in December 2000.

Linkages with Other Networks

The PPRU Network has established linkages with the Collaborative Antiviral Study Group of NIAID and the Research Units in Pediatric Psychopharmacology of NIMH. These linkages are developing common protocols for new study designs. The groups also have ongoing discussions with members of the Pediatric Network of the Cystic Fibrosis Foundation, the European Pediatric Pharmacology Research Units, and the Canadian Pediatric Pharmacology Unit Network to facilitate pediatric drug trials, coordinate activities, and avoid duplicative studies.

Advocacy Role

The PPRU Network was created to be the gold standard for performing pediatric drug trials. Its major concern is to insure that these trials are properly done. The PPRU Network's investigators are actively involved in organizing workshops geared to help pharmaceutical companies and contract research organizations that develop pediatric drug

testing programs. The Network has also developed position statements on specific aspects of drug trials, such as position paper on the exclusion criteria for febrile children in clinical trials of antipyretic drugs that was recently published in *Current Therapeutic Research* (61:31,2000). Another position paper on the testing of antihypertensive drugs in children is slated for publication in *Pediatrics*.

Interface with the FDA

NICHD's-PPRU Network serves as a liaison to the Pediatric Drug Development Organization within the FDA's Center for Drug Evaluation and Research (CDER), which is empowered to implement the pediatric provisions of FDAMA. The NICHD staff meets monthly with CDER's Associate Director for Pediatrics and staff to address issues related to the implementation of FDAMA and the determination of the research needs in pediatric trial methodology. Investigators from the PPRU Network will address the research needs identified by staff of NICHD and CDER.

In addition, the FDA awarded the PPRU Network a contract to develop industry guidelines for the performance of clinical trials in newborns. The Network's Newborn Group is currently involved in this task.

Workshops

The PPRU Network also sponsored a number of workshops since 1998, including one on biomarkers and surrogate endpoints in pediatrics to advance this novel area of research in clinical trial methodology, and one on developmental pharmacology to provide a forum for identifying research needs and investigative strategies that incorporate new technologies in molecular biology. The major focus of the latter workshop was the ontogeny of drug metabolizing enzymes, ion channels, drug receptors, and drug transport proteins.

A workshop will be held September 25-26, 2000, on the use of drugs in pregnancy. Agenda items include: summarizing the evidence that supports the need to label drugs used during pregnancy; determining research needs; and developing recommendations for the design of drug trials during pregnancy. On December 4-5, 2000, the FDA and the NICHD will co-sponsor a conference entitled, *Clinical Pharmacology during Pregnancy: Addressing Clinical Needs through Science*. The goals of the conference are to raise awareness among clinician researchers and leaders about clinical research, encourage collaboration in these areas, and garner support for such research from health advocacy groups and others.

THE CHILD HEALTH RESEARCH CAREER DEVELOPMENT PROGRAM

The Child Health Research Career Development Award Program, now in its tenth year, emerged through Congressional action taken in response to efforts by the National Association of Children's Hospitals and the Pediatric Research Societies. The goal of the Child Health Research Career Development Award Program is to establish Centers of Excellence in pediatric research. The Program began in 1990, with the funding of six hospitals. The number increased to 19 Centers by 1992, with an additional Center added in 1997 (Figure 6). Funding for these 20 Centers comes mainly from P30 grants, which provide five-year funding for new research projects by nascent pediatric investigators, as well as core support for laboratory and administrative resources.

The Child Health Research Career Program divides its resources between the support of well-funded pediatric departments and the development and stimulation of new Centers that demonstrate great potential. The P30 funding mechanism was recently changed to the K12 mechanism, or the Mentored Clinical Scientist Development Award, to shift the focus back to the original programmatic mission of training outstanding pediatrician scientists. Program funds are meant to ensure that the NICHD is able to provide an ideal environment for learning, while nurturing young scientists into the forefront of pediatric research. In each Center, established investigators make available their expertise and laboratory facilities for use by junior investigators on research projects to enhance their research skills. This experience also enables the junior researchers to generate preliminary data to include in grant applications for independent funding. The Program is an outstanding investment for the future of pediatric research and physician scientist development.

Over the past decade, 26 pediatric departments have received funding through the Child Health Research Career Development Program. In addition, 355 young pediatric investigators have worked in 14 different subspecialty areas. The areas of greatest concentration include hematology/oncology, neonatology, genetics, and infectious diseases. Further, 36 percent of these investigators are female, with 16 percent from minority groups. The group also contains five tenured professors and 59 tenure-track associate professors. Only 11 percent of this group report spending less than ten percent of their time in research.

One measure of success of the Child Health Research Career Development Award Program is attaining competitive NIH research grants. The most successful centers, as measured by their ability to produce investigators able to obtain independent NIH funding, include Yale University, the University of Michigan, Children's Hospital of Philadelphia, and the Cincinnati Children's Hospital. Subspecialty areas that yielded the highest NIH-funding success rates are infectious diseases, endocrinology, and hematology/oncology. Further details of this program analysis appear in the Appendix section of this report.

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FIGURES AND TABLES

Figure 1: ENGB-Supported Projects: 1999

(In Millions of US Dollars)

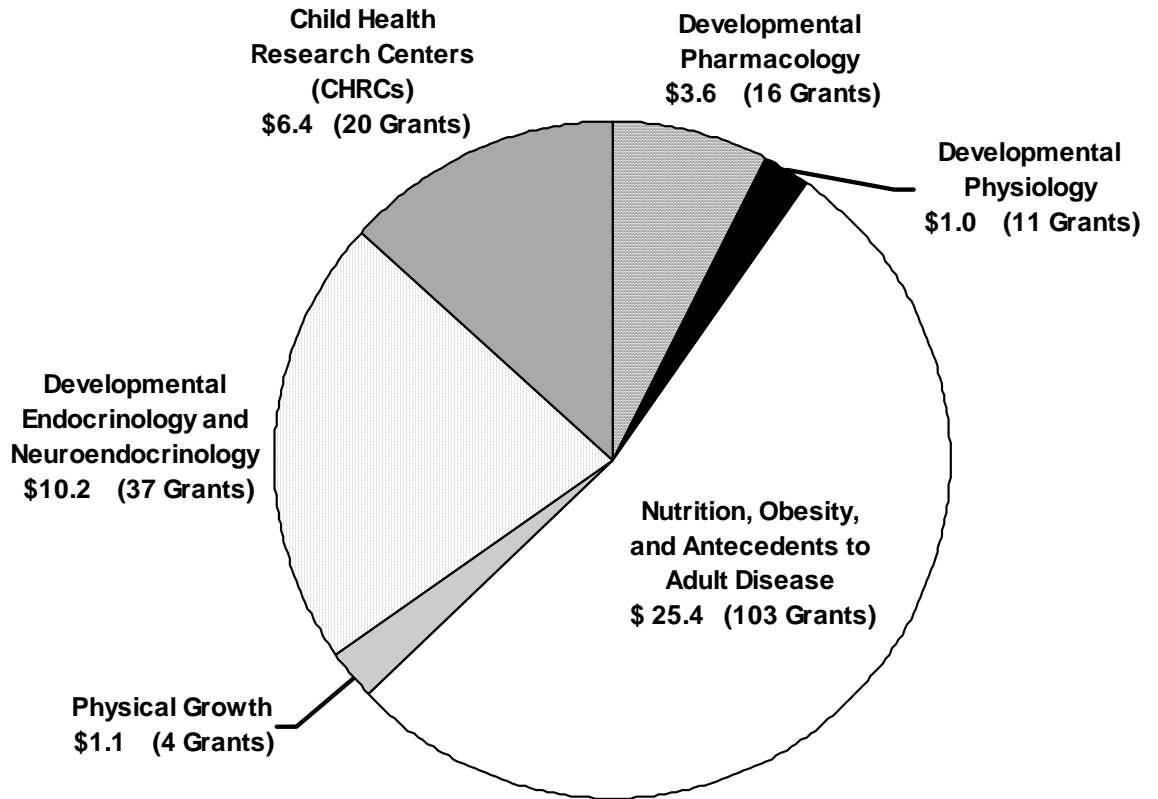


Figure 2: Active PPRU Protocols: 1994-2000

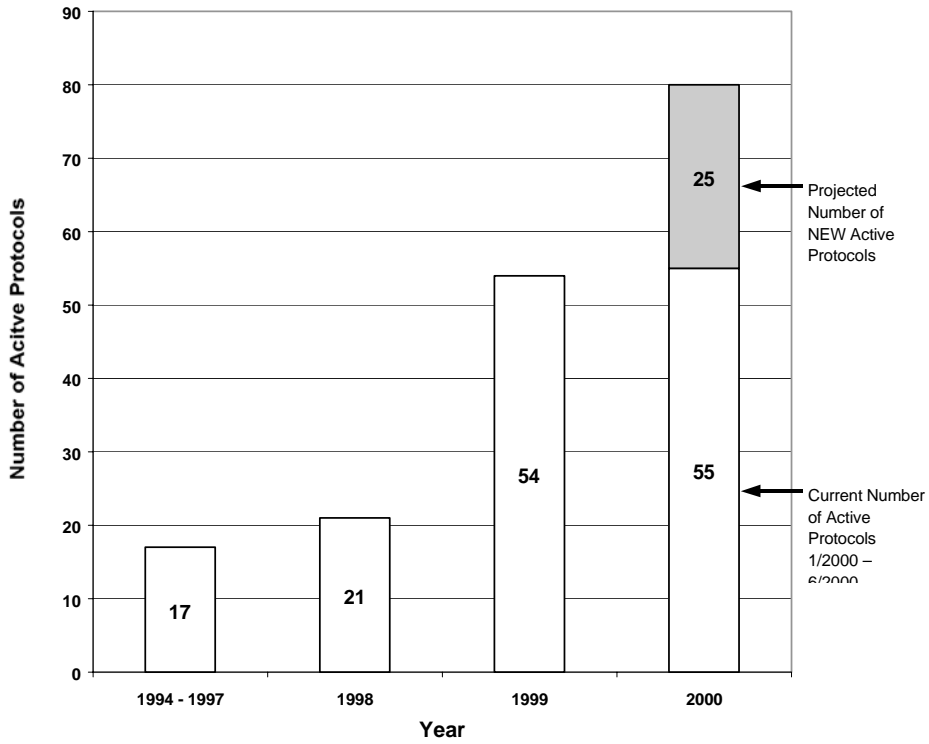


Figure 3A: Focus of Therapeutic Categories in PPRU Protocols: 1998-2000

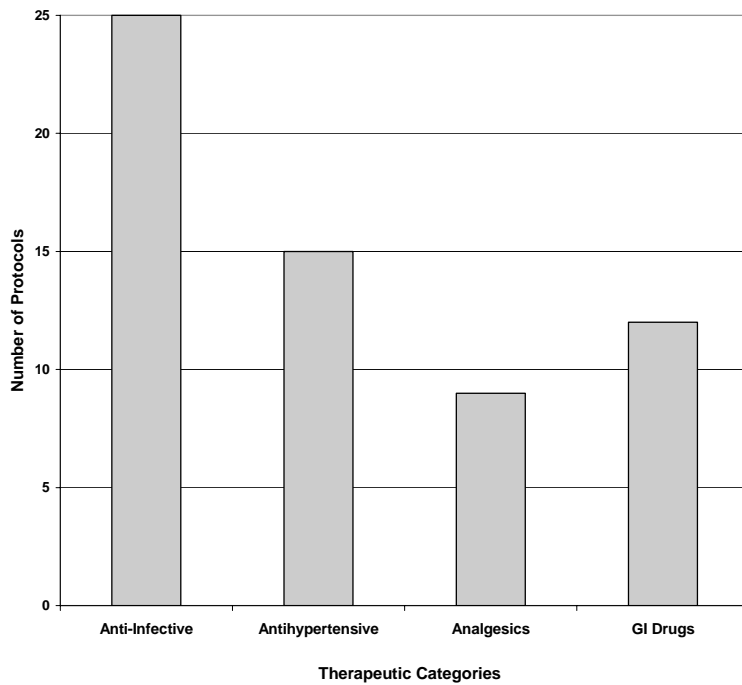


Figure 3B: Other Therapeutic Categories in PPRU Protocols: 1998-2000

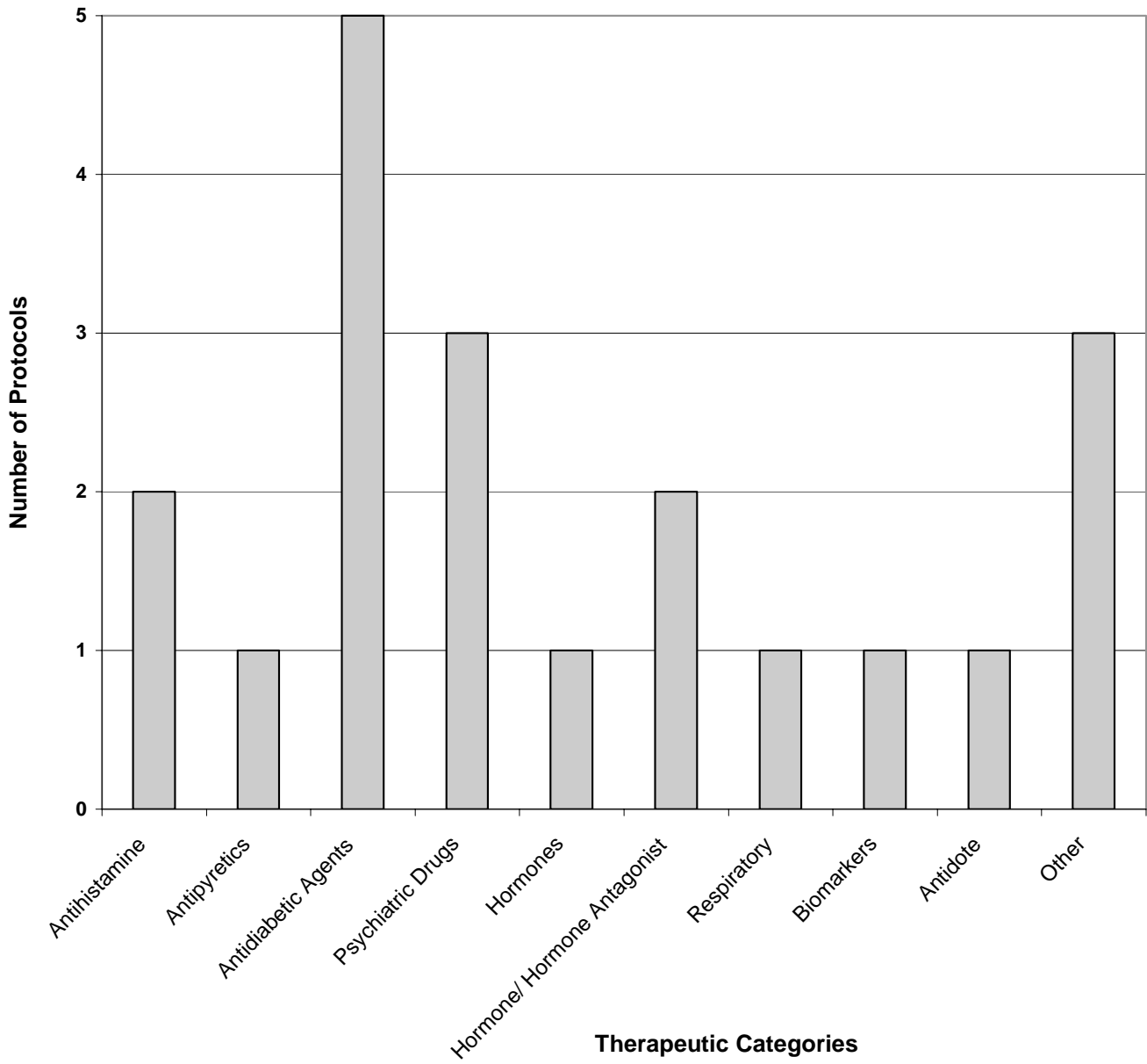


Figure 4: PPRU Protocol Populations by Age Groups: 1998-2000

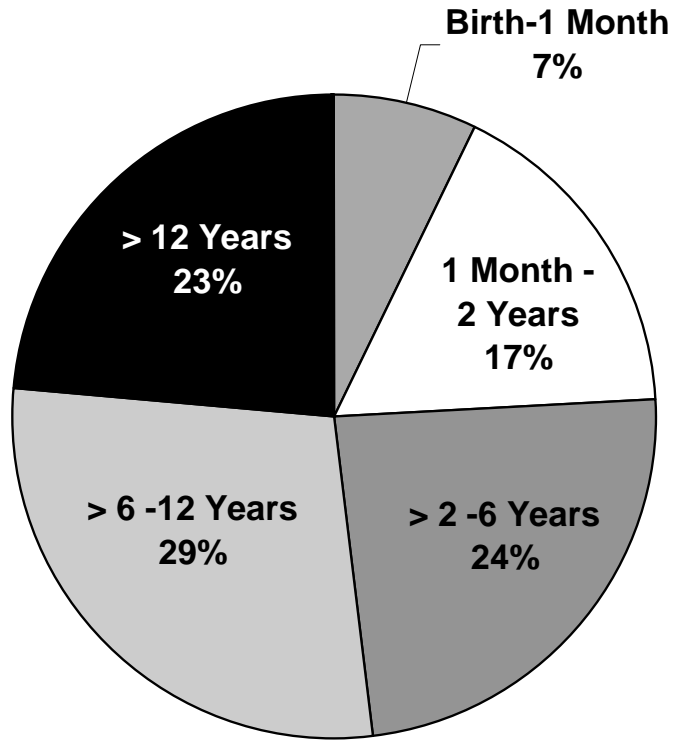
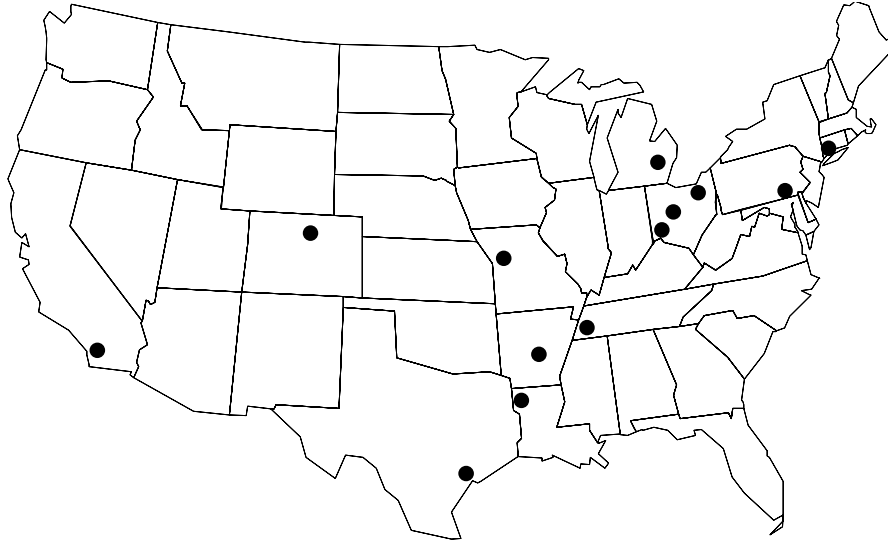


Figure 5: Pediatric Pharmacology Research Unit (PPRU) Network

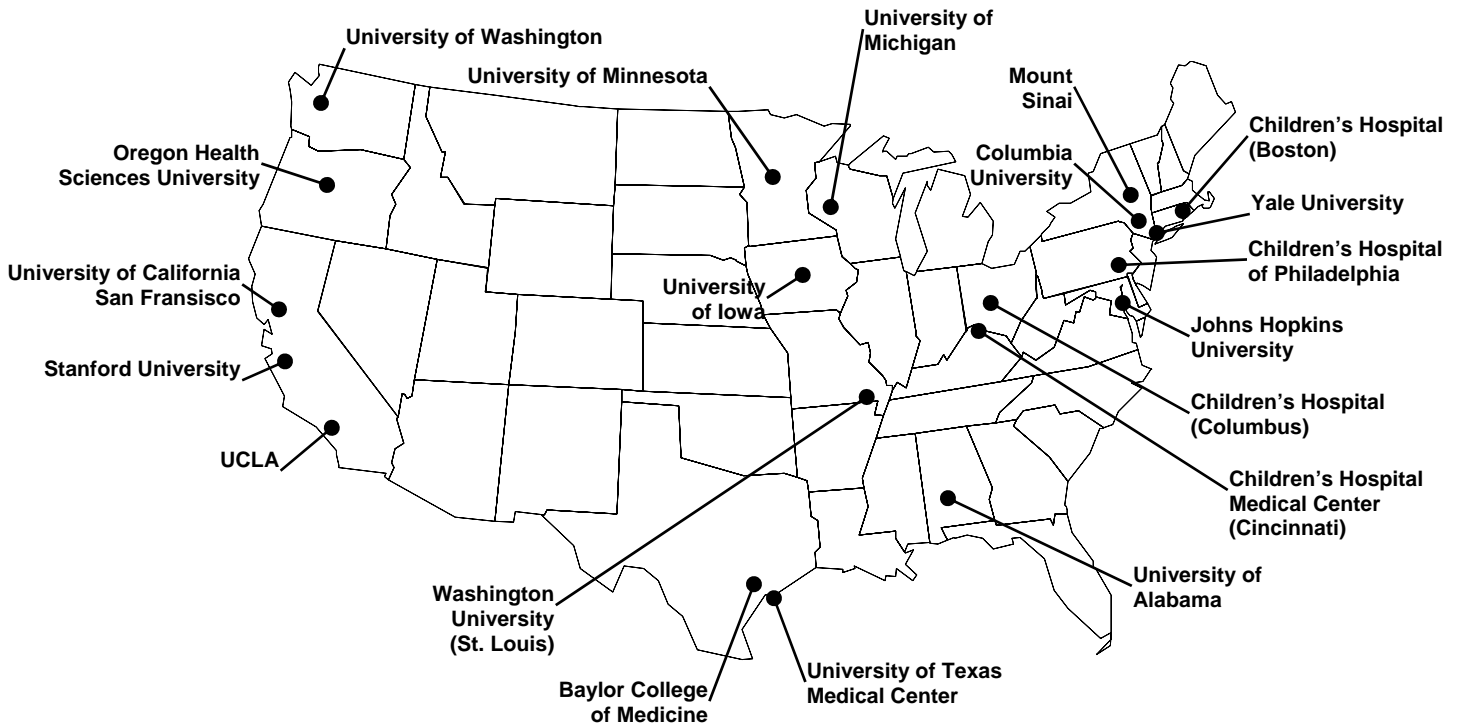


Unit Name and Principal Investigators

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- Baylor College of Medicine—David Poplack, M.D.
- Children’s Mercy Hospital, Kansas City—Gregory Kearns, Pharm.D.
- Children’s Hospital of Philadelphia—Beverly Lange, M.D.
- Children’s Hospital Research Foundation, Columbus, OH—Dr. John Van Der Aken
- Louisiana State University Medical School—John Wilson, M.D.
- National Jewish Medical and Research Center/The Children’s Hospital, Denver, CO—Stanley Szeffler, M.D.
- Rainbow Babies and Children’s Hospital, Cleveland, OH—Jeffrey Blumer, M.D., Ph.D.
- University of California at San Diego—James Connor, M.D.
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- University of Tennessee—Russell Chesney, M.D.
- Wayne State University—Jacob Aranda, M.D., Ph.D.
- Yale University—William Tamborlane, M.D.

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Figure 6: NICHD Child Health Research Centers (CHRCs) 1999



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Table 1: Pharmaceutical Company-Sponsored Studies: 1998-2000

Company	Number of Studies
Aronex Pharmaceuticals	1
Astra Zeneca LP	8
Aventis Behring, LLC	3
BMS	2
Bristol-Myers Squibb	7
Byk Gulden Pharmaceuticals	1
Dura Pharmaceuticals Inc.	1
Fujisawa Health Care, Inc.	1
Glaxo Wellcome, Inc.	6
ICN	1
Janssen Pharmaceuticals	5
Laboratoires UPSA	1
MedImmune, Inc.	1
Merck and Co., Inc.	12
MiniM	1
Novartis	2
Orphan Medical, Inc.	1
Pan American Laboratories, Inc.	1
Parke Davis	2
Parmacon-IL, LLC	1
Pfizer, Inc.	4
Pharmacia & Upjohn Company	2
R.W. Johnson Pharmaceutical Research Institute	11
Roche Laboratories	1
Sage	2
Shering-Plough Research Institute	1
SmithKline Beecham	3
TAP Pharmaceuticals	1
ViroPharma	4
Whitehall-Robbins Healthcare	1
Zara, Inc.	1

APPENDIX: THE CHILD HEALTH RESEARCH CENTERS (CHRCs) PROGRAM: 1990-1999

In 1990, the Branch initiated a Congressionally mandated program of CHRCs, intended to provide resources to pediatric departments and Children's Hospitals. Over the past decade, 26 Centers have received funding through the P30 grants, which provide five-year funding for new research projects by junior pediatric investigators (also referred to as scholars), as well as core support for laboratory and administrative resources. Scholars usually receive funding through this program in one-year increments, up to five years. Most scholars receive two years of support.

One measure of success is the attainment of fundable priority scores on grant applications submitted to the NIH. Approximately one-half (169) of the 355 scholars have applied to the NIH for funding during or after participation in this Program. The group has generated a total of 396 grant applications, receiving 136 NIH research grants. Two-thirds of these grants represent R awards, while approximately one-third are K awards; two percent of the grants represent other award mechanisms, such as cooperative agreements.

The overall success rate for applications submitted to the NIH by individuals who have received CHRC Program funding is 34 percent. Approximately one-half of the 244 CHRC scholars from the more established programs (13 centers with CHRC funds ranging from seven-to-nine years) have submitted applications during or after their training. This group has generated 298 grant applications, yielding 106 successful awards, a success rate of 36 percent. In addition, 70 percent of the applicants from this cohort have been successful in obtaining some form of NIH funding. For instance, these individuals have received a total of 40 R01 grants, mostly within the past five years.

SUBSPECIALTY AREAS

In the past decade, a total of 355 (128 women, 56 minority) pediatric investigators have pursued research training and career development in 14 different subspecialty areas of pediatrics. Half of this group (183 scholars) is made up of four subspecialty areas, namely hematology/oncology, neonatology, genetics, and infectious diseases. Because the Program ensures that no particular subspecialty receives a disproportionate amount of funding, scholars usually come from a variety of subspecialties. The hematology/oncology, genetics, and infectious disease areas have produced the greatest number of NIH grant applications (182). Subspecialty areas with the highest success rates for receiving R awards are immunology, infectious diseases, and hematology/oncology.

ACADEMIC RANK

Among the 355 scholars that received funding through the year 1999, five have reached the level of full professor, 58 have been promoted to associate professors, 233 are assistant professors, and 36 are research associates or instructors. Nine individuals are in

private practice, while 14 are in other settings with alternative ranking systems, such as US- or foreign-government employees, employees of foreign academic institutions, or individuals working in the pharmaceutical industry. Although women investigators make up 36 percent of the CHRC scholars, only 14 (ten percent) of the female investigators have been promoted beyond the level of assistant professor. None of the five scholars that have reached the level of professor are women or minorities.

TIME IN RESEARCH

Evaluating the amount of professional time CHRC-supported individuals devote to research represents another important measure of Program success. The current distribution of percent time in research is shown in Appendix Figure 1, with the analysis limited to those individuals whose first year of funding was prior to 1998. Of the total 286 investigators in this category, 160 scholars (56 percent) report spending greater-than-half their professional time in research, while 58 individuals (20 percent) report spending less than 30 percent time in research.

NIH RESEARCH GRANT SUPPORT (R AWARDS)

The acquisition of independent funding by CHRC-supported investigators is the most important outcome variable for evaluating the success of the program. For the entire cohort of 355 investigators, there have been a total of 366 applications for NIH Research Grant support yielding 92 successful awards to 65 investigators. Women investigators comprise 18 percent of the applicant pool and 17 percent of the successful grantees. Minority investigators comprise 12 percent of the applicant pool and 11 percent of the successful grantees.

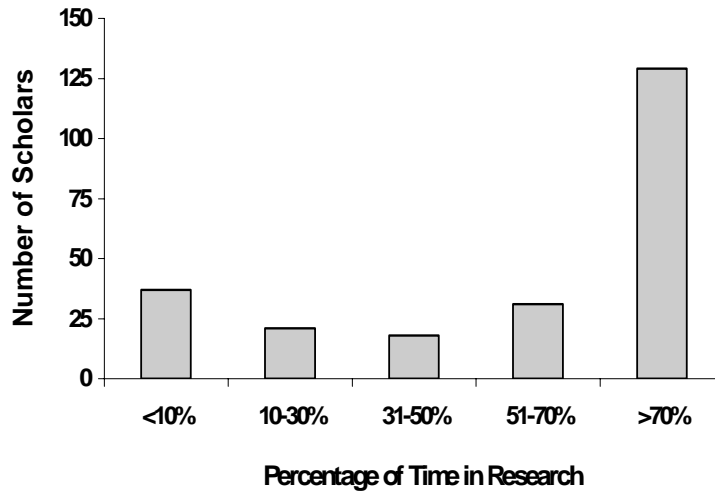
Limiting the analysis to the 13 more established CHRC programs (funding ranging from seven-to-nine years) and to individuals whose first year of funding was prior to 1998, the overall success rate is 22 percent, defined here by the number of grantees who successfully obtained one-or-more R awards when compared to the total number of trainees. Institutions receiving CHRC funding for five years or less have not had sufficient time to train adequate numbers of scholars. It is therefore premature to evaluate the success of their programs based upon obtaining these highly competitive R awards.

NON-NIH FUNDING SOURCES

Scholars have also vigorously pursued non-government sources of financial support for their research endeavors and have received funding from sources, such as the American Cancer Society, the American Heart Association, the March of Dimes Foundation, and the Robert Wood Johnson Foundation. These awards range in size from \$7,000 to \$350,000. A total of 209 scholars received a total of 462 awards from private sources. These data indicate that the most successful scholars hold multiple grants from both government and private foundations.

APPENDIX FIGURE 1

CHRC Scholars' Time Spent Doing Research



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