

# XII.

## National Institute of Diabetes and Digestive and Kidney Diseases

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### INTRODUCTION

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) conducts and supports both fundamental and clinical research and research training focused on several diseases characterized by chronicity and long-term disabling effects. NIDDK provides leadership for a national program in diabetes and in diseases resulting from inherited errors of metabolism, including cystic fibrosis; endocrine disorders; diseases of the gastrointestinal tract (e.g., diseases of the liver and gallbladder); diseases of the blood and bone; and kidney and urologic diseases. The Institute also conducts and supports research in nutrition and nutrition-related disorders. The major objective of this national program is to identify and pursue scientific opportunities yielding fundamental, innovative, and valuable contributions to human health.

NIDDK acquires new biomedical knowledge through grant-supported research, field studies, centrally directed collaborative research contracts, and research at NIDDK facilities in Bethesda, Maryland, and Phoenix, Arizona. The Institute's extramural programs support fundamental and clinical research conducted at universities, medical schools, and other research centers throughout the United States and abroad.

### HIGHLIGHTS OF RECENT SCIENTIFIC ADVANCES RESULTING FROM INTERNATIONAL ACTIVITIES

The Laboratory of Bioorganic Chemistry is responsible for numerous scientific advances stemming from its international activities. These advances include the following:

- elucidation of the structure of biologically active alkaloids from amphibians, birds, and insects (specimens supplied by scientists in Argentina, Brazil, Chile, Costa Rica, Madagascar, Mexico, New Guinea, Panama, and Venezuela);
- illumination of routes and mechanisms

of metabolism that lead to carcinogenic activity, as shown in studies of polycyclic aromatic hydrocarbons and their aza analogues (collaboration with researchers in Australia, Germany, and Northern Ireland);

- insights into structural modifications affecting activity at receptors for peptide, histamine, and adenosine triphosphate, as well as adenosine muscarinic and adrenergic receptors (cooperation with investigators in countries including Germany, Israel, Sweden, and the United Kingdom); and

- synthesis of a series of perfluoroalkylpyrimidines to be used as affinity labels for viral enzymes and for pyrimidine reductase (joint research with scientists in Japan).

### SUMMARY OF INTERNATIONAL PROGRAMS AND ACTIVITIES Country-to-Country Activities and Bilateral Agreements

#### Japan

In fiscal year 1999 (FY 99), the Joint U.S.-Japan Nutrition and Metabolism Panel remained focused on three major priority areas: (1) developing close interactions and new productive efforts in obesity, diabetes, and related metabolic disorders; (2) metabolic bone disease; and (3) nutrition and host defense. Within each of these areas, emphasis continues to be on problems of common concern to the United States, Japan, and other countries in Asia. To advance these goals, the joint panel continues to promote studies of nutritional epidemiology, molecular genetics, cellular biology, and clinical research.

The joint panel has established a number of collaborative efforts among its members, and these activities continue to be fostered by the annual meetings and conferences. The 1999 joint meeting and symposium on Diabetes, Obesity, and Carbohydrate Metabolism were held at the Kaazusa Akademia center, in Kisarazu, Chiba, Japan, on December 12–13, 1999. The conference fostered

significant interaction between Japanese and U.S. participants. Topics included prevention and treatment of obesity; comparative studies on complications of diabetes and obesity in Japan and the United States; diabetes, obesity, and related genetic defects; and insulin resistance and its molecular mechanisms. A highlight of the conference was a poster session of nearly 20, primarily young, Japanese investigators. Such a session offered opportunities for interactions with senior investigators.

### Activities With International and Multinational Organizations World Health Organization

#### *Division of Diabetes, Endocrinology, and Metabolic Diseases*

The Division of Diabetes, Endocrinology, and Metabolic Diseases, an extramural research program, is a World Health Organization (WHO) Collaborating Center for Diabetes Research, Information, and Education. The center conducts research on diabetes in representative samples of adults in the U.S. population. Data from the U.S. studies are included in analyses of diabetes developed by WHO.

The Division's Diabetes Research Program supports a contract for analysis of the WHO Multinational Study for Vascular Disease and Diabetes. Morbidity and mortality for diabetes in 10 countries are the focus of this research. Goals of the study include (a) identification of risk factors and risk markers for complications and (b) quantitation of causes of interpopulation differences in microvascular and macrovascular disease in diabetes.

#### *Phoenix Epidemiology and Clinical Research Branch*

The Phoenix (Arizona) Epidemiology and Clinical Research Branch, Division of Intramural Research, continues to serve as a WHO Collaborating Center for the Design,

Methodology, and Analysis of Epidemiologic and Clinical Investigations in Diabetes. This center collaborates with WHO in implementing the action program of WHO and the International Diabetes Federation and provides advice, consultation, and collaboration for investigators. The WHO collaborating center assists (a) in development and application of study design and of standardized methods for epidemiologic and clinical investigations and (b) in analysis of data on the causes and pathogenesis of non-insulin-dependent diabetes mellitus (type 2 diabetes) and its complications. The center provides investigators with short- or long-term training experience in the methods and application of epidemiologic and clinical research on type 2 diabetes.

The center participates in the WHO Multinational Study for Vascular Disease and Diabetes, which is examining the incidence and mortality related to vascular complications of diabetes among ethnic groups in different countries.

### **Extramural Programs Division of Diabetes, Endocrinology, and Metabolic Diseases**

#### *Australia*

Using the NOD mouse, an animal model that replicates many aspects of insulin-dependent diabetes mellitus (type 1 diabetes) in humans, researchers at Royal Melbourne Hospital, Victoria, are expressing proinsulin in hematopoietic stem cells present in the bone marrow. They hypothesize that this procedure will allow induction of immune tolerance by hematopoietically derived antigen-presenting cells. This research may lead to a method to prevent type 1 diabetes in persons at risk for the disease.

An investigator at the International Diabetes Institute, Melbourne, is evaluating the emergence of diabetes mellitus as a major chronic disease associated with social, economic, lifestyle, and environmental changes in populations in the Indian Ocean and the South Pacific. Marked differences in susceptibility to diabetes among various ethnic groups occur, and the differences have a bearing on the causes of diabetes in specific U.S. subpopulations. These longitudinal studies address (a) the contributions of specific environmental and lifestyle factors to the causes of diabetes and (b) the interac-

tions of these factors with genetic factors that determine susceptibility to diabetes.

#### *Canada*

The University of Toronto, Ontario, and the University of Western Ontario, London, are participating in the Epidemiology of Diabetes Interventions and Complications Study—a multicenter, longitudinal, epidemiologic study of the 1,441 patients who participated in the Diabetes Control and Complications Trial. This 10-year follow-up study is focusing on the development of microvascular and macrovascular disease.

Several Canadian institutions are involved in the Diabetes Prevention Trial for Type 1 Diabetes. The participating institutions are the University of Calgary, Alberta; the University of Alberta, Edmonton; British Columbia Children's Hospital, Vancouver; the University of Manitoba, Winnipeg; the Grace Health Center, Halifax, Nova Scotia; the University of Western Ontario, London; the Hospital for Sick Children, Toronto; and the Montreal Children's Hospital, Quebec. The major objective of the trial is to determine whether antigen-based therapies (parenteral or oral insulin) in nondiabetic relatives of persons with type 1 diabetes can prevent or delay the onset of clinical diabetes. The study has recruited approximately 65% of its goal of 830 volunteers, and the plan is to perform approximately 6 years of follow-up.

Researchers at the University of Toronto are examining insulin secretion from the insulin-secreting cells (beta cells) of the pancreas. Specifically, they are attempting to elucidate the mechanism by which exocytotic proteins interact with plasma membrane ion channels. They will use novel techniques for gene transfer to explore these interactions.

Investigators at the Hospital for Sick Children, Toronto, played a key role in identification of the gene responsible for cystic fibrosis (CF). These scientists are leading an international consortium that has identified more than 500 specific gene mutations capable of causing this clinical disorder. These investigators have identified mutations associated with pancreatic sufficiency or insufficiency, allowing physicians to predict which children with CF will require pancreatic enzyme replacement therapy. They are characterizing the promoter region of the

CF gene (CFTR), which regulates gene expression, and have developed model systems to study the function of the protein coded for by the CF gene.

The NIDDK-supported Cystic Fibrosis Research Center, Hospital for Sick Children, Toronto, has made major strides in correlating clinical phenotype with genetic mutations underlying the disorder and in identifying a broad array of testicular defects that cause infertility in CF. In addition, the researchers are studying the role of mutations of the CFTR gene in the development of idiopathic pancreatitis. These studies have greatly expanded understanding of the biological importance of the CFTR protein encoded by the CFTR gene. Roles of the CFTR protein that have been demonstrated by this study may suggest avenues for future treatment of CF, male infertility, and pancreatitis.

Under a research grant awarded to McGill University, Montreal, the role of phosphorylation in regulation of the CFTR protein is being investigated. This project addresses practical aspects of the function of the protein that would be used to improve the effectiveness of new therapies for CF.

Investigators from Inotek Corporation, Cincinnati, Ohio, are collaborating with an investigator at the University of Alberta, Edmonton, to determine whether a novel, potent, orally active inhibitor of the enzyme poly(adenosine triphosphate-ribose) synthetase can be used to prevent free-radical destruction of insulin-producing beta cells in the pancreas.

#### *China*

Childhood diabetes in China is the focus of two projects in which scientists at the University of Pittsburgh, Pennsylvania, are collaborating with scientists at Beijing Hospital. In the first project, an epidemiologic study has established registries of children with diabetes who reside at different sites in China. This study will be used to define geographic, ethnic, and temporal variations in these groups. These data will be compared with data from other international childhood diabetes registries. In the second project, researchers are comparing characteristics of patients who have new onset of diabetes with characteristics in other populations, in an effort to determine whether childhood diabetes in China is associated

with specific genetic alleles, immune markers, and biochemical factors.

Researchers at the Joslin Diabetes Center, Boston, Massachusetts, are collaborating with researchers at the People's Hospital, Beijing Medical University, to identify susceptibility genes involved in the development of type 2 diabetes. They have obtained results suggesting that allelic variation at a particular gene locus contributes to the development of type 2 diabetes in a significant subset of families.

#### *Denmark*

Scientists at the Hagedorn Research Institute, Copenhagen, are studying the role of specific genes that code for proteins in the Notch signaling system. They are investigating how these genes contribute to regulation of endocrinogenesis of the pancreas. The purpose of the study is to determine whether in vitro modulation of the Notch system can lead to indefinite propagation of precursor pancreatic cells and whether these cells can be converted to beta cells.

Another group of researchers at the Hagedorn Research Institute are investigating the role of growth hormone and prolactin in the stimulation of beta cell proliferation and insulin production. The team will examine the pathways involved in the beta cell response to these hormones, with the goal of identifying and determining the function of growth hormone-regulated and prolactin-regulated beta cell genes.

#### *Israel*

Investigators at the Washington University School of Medicine, St. Louis, Missouri, are seeking to clarify the genetic basis of the metabolic defects of type 2 diabetes. The focus of their studies is the Ashkenazi Jewish population in Israel, a relatively homogeneous population that makes it easier to detect genes for diabetes. Clinical and demographic data will be collected on 400 sibling pairs with type 2 diabetes, through the Diabetes Clinics at Hadassah Hospital and the Histadrut, Jerusalem. A whole-genome search will be used to identify genes that are associated with the diabetes phenotype.

Researchers at the Albert Einstein College of Medicine, Yeshiva University, Bronx, New York, are working with an investigator at Tel Aviv University, Israel, to generate lines of pancreatic beta cells for use as a cell therapy

for diabetes. They will examine the effects of cell proliferation and growth arrest on differentiated functions of beta cells, such as the secretion of insulin.

#### *Sweden*

A scientist at the University of Washington, Seattle, is using a nationwide Swedish diabetes registry to define genetic, autoimmune, and viral antibody markers for type 1 diabetes in patients with disease onset at ages 15–34 years.

An investigator at Case Western Reserve University, Cleveland, Ohio, is collaborating with investigators at Karolinska Hospital, Stockholm, to develop and apply methods for quantifying pathways of carbohydrate and lipid metabolism in humans. These studies, which use stable isotope-labeled compounds, are expected to elucidate the role of the liver in causing hyperglycemia in patients with diabetes.

#### *Switzerland*

A researcher at the Salk Institute for Biological Studies, La Jolla, California, is working with a researcher at the Institute of Pathology, University of Bern, to develop agonists and antagonists for somatostatin that can differentially affect receptor subtypes and thus affect only particular actions of somatostatin. This peptide, which is produced in the brain, inhibits the release of growth hormone and has numerous other functions as well. It is used therapeutically for the treatment of acromegaly and as an imaging tool for certain kinds of tumors. There are five subtypes of somatostatin receptors; their presence in the brain and other tissues subserves the multiple functions of somatostatin.

#### *United Kingdom*

Workers at Anergen, Inc., Redwood City, California, together with an investigator at University College, London, England, have developed a peptide vaccine that protects against the development of spontaneous diabetes in NOD mice. They are testing this vaccine to determine its efficacy in preventing diabetes in NOD mice with early symptoms of diabetes. If efficacy can be demonstrated in NOD mice with late-stage prediabetes, Anergen, Inc., will initiate clinical development of the peptide vaccine against type 1 diabetes in humans.

#### *Multinational Studies*

Fifteen research groups from Europe and the United States have participated in a consortium to combine the data for all the genetic markers on chromosome 20. Subcontracts have been issued to groups in France, Sweden, and the United Kingdom for participation in this effort. The combined data consist of 24 individual data sets, including 2 on African Americans; 13 on whites (6 on residents of Europe and 7 on residents of the United States); 2 on Japanese; 6 on Mexican Americans; and 1 on Native Americans. This is the largest number of data sets ever analyzed for a single disease, and it will be used to map genes for diabetes.

A registry of patients who have had pancreas and islet transplantation, which contains data collected worldwide, is being supported by a contract at the University of Minnesota, Minneapolis. This information addresses the capability of transplantation to prevent, improve, or stabilize the complications of diabetes.

Investigators at University Hospital, Boston, Massachusetts, are collaborating with investigators at the University of Montreal and Karolinska Institute, Stockholm, to study the role of acyl coenzyme A in insulin secretion and in type 2 diabetes and obesity. The investigators are studying an important hypothesis that has the potential of linking changes in fatty-acid metabolism that occur in obesity and type 2 diabetes to beta cell dysfunction.

The National Hormone and Pituitary Program of NIDDK supports preparation and distribution of highly purified hormones and antisera against these hormones. The hormones and antisera are provided for use to qualified investigators in the United States and abroad.

#### **Division of Digestive Diseases and Nutrition**

##### *Australia*

The Liver and Biliary Diseases Program, Division of Digestive Diseases and Nutrition, supports several clinical trials and research projects in the basic sciences. A joint project at the University of California, Davis, and the Walter and Eliza Hall Institute of Medical Research, Melbourne, is aimed at elucidation of antigen receptors in the autoimmune pathogenesis of primary biliary cirrhosis. A project at the University of Sydney, New

South Wales, promises to have a major scientific impact on understanding of the interaction between hepatitis C virus and the liver. The project will provide prognostic indicators for predicting disease outcome in individual patients and a noninvasive test of hepatic fibrosis and function.

A project at the University of Adelaide is aimed at revealing the mechanism for hepatic uptake, hepatocellular transport, and transmembrane transfer of small molecules in the endoplasmic reticulum.

#### **Bangladesh**

A small, 2-year, clinical trial, entitled *H. pylori*: a Cause and Treatment Failure of Iron Deficiency Anemia, is based at the International Center for Diarrhoeal Disease Research, Dacca. This study is a prospective, randomized, double-blind, placebo-controlled trial to test the hypothesis that the poor response to iron therapy seen in iron-deficient children of Bangladesh is related to low gastric acid output caused by *Helicobacter pylori* infection. The intervention in this trial is iron therapy combined with elimination of *H. pylori* by administration of antibiotics.

#### **Brazil**

A clinical investigator at the University of Iowa, Iowa City, will determine the presence of liver-related immunosuppressive factors in human lymphocytes in patients with or without *Leishmania*. These studies will be performed in collaboration with investigators at Universidade Federal do Rio Grande do Norte, Natal.

#### **Canada**

The North American Study for the Treatment of Refractory Ascites, based at the Medical College of Virginia, Richmond, has one foreign site—Toronto Hospital. This study is a multicenter, prospective, randomized clinical trial designed to test whether transjugular intrahepatic shunt of the portal system is more effective for refractory ascites than a standard therapy—total paracentesis, sodium restriction, or diuretic agents. Refractory ascites is a serious complication of cirrhotic portal hypertension and is associated with considerable morbidity, increasing health care costs, decreasing quality of life, and eventually, death or increased risk of death after orthotopic liver transplantation.

A clinical trial on functional bowel disorders is based at the University of North Carolina, Chapel Hill, and has one foreign component at the Clarke Institute of Psychiatry, Toronto Hospital. It is hoped that this study will enroll at least 300 female patients with functional bowel disorders (irritable bowel syndrome, painful constipation, or functional abdominal pain) to gain further information on this complicated syndrome. Functional bowel disorders lower quality of life and reduce individual productivity.

#### **Chile**

A clinical researcher at the University of Maryland Medical Center, Baltimore, in collaboration with the Center for Vaccine Development, Baltimore, is the principal investigator for a project that will determine the age-specific seroprevalence and seroincidence of hepatitis A infection in Santiago. In addition, the age-specific incidence of fulminant hepatic failure will be determined, to detail the incidence of fulminant hepatic failure secondary to hepatitis A infection.

#### **China**

A clinical trial, entitled Relative Effects of Diet and Exercise on Body Composition, is based at Tufts University, Boston, Massachusetts, and has two foreign sites—the Chinese Academy of Preventive Medicine, Beijing and Shanghai. This is a cross-sectional study of 112 healthy, adult men and women, aged 37–47 years. Two hypotheses will be tested: (1) Physical activity but not dietary fat intake is a significant predictor of individual variability in total body fat. (2) The proportion of body fat located centrally is negatively associated with physical activity and not significantly associated with dietary fat intake.

#### **Mexico**

NIDDK is supporting a grant to study the natural history of *Helicobacter* infection in infants of low-income families living on the border of the United States and Mexico. A group of infants from the day-care centers operated by the Instituto Mexicano del Seguro Social, in Juárez, will be examined. This study will improve understanding of the epidemiology of *Helicobacter* infection by determining the incidence of *H. pylori* infection in this population compared with a U.S. population in San Elizario, El Paso County,

Texas. In addition, the scientists will determine the frequency with which *H. pylori* infections spontaneously clear during the 1st 3 years of life. The studies will also examine the impact of socioeconomic indicators, hygiene, and diet on the incidence, recurrence, and persistence of *H. pylori* among this population.

#### **Multinational Studies**

A clinical trial, entitled Prevention of Esophageal Varices by Beta-adrenergic Blockers, is based at Yale University, New Haven, Connecticut, and has two foreign sites: the Royal Free Hospital, London, England, and the University of Barcelona, Spain. This clinical trial is a prospective, randomized, double-blind, placebo-controlled study designed to investigate whether early therapy with a nonselective  $\beta$ -adrenergic blocker can prevent or delay the development of gastroesophageal varices in patients with cirrhosis and portal hypertension.

#### **Division of Kidney, Urologic, and Hematologic Diseases**

##### **Canada**

A clinical center at Queen's University, Kingston, Ontario, is one of the major participating centers in the NIDDK-funded, collaborative, clinical study on chronic prostatitis. In addition to the principal investigator at Queen's University, that center includes investigators at the University of Toronto and the University of Calgary, Alberta.

A researcher from Boston Children's Hospital, Massachusetts, is performing genetic mapping of the zebra fish and is collaborating with a researcher from Ottawa Civic Hospital, Ontario, to generate molecular reagents.

An investigator at the University of Vermont, Burlington, is working with an investigator at the University of British Columbia, Vancouver, to elucidate structure–function relationships in recombinant transferrins. The results of this work are essential to understanding the molecular basis of certain defects in iron delivery and to the design of rational interventions in the delivery of iron to neoplasms.

##### **Germany**

A scientist from Washington University, St. Louis, who is also performing genetic mapping of the zebra fish, is collaborating with

a scientist from Max Planck Institute, Tübingen, who is providing mutant zebra fish embryos for study. A new award to the Max Planck Institute has been made to support a project headed by a Nobel Laureate, which is intended to improve the genetic map of the zebra fish.

#### **Malawi**

In collaboration with the University of North Carolina, studies will be undertaken on men infected with human immunodeficiency virus (HIV) and other sexually transmitted diseases, to determine the effects of *Trichomonas* on excretion of HIV in semen; whether recommended treatment of *Trichomonas* is effective in HIV-infected men; whether such treatment reduces the excretion of HIV in semen; and which inflammatory cytokines are associated with excretion of HIV in semen in patients infected with *Trichomonas*. This research will help to determine the efficacy of current therapy and to improve the guidelines for treatment of sexually transmitted diseases in Malawi and will also prove germane to prevention and treatment of HIV in the United States. The work is conducted in Malawi because of the high prevalence of *Trichomonas* and HIV in the study population. The principal foreign investigators are from Lilongwe Central Hospital.

#### **New Zealand**

A scientist at the University of Auckland is studying human embryonic hemoglobin, to obtain a detailed understanding of the structure and functioning of the three human embryonic hemoglobins and to gain insight into the "normal" behavior of the oxygen transport system that operates at the earliest stages of human development. Human embryonic hemoglobins will be produced in a system for expression of recombinant yeast, without recourse to use of human embryonic tissue.

#### **Sweden**

A recently funded grant addresses the role of a newly identified protein in the pathogenesis of nephrotic syndrome. This discovery may also help researchers to understand normal kidney function and to find new targets for therapies for certain types of acquired kidney diseases, such as diabetic nephropathy.

The principal investigator is at Karolinska Institute, Stockholm.

#### **United Kingdom**

A study at University College, London, aims to determine the role of unbound body iron in the pathogenesis of iron overload.

#### **Multinational Studies**

A grant addressing the mapping of genes for nephropathy in type 1 diabetes involves a major collaborative effort among researchers at the Joslin Diabetes Center, Boston, Massachusetts; the National Public Health Institute, Helsinki, Finland; and Karolinska Institute, Stockholm. Results from Finnish populations will be used to validate the U.S. data. Karolinska Institute will coordinate the results from the two populations.

The Division of Kidney, Urologic, and Hematologic Diseases is collaborating with the European Institute of Oncology in the study of Cancer in End-Stage Renal Disease. The Division provided data from the U.S. Renal Data System and has been taking part in this study comparing the development of cancer in patients with end-stage renal disease, in Australia, New Zealand, Europe, and the United States.

#### **International Meetings**

##### **Division of Digestive Diseases and Nutrition**

The Division of Digestive Diseases and Nutrition sponsored the 6th International Symposium on Hepatitis C Virus and Related Viruses, in Bethesda, Maryland, on June 6-9, 1999. The purpose of this meeting was to convene international scientists from various disciplines with a research interest in hepatitis C virus and related viruses and to provide an open forum for the exchange of up-to-date knowledge on the basic science and translational research of hepatitis C virus and its associated diseases.

A workshop entitled Complementary and Alternative Medicine in Chronic Liver Disease was held in Bethesda, on August 22-24, 1999. The workshop was cosponsored by the Office of Dietary Supplements and the Division of Digestive Diseases and Nutrition, of the National Center for Complementary and Alternative Medicine, National Institutes of Health (NIH), and by the American Association of Naturopathic Physicians. Invited speakers included physician-scientists from Austria, China, Japan, Korea, Nigeria, and the United States.

The aim of the workshop was to assess the current knowledge on complementary and alternative medicine for chronic liver diseases. The meeting focused on the available scientific evidence for efficacy and safety of these agents and on identification and prioritization of research needs that will more fully define the efficacy and safety of complementary and alternative medicine for the treatment or amelioration of liver diseases.

##### **Division of Kidney, Urologic, and Hematologic Diseases**

The Division of Kidney, Urologic, and Hematologic Diseases organized and convened a workshop on Women and Renal Disease, which was held in Bethesda, on September 14-17, 1999. The meeting provided a forum for discussing differential outcomes in progressive renal disease and complications in women with chronic renal disease. Participants in the poster session and meeting discussions came from Canada, Germany, Israel, Spain, and Switzerland.

The Division supported meetings convened by the European Society for Pediatric Hematology and Immunology and the World Congress on Iron Metabolism, in Washington, D.C., on May 11-15, 1999, to examine current information on maturational aspects of the developing immunologic and hematologic systems and the clinical application to human disease.

The Division also supported the 5th Banff Conference on Allograft Pathology, in Alberta, on June 7-12, 1999. The purpose of this meeting was to refine criteria for renal pathologic parameters in patients who have had transplants, including classification of the process of organ rejection.

##### **Intramural Programs and Activities Clinical and Endocrinology Branch**

###### **Molecular and Cellular Physiology Section**

The Molecular and Cellular Physiology Section, Clinical and Endocrinology Branch, has two international studies. One study, with investigators at the Weizmann Institute of Science, Rehovot, Israel, is focusing on the signaling pathways of insulin-like growth factor I (IGF-I) and insulin receptor. The second study involves scientists in

Barcelona, Spain, and examines the role of the sulfonylurea receptor in the functions of the pancreatic beta cell and the central nervous system. Ten foreign investigators are working in this Section: one Visiting Fellow from Canada; one Visiting Fellow and one Visiting Associate from China; one Visiting Associate from Colombia; two Visiting Fellows from France; two Visiting Fellows from Israel; and one Visiting Associate and one Visiting Fellow from Spain.

#### ***Molecular Regulation and Neuroendocrinology Section***

The Molecular Regulation and Neuroendocrinology Section is initiating several pilot studies to determine whether future joint studies of thyroid function are feasible. In one pilot project, expression of thyroid hormone and retinoid receptors in bone will be investigated in a joint project with scientists at the General Hospital, Southampton, England. The study will examine the expression of these receptors in various bone diseases, by using antibodies previously generated in the Molecular Regulation and Neuroendocrinology Section. In research using cDNA (complementary DNA) microarray studies of thyroid hormone receptor knockout mice, scientists from the Ecole Normales Supérieure, Lyon, France, will focus on the regulation of gene expression for thyrotropin receptor isoforms in specific tissues of the thyrotropin receptor knockout mouse. Also, scientists at the University of Haifa, Israel, will cooperate in studies on the role of thyrotropin-stimulating hormone (TSH) in migration of the embryonic thyroid primordium for development of the mature thyroid gland. The project will examine thyroid function, structure, and location in patients with mutant TSH receptor. Three foreign scientists are working in the Section's laboratory: one Visiting Scientist from Greece, one Visiting Associate from Israel, and one Special Volunteer from Japan.

#### **Diabetes Branch**

##### ***Clinical and Cellular Biology Section***

The Clinical and Cellular Biology Section, Diabetes Branch, collaborates with a researcher at the Institute of Histology and Embryology, University of Geneva School of Medicine, Switzerland. In a long-standing joint research effort between NIDDK and researchers in Geneva, morphological tech-

niques have been used to investigate receptor-mediated endocytosis of insulin and related peptides.

##### ***Experimental Diabetes, Metabolism, and Nutrition Section***

The Experimental Diabetes, Metabolism, and Nutrition Section is continuing to work with institutions in two countries (England and Japan) and has initiated studies in two additional countries (Germany and Sweden). In an ongoing project with the University of Bath, researchers are investigating the development of insulin resistance in adipose cells in culture. A study with scientists at Yokohama City University and Tokyo University is examining the role of insulin receptor substrates in the glucose transport response to insulin and the effects of steroid hormones on insulin action. In a joint project with the University of Cologne and the University of Göteborg, investigators are studying components of the insulin receptor signaling pathway that regulate the subcellular trafficking of glucose transporters in isolated adipose cells.

Four scientists from abroad are working in this Section: two Visiting Fellows (one from Canada and one from Romania), one Fogarty Scholar-in-Residence (from Sweden), and one Visiting Scientist (from the United Kingdom).

##### ***Molecular Biology and Gene Regulation Section***

In the Molecular Biology and Gene Regulation Section, collaboration has been initiated with investigators at Kyoto University, Japan, to study the role of leptin in insulin resistance in mice, particularly as applied to lipotrophic diabetes. The Section currently has no foreign investigators, but two Visiting Fellows (one from the Czech Republic and one from Italy) are scheduled to start in early 2000.

##### ***Receptors and Hormone Action Section***

The Receptors and Hormone Action Section serves as host to one Visiting Scientist from Colombia; one Visiting Scientist from France; one Visiting Scientist and one Visiting Fellow from Italy; one Visiting Fellow from Spain; and one Visiting Associate from Turkey.

#### **Genetics and Biochemistry Branch**

The Genetics and Biochemistry Branch is host to 14 visiting scientists from eight countries. There are eight Visiting Fellows in this Branch: Canada (one), the Czech Republic (one), France (two), Russia (one), Slovakia (one), the United Kingdom (one), and Yugoslavia (one). The Branch also serves as host for six Visiting Scientists—three Staff Scientists from Russia and three Research Fellows (one from China and two from Russia). Work includes research in the following areas:

- a novel transcription factor found predominantly in testis;
- the mechanism(s) by which the transcription factor NURR1 regulates expression of tyrosine hydroxylase;
- structural, biochemical, and mechanistic studies of DNA mismatch repair;
- the role of chromatic structure in regulating the late steps of homologous recombination involving DNA branch migration and Holliday junction resolution;
- crystallization and characterization of several recombination and DNA repair proteins;
- investigation of the role of specific amino acid residues in the hydrolysis of nucleotide triphosphates;
- identification, purification, and characterization of proteins comprising a ribonucleotide particle essential for processing of pre-rRNA (preribosomal RNA) in *Xenopus*;
- gene expression in bacteria and yeast as a function of DNA damage;
- mechanisms by which proteins are secreted from cells; and
- mechanisms by which proteins are transported into cell membranes.

#### **Genetics of Development and Disease Branch**

During FY 99, the Genetics of Development and Disease Branch hosted investigators from three countries: Argentina (one), China (five), and Japan (four). Five of the researchers are Visiting Fellows, three are Visiting Associates, and two are Special Volunteers. The Branch also conducted the following collaborative efforts with other countries:

- investigation of the pathogenesis of type C Niemann-Pick disease, with scientists at Lyon-Sud School of Medicine, Oullins, France;

■ studies on the functions of glycosphingolipids, with investigators at the Kekulé Institut für Organische Chemie und Biochemie der Universität Bonn, Bonn, Germany; and

■ research on generation of a mouse model for achondroplasia, with investigators at the Weizmann Institute of Science, Rehovot, Israel.

### **Mathematical Research Branch**

Scientists in the Mathematical Research Branch maintain joint research efforts with groups in Canada, England, and Germany. In FY 99, Branch scientists collaborated with researchers at the University of Toronto, on models of synaptic transmission in the crayfish neuromuscular junction, and with researchers at the University of Hannover Medical School, Germany, on the theory of protein folding. Branch researchers are investigating hypothalamic neurons and analysis of integrate-and-fire models of neuronal electrical activity, in cooperative studies with scientists at the Babraham Institute, Cambridge, England.

During FY 99, the Branch hosted a senior sabbatical visitor from the Center for Scientific and Industrial Research, Hyderabad, India. A Visiting Fellow from China is working on models of actin-myosin binding in skeletal muscle. A Visiting Fellow from New Zealand is working on mathematical models of electrical activity and hormone secretion in hypothalamic neurons.

The Chief of the Mathematical Research Branch was an invited speaker at the Bioinformatics '99 meeting, in Lund, Sweden.

### **Metabolic Diseases Branch**

#### *Cell Regulation Section*

The Cell Regulation Section, Metabolic Diseases Branch, is concerned with the hormonal regulation of growth versus differentiation and the simultaneous preservation of self-tolerance. Self-tolerance prevents development of autoimmune diseases of the thyroid and development of diabetes, systemic lupus, and other organ-specific immune diseases caused by dysregulation of major histocompatibility gene expression in target tissues. In FY 99, the Section had five Visiting Fellows (two from Italy, two from Japan, and one from Korea) and two Special Volunteers (one each from Italy and Japan).

This Section has six collaborative projects with foreign laboratories in Italy and Japan.

Investigators in the Section and at Università degli Studi "G. D. Annunzio" Faculty of Medicine and Surgery, Palazzina Scuole di Specializzazione, Chieti, Italy, are collaborating to study the role of a 90-kilodalton immunomodulator as a protective factor in autoimmunity, cancer, and acquired immunodeficiency syndrome (AIDS). They are also working to develop new drugs related to methimazole for treatment of diverse diseases such as lupus, diabetes, and rheumatoid disease. These projects have developed drugs that can suppress the development of diabetes in the NOD mouse and systemic lupus erythematosus in other experimental animal models. Investigators at the Center for Endocrinology and Experimental Oncology, the Center for Nursing Research, and the Federico II Medical School, Naples, are studying the mechanism by which thyroglobulin acts to suppress transcription. Researchers at the Institute of Internal Medicine, Infectious Diseases, and Immunopathology, Polyclinic Hospital, Padiglione Granelli, Milan, are also working to identify normal human T cells that can process thyroid autoantigens to yield diverse responses of humoral autoimmunity against cytotoxicity.

The project at Chiba University Medical School, Japan, is exploring the mechanism by which TSH, insulin, and IGF-I control thyroid cell growth through 3-hydroxy-3-methylglutaryl coenzyme A reductase and small G proteins. In another project, scientists investigating the mechanism of the development of thyroid autoimmune disease have uncovered a new experimental model of Graves' disease and have defined, for the first time, the role of aberrant expression of major histocompatibility genes in development of thyroid autoimmunity. They have also identified thyrotropin receptor epitopes important for the development and expression of autoantibodies to the thyrotropin receptor in thyroid autoimmune diseases.

#### *Genetics and Endocrinology Section*

The Genetics and Endocrinology Section conducts studies on genetic disorders, emphasizing overfunction in parathyroid and other endocrine tissues. In FY 99, the Section had one Special Volunteer from Brazil, one Visiting Fellow from Germany, and one Visiting Scientist from Kenya.

The Chief of the Section was appointed

to the International Scientific Committee of the Multiple Endocrine Neoplasia Workshops.

Scientists from the Section presented work at the 7th Annual Scientific Meeting of GEMEN (Group for Studies of Multiple Endocrine Neoplasia), in Dijon, France, on December 3–4, 1998; at the International Symposium on Thyroid and Parathyroid Tumors, in Pisa, Italy, on March 25–27, 1999; and at the 7th International Workshop on Multiple Endocrine Neoplasia, in Gubbio, Italy, on June 30–July 2, 1999. A student at Howard Hughes Medical Institute, Chevy Chase, Maryland, also presented work at the 7th International Workshop on Multiple Endocrine Neoplasia, where she was awarded the juried prize for the best abstract of the meeting.

The Section has joined an international consortium to clone the gene for hyperparathyroidism–jaw tumor syndrome. This consortium is headed by a scientist from the National Human Genome Research Institute, NIH, and includes groups from England, the Netherlands, Sweden, and the United States.

#### *Kidney Diseases Section*

The Kidney Disease Section conducts research on the pathogenesis and treatment of glomerular diseases, especially lupus nephritis, membranous nephropathy, and focal segmental glomerulosclerosis. In FY 99, three foreign scientists worked on these projects; these scientists included a Chinese virologist (Visiting Associate), a Hungarian physician (Courtesy Associate), and a Japanese nephrologist (Visiting Associate). Abstracts were presented at the XVth International Congress of Nephrology, in Buenos Aires, Argentina, on May 2–6, 1999.

#### *Molecular Pathophysiology Section*

The Molecular Pathophysiology Section is host to one Visiting Associate (from China); three Visiting Fellows (one from China, one from Russia, and one from the United Kingdom); and one Special Volunteer (from Japan). Two supplemental Visiting Fellows (one from Brazil and one from Mexico) are also working in the Section. In addition, the Cellular Signaling Unit has an ongoing collaboration with a Turkish scientist who previously worked as a Visiting Fellow in the laboratory. This work is supported in part by

a grant from the Turkish Scientific and Technical Research Council. The researchers are focusing on the role of palmitoylation in intracellular targeting of proteins, and they found that multiple sites of palmitoylation and a proline-rich region provided signals for targeting the Golgi apparatus.

In FY 99, the Section completed a joint project with investigators at the University of Montreal. The findings demonstrated that nitric oxide could depress the palmitoylation of a cell-surface receptor and signaling through this receptor. This work was presented at the 5th International Dahlem Symposium on Cellular Signal Recognition and Transduction, in Berlin, Germany, on October 14–16, 1999.

In addition, the Section is working with researchers at the Weizmann Institute of Science, Rehovot, Israel, to study the mechanism of superextraction of cyclase by opiates.

#### **Molecular and Clinical Hematology Branch**

The Molecular and Clinical Hematology Branch is collaborating with scientists in China and Thailand in a clinical trial on the effects of hydroxyurea on the level of effective erythropoiesis, transfusion requirements, and the red blood cell production rate in  $\beta$ -thalassemia intermedia. This investigation is being conducted at Shanghai Institute of Medical Genetics, China, and Mahidol Hospital, Bangkok, Thailand. The preliminary data indicate that hydroxyurea may improve the effectiveness of erythropoiesis in a substantial proportion of patients with  $\beta$ -thalassemia intermedia.

On the basis of these encouraging results, this investigation was expanded to determine whether genotypes that account for diminished synthesis of specific beta chains of hemoglobin in  $\beta$ -thalassemia may predict response to hydroxyurea. To accomplish this goal, the Branch will organize a working group of investigators from Greece, Israel, Italy, Sardinia, Sicily, and Thailand to begin this trial, using an NIH protocol. Initial review board approval at European and Middle Eastern sites is anticipated in the first quarter of 2000, and accrual of patients is expected to begin by May 2000.

#### **Phoenix Epidemiology and Clinical Research Branch**

The Phoenix Epidemiology and Clinical Research Branch serves as a WHO Collaborating Center for the Design, Methodology, and Analysis of Epidemiologic and Clinical Investigations in Diabetes. During FY 99, the Chief of the Branch served as a faculty member in the joint training course of WHO and the International Diabetes Federation, in Epidemiological and Public Health Aspects of Diabetes Mellitus. The course was conducted in Cambridge, England, on July 18 and 24, 1999. In addition, a series of reports on the WHO Multinational Study for Vascular Disease and Diabetes, in which the Branch is a collaborating center, were completed.

The Branch has continued its long-standing, joint research with the China-Japan Friendship Hospital, Beijing, China. Using data from the completed Da Qing clinical trial on the effects of lifestyle intervention and impaired glucose tolerance, analyses were conducted to determine factors that predict responsiveness to lifestyle intervention in the prevention of diabetes. The results of these investigations were presented as an invited lecture at the Steno Symposium, in Copenhagen, Denmark, in June 1999.

The Branch Chief served as an advisor to the Joint Arizona-Sonora-U.S. Border Initiative. In collaboration with investigators from the University of Arizona, Arizona State Health Department, and the Health Department of the State of Sonora, Mexico, a survey of diabetes and its risk factors was conducted in the town of Douglas, Arizona. A parallel study, using the same methods, is now being conducted in the sister town of Agua Prieta, Sonora.

#### *Clinical Diabetes and Nutrition Section*

The Clinical Diabetes and Nutrition Section has one Research Fellow (from Italy); four Visiting Fellows (one each from China, India, Korea, and Slovakia); and one Special Volunteer (from China). These scientists are studying the genetic causes of diabetes among the Pima Indians of Arizona.

A Staff Fellow attended the 5th International Human Chromosome 1 Workshop, in Cambridge, England, on August 4–9, 1999. The investigator arranged research projects with investigators at the University of Leices-

ter and at Queen Charlotte's and Chelsea Hospital, London, England. These collaborations involve sharing data about chromosome 1, including physical mapping, gene markers, and gene localization within a region on chromosome arm 1q that is linked with diabetes in the Pima Indians. The studies should facilitate positional cloning of the underlying disease gene.

In a joint effort with the Section, scientists from the University of Sydney, Australia, measured fatty-acid composition of plasma membrane in muscle and fat samples from Pima Indians.

#### *Diabetes and Arthritis Epidemiology Section*

During FY 99, Visiting Fellows, Visiting Associates, and Special Volunteers, from Romania, Sierra Leone, Somalia, and the United Kingdom have worked in the Diabetes and Arthritis Epidemiology Section. Researchers have performed studies of the epidemiology and genetics of type 2 diabetes mellitus in the Pima Indians; on diagnostic criteria for diabetes; and on methods for diabetes screening in children. They investigated the relationship of dietary characteristics to the development of type 2 diabetes; the frequency and pathogenesis of type 2 diabetes in Pima Indian children and adolescents, including the long-term effects of the diabetic pregnancy on the early appearance of type 2 diabetes; and the effects of parental diabetes on birth weight and diabetes risk in children.

A Staff Scientist in this Section is a member of the analysis committee of the International Type 2 Diabetes Linkage Consortium. He has a leading role in comparative analyses of genes for susceptibility to diabetes across 26 populations in eight countries.

#### **Laboratory of Biochemistry and Genetics**

##### *Enzyme Structure and Function Section*

The Enzyme Structure and Function Section, Laboratory of Biochemistry and Genetics, hosted three Postdoctoral Fellows: one from China and two from Korea. These investigators conducted research on the structure and function of a yeast protein (cystathionine b-synthase) and on the regulation of a protein complex (tryptophan synthase a<sub>2</sub>b<sub>2</sub> complex) by pH, temperature, guanidine

hydrochloride or urea, and mutations. The Section has also collaborated with scientists at the University of Parma, Italy, on single-crystal studies of the tryptophan synthase  $\alpha 2\beta 2$  protein complex.

#### *Genetics of Simple Eukaryotes Section*

The Genetics of Simple Eukaryotes Section is hosting five Visiting Fellows: one each from China, France, Japan, the Netherlands, and the United Kingdom. Research is being conducted in the following areas:

- identification of chromosomal genes necessary for the propagation of the yeast prion;
- examination of the regions of the Ure2 protein involved in prion generation and propagation;
- mutagenesis studies to elucidate molecular mechanisms related to biogenesis, maintenance, and elimination of a yeast prion-like element; and
- characterization of the Mks1 protein involved in regulation of nitrogen catabolism.

#### *Morphogenesis Section*

The Morphogenesis Section is hosting four Visiting Fellows: one each from Brazil, Germany, India, and Korea. The Visiting Fellows are conducting research in the following areas:

- isolation of mutants of the guanosine triphosphate-binding protein Rho1p, to assess known functions of the protein, including regulation of  $\beta$ -1,3-glucan synthesis;
- study of mutants involved in chitin synthase II function, to identify proteins involved in septum formation; and
- identification of proteins responsible for the formation of cross-links in the yeast cell wall.

#### *Physical Biochemistry Section*

The Physical Biochemistry Section hosts two Visiting Fellows from Australia. One investigator is engaged in a study of the effect of macromolecular crowding on the formation of microtubules. The other investigator is studying the effect of macromolecular crowding on specific and nonspecific DNA-protein interactions.

The Section is collaborating with scientists at the Biological Research Center, Spanish Council for Higher Research, Madrid, in research on fiber formation by the bacterial

septation protein FtsZ and on the development of new methods for measuring macromolecular associations in highly crowded media.

#### **Laboratory of Bioorganic Chemistry**

The Laboratory of Bioorganic Chemistry continues to serve as a leader in the fields of biochemical pharmacology and medicinal chemistry, because of constant research innovations and introduction of novel concepts and agents. Scientists from many nations work in the Laboratory, which collaborates with scientists from institutions and universities around the world. Through these international research efforts, new natural products with potentially useful biological activities have been identified and are being used worldwide in studies of the roles of ion channels and second messengers in the function of numerous physiological systems. These natural products include the following:

- forskolin, an activator of adenylate cyclase;
- batrachotoxin, an activator of sodium channels;
- histrionicotoxin, a noncompetitive blocker of nicotinic receptor-regulated channels;
- pumiliotoxin, a potent myotonic and cardiotoxic agent;
- epibatidine, a nicotinic agonist that is many times more potent than morphine as an analgesic agent;
- maitoxin, a potent activator of calcium influx and phosphoinositide breakdown; and
- ibogaine, an extremely potent noncompetitive blocker of ganglionic and neuronal nicotinic receptors.

Through joint research efforts in Costa Rica, Panama, and Venezuela, insects have been identified as dietary sources for some of the biologically active alkaloids found in the skin of frogs. Alkaloids were also discovered in the skin and feathers of certain birds from New Mexico and Papua New Guinea. In addition, structures of new biologically active natural products from plants and marine organisms from New Zealand were determined.

Many of the international research efforts have involved programs (a) for discovery of new natural and synthetic compounds and their evaluation as antiviral agents or as

agents for treatment of cardiovascular disease or diseases of the central nervous system and (b) for evaluation of the activity of adenosine, adenosine triphosphate, peptide, histamine, dopamine, serotonin, and cholinergic and adrenergic receptors. These collaborative studies have led to the development of new synthetic routes for preparation of novel analogues of biologically active substances and novel techniques for determination of post-translational modifications of proteins.

The Laboratory of Bioorganic Chemistry consists of five sections (Drug-Receptor Interactions, Molecular Recognition, Molecular Signaling, Oxidation Mechanisms, and Pharmacodynamics) and four work groups (Mass Spectrometry, Natural Products, Neuroscience, and Nuclear Magnetic Resonance Spectroscopy). The Laboratory continues to have major international commitments, both with foreign scientists who are training in the Laboratory and through collaboration with scientists in many countries. Scientists from Canada, China, Germany, Israel, Italy, Japan, Korea, New Zealand, and Vietnam are receiving postdoctoral training in the Laboratory.

Numerous scientific advances stem from the international activities of the Laboratory of Bioorganic Chemistry. (See also the section on "Highlights of Recent Scientific Advances Resulting From International Activities.") Current and continuing cooperative efforts with foreign countries include the following:

- Argentina—with the University of Buenos Aires on studies of biologically active alkaloids from Argentinean toads and dietary arthropods.
- Australia—with the Pharmaceutical Research Institute, Queensland, on novel nicotinic agonists from endemic plants; with the University of Adelaide on dietary origin of alkaloids from myobatrachid frogs; and with the University of Sydney on studies of metabolism and tumorigenicity of benzacridines and the mechanism of action of human epoxide hydrolase.
- Brazil—with Universidade Federal Rio de Janeiro on discovery and investigation of biologically active products.
- Canada—with the University of Western Ontario, London, on development of xanthines for improvement of tolerance to cold; with the Montreal Research Institute

on evaluation of analogues of opioid peptides in the central nervous system; with the University of British Columbia, Vancouver, on xanthenes as inhibitors of mitosis; and with the University of Calgary, Alberta, on involvement of excitotoxic mechanisms in neuronal death caused by neurovirulent strains of HIV macrophages.

■ Chile—with the University of Chile, Santiago, on the effects of pumiliotoxin on ryanodine receptors.

■ Germany—with the Free University of Berlin on genetic analysis of G protein-coupled receptors; with the University of Mainz on mechanisms of polycyclic aromatic hydrocarbon-elicited carcinogens; with the University of Münster on stereoselective syntheses of fluorinated dihydroxyphenylserines; with the University of Frankfurt on inhibition of ectonucleotidases; and with the University of Leipzig on safety of drugs, including biopolymeric drugs.

■ Israel—with Haifa University, on thio analogues of ultimate carcinogens from polycyclic aromatic hydrocarbons; with the Institute for Biological Research, Nes Ziyvona, on molecular probes for cholinergic receptors; and with Bar-Ilan University, Ramat Gan, on the development of novel systems for drug delivery and the action of adenosine agonists and antagonists on cardiac myocytes.

■ Italy—with the University of Bari on renal effects of adenosine receptor agonists and antagonists; with the University of Ferrara on the design of selective adenosine agonists; with the University of Milan on second-messenger systems associated with purinoceptors and on mechanisms of apoptosis induced by adenosine; with the University of Parma on the biological activity of histamine analogues; and with the University of Rome on investigation of biologically active alkaloids and amines from amphibians.

■ Japan—with Osaka City University on isolation and elucidation of the structure of alkaloids from amphibians; with Kumamoto University on the development of affinity labels for receptors and ion channels; with Tohoku University and the University of Tokyo on structural and biological activity of marine toxins; with the National Research Institute, Nagoya, on perfluoroalkylation of aromatic and heterocyclic rings; with Nagoya City University on metabolism of

polycyclic aromatic hydrocarbons; with Toyama Medical and Pharmaceutical University on enantio-selective electrophilic fluorination; with Gifu University on the use of cytokine knockout mice in research on dementia secondary to AIDS; and with Tokushima Bunri University on the synthesis of fluorinated chalcones as potential inhibitors of 5-lipoxygenase and cyclooxygenase.

■ Korea—with the Korea Advanced Institute of Science and Technology, Taejon, on the structure of tetrodotoxin analogues from amphibians.

■ Madagascar—with Service de Chimie on investigation of biologically active alkaloids from amphibians and arthropods.

■ Mexico—with Universidad de Mexico, Mexico City, on centrally active alkaloids from birds.

■ the Netherlands—with the Academic Medical Center, Amsterdam, on pathogenesis of hepatic encephalopathy, and with the Center for Biopharmaceutical Sciences, Leiden, on molecular modeling and site-directed mutagenesis of purinergic receptors and on pharmacokinetics of adenosine receptor ligands.

■ New Zealand—with the University of Canterbury on the identification of biologically active compounds from natural products.

■ Northern Ireland—with Queens University of Belfast on synthesis of optically active arene oxides.

■ Panama—with the Smithsonian Tropical Research Institute, Panama City, on insect origins for biologically active alkaloids in skin of certain neotropical frogs.

■ Portugal—with the Gulbenkian Institute, Lisbon, on biological activity of xanthenes in neuromuscular preparations.

■ Sweden—with Karolinska Institute, Stockholm, on evaluation of purine nucleosides and nucleotides, the biological activity of caffeine and related xanthenes, and the biological activity of adenosine analogues, and with Uppsala University on synthetic incorporation of fluorinated purines and pyrimidines into polynucleotides and on studies of the biological activities of these analogues.

■ Thailand—with Kasetsart University, Bangkok, on identification, structure, and biological activity of natural products.

■ Turkey—with the University of Ankara

on involvement of oleic amide in manifestations of hepatic encephalopathy.

■ United Kingdom—with University College, London, on evaluation of adenosine triphosphate analogues and purine antagonists; with the University of Manchester, England, on evaluation of neuropeptide hormone analogues in the cardiovascular system and the central nervous system; with the University of Nottingham, England, on electrophysiological effects of antibodies to the  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor elaborated during the course of HIV infection; with Kings College, London, and Victoria Hospital, Newcastle upon Tyne, England, on mechanisms of pathogenesis of hepatic encephalopathy; and with the University of Dundee, Scotland, on immunohistochemical localization of protein kinase C isozymes in transfected cells.

■ Venezuela—with Internacional Instituto de Estudios Avanzados on investigation of effects of toxins on ion channel function.

#### **Laboratory of Cell Biochemistry and Biology**

The Laboratory of Cell Biochemistry and Biology is hosting four investigators from three countries: two Visiting Fellows (one from Germany and one from Italy); and two Special Volunteers (one from China and one from Italy). The Laboratory is involved in eight studies with investigators in seven countries: Canada, China, France, Hungary, Italy, Japan, and the United Kingdom.

#### *Cell Biochemistry Section*

The Cell Biochemistry Section, Laboratory of Cell Biochemistry and Biology, is collaborating on studies of the structural characteristics of nuclear proteins with an investigator at the Medical Research Center, Cambridge, England. Other projects on nuclear proteins involve investigators in Denmark and at the Max Planck Institute, Tübingen, Germany.

#### *Lipid Cell Biology Section*

The Lipid Cell Biology Section supports four international research activities. Work with a Canadian investigator at the Royal Victoria Hospital, Montreal, focuses on lipoprotein metabolism. Genetic diseases of cholesterol metabolism are the subject of the joint work with a scientist at the Faculté de Médecine,

Lyon, France. Two Japanese collaborative efforts, one with Ehime University and one with the Nippon Medical School, Tokyo, involve imaging studies of adipocytes and cultured blood cells.

### **Laboratory of Cellular and Developmental Biology**

The Laboratory of Cellular and Developmental Biology is host to 20 international researchers.

#### *Developmental Biochemistry Section*

In the Developmental Biochemistry Section, Laboratory of Cellular and Developmental Biology, three Visiting Fellows (one from Australia, one from China, and one from Poland) are investigating the developmental genetics of germline sex determination in *Drosophila melanogaster*. A tenure-track Senior Staff Scientist (from the United Kingdom) has established a research group with three Visiting Fellows (one from Canada, one from Korea, and one from Taiwan) who are using minichromosomes in *Saccharomyces cerevisiae* to study the activation of gene expression. One Visiting Fellow from Slovakia is studying sequence-directed protein folding, by using site-directed mutagenesis in bacteria.

#### *Mammalian Developmental Biology Section*

In the Mammalian Developmental Biology Section, two Visiting Fellows (one from Canada and one from China), one Staff Fellow (from Italy), and one Special Volunteer (from Japan) are investigating the molecular basis of female gonadogenesis, mechanisms of oocyte-specific gene expression, and the structural basis of species-specific fertilization.

### **Membrane Regulation Section**

In the Membrane Regulation Section, two Visiting Fellows (from China) and two Special Volunteers (one from France and one from Japan) are investigating the role of intrinsic lipid proteins in the formation and hydrolysis of neutral lipid-storage droplets. In addition, one Visiting Fellow from China is studying enhancer-dependent expression of globin genes in K562 cells.

### **Molecular Mechanisms of Development Section**

In the Molecular Mechanisms of Development Section, one Research Fellow (from China) and two Visiting Fellows (one from China and one from Korea) are investigating the role of signal transduction in controlling pattern formation and cell fate in *Dictyostelium discoideum*.

#### *Biotechnology Unit*

In the Biotechnology Unit, one Visiting Fellow (from Taiwan) is investigating the optimization of large-scale expression systems for clinically relevant proteins.

### **Laboratory of Chemical Physics**

A collaboration of the Laboratory of Chemical Physics with a researcher at the Institut Laue-Langevin, Grenoble, France, has resulted in a very simple model that illustrates and clarifies a surprising number of basic concepts needed for a fundamental understanding of protein folding. A diffusion equation is used to describe the kinetics of folding, an approach that provides a crucial part of the theoretical foundation for the statistical mechanical model, which was developed in the Laboratory of Chemical Physics.

A scientist at the Karpov Institute of Physical Chemistry, Moscow, Russia, has worked with the Laboratory to create software that has the potential to revolutionize methods for performance of cutting-edge laser experiments. The computer code, which is being developed by use of LabVIEW, is modular, hierarchical, extensible in structure, and accessible across the Internet. With this new paradigm, multiple experiments can be conducted virtually simultaneously, from any location, by using the same computer hardware. Because the paradigm is adaptable, sophisticated state-of-the-art experiments can be set up and executed with ease.

A scientist from Spain who is working at the European Molecular Biology Laboratories, Heidelberg, Germany, in collaboration with the Laboratory of Chemical Physics, has developed statistical mechanical models for explaining the kinetics and thermodynamics of formation of the secondary structure in proteins. He has applied these models to small, single-domain proteins, quantitatively explaining, for the first time, the wide range of folding rates. This work represents

a major breakthrough in understanding of the mechanism of protein folding.

A scientist from Lulea University of Technology, Sweden, has spent 1 year in the Laboratory (July 1998–July 1999) on a fellowship from the Swedish Foundation for International Cooperation in Research and Higher Education. This scientist played a pivotal role in the development of new “multiple quantum,” solid-state, nuclear magnetic resonance spectroscopy techniques and in the successful application of these techniques to structural studies of  $\beta$ -amyloid fibrils in patients with Alzheimer’s disease.

### **Laboratory of Medicinal Chemistry**

The Laboratory of Medicinal Chemistry hosted eight scientists from five countries and Taiwan. These scientists consisted of six Visiting Fellows (one from Austria, two from China, one from France, one from Russia, and one from Taiwan); one Research Fellow (from China); and one Special Volunteer (from Japan).

#### *Section on Carbohydrates*

The Section on Carbohydrates, Laboratory of Medicinal Chemistry, has two Visiting Fellows (one from China and one from France) and one Visiting Scientist (from Russia). They are involved in (1) mapping the interaction of *Vibrio cholerae* 0:1 antigens and their homologous antibodies and (2) design, synthesis, and analysis of conjugate vaccines against cholera.

The Section has a Cooperative Research and Development Agreement with Croatia, for work on the development of compounds that enhance the immune response. The Section is engaged in joint research with scientists at the Massachusetts Institute of Technology on the development of solid-phase synthesis of the carbohydrate antigen of *V. cholerae* 0:1 and works with scientists at the Institute of Chemistry, Slovak Academy of Sciences, on new strategies in oligosaccharide synthesis and their application in syntheses of oligosaccharides of biological and industrial importance.

#### *Drug Design and Synthesis Section*

In the Drug Design and Synthesis Section, a Visiting Fellow from Austria is synthesizing agents that act on serotonin type 2a receptors, to develop agents for positron emis-

sion tomography imaging of the human brain in various disease states. A Research Fellow and a Visiting Fellow from China are synthesizing agents that act on the dopamine, serotonin, and norepinephrine transporter, to develop potential medications for the prevention and treatment of cocaine and methamphetamine abuse. A Visiting Fellow from Taiwan synthesized iodo and fluoro derivatives of a corticotropin-releasing hormone antagonist, as potential imaging agents for single photon emission computed tomography studies and positron emission tomography scans, respectively.

Collaborative work continues with researchers at the Centre Hospitalier, Universitaire de Bicetre, Assistance Publique Hôpitaux de Paris, France. The purpose is to investigate the role of a novel, haloperidol-insensitive binding site in modulation of non-*N*-methyl-D-aspartate receptors by  $\sigma$  ligands. The Section continues to work with researchers at the Laboratory of Endocrinology and Cellular Communication, Institut National de la Santé et de la Recherche Médicale (INSERM), France. The researchers are investigating the relationship of antiestrogen binding sites and  $\sigma$  receptors, with respect to cytotoxicity of various compounds, particularly those related to tamoxifen.

This Section is also involved in work with investigators from the Polish Academy of Sciences in a program designed to gain further insight into the mechanism of action of cocaine and other stimulant drugs in the central nervous system.

In addition, the Unit of Receptor Biochemistry and Pharmacology, Drug Design and Synthesis Section, has a Visiting Scientist from Russia, who is studying  $\sigma$  receptor mechanisms with scientists at Hadassah Medical School, Hebrew University, Jerusalem, Israel. This project is aimed at determining the role of  $\sigma$  receptors in regulation of intracellular calcium and the possible use of calcium as a second messenger. Furthermore, the scientists are investigating the role of  $\sigma$  receptors in regulation of cell growth and induction of apoptosis. An ongoing joint effort with a research group at the Institute for Pharmaceutical Chemistry, University of Vienna, Austria, involves studies of the structure and activity of  $\sigma$  ligands. This project is aimed at determining the effect of variousazole and aminoazole systems on  $\sigma$  receptor binding affinity. The research will involve

quantitative molecular modeling techniques to devise a pharmacophoric model predictive of ligand affinity at  $\sigma$  receptors.

The Chief of the Unit on Receptor Biochemistry and Pharmacology presented an invited talk entitled Sigma Receptors: Recent Advances and New Clinical Potentials, at the 12th Camerino-Noordwijkerhout Medicinal Chemistry Symposium, in Camerino, Italy, on September 5–9, 1999.

#### **Laboratory of Molecular Biology**

The Laboratory of Molecular Biology continued extensive collaboration with 10 institutions in seven countries. The countries are Belgium (one), Canada (two), Germany (one), India (one), Japan (one), Korea (one), and the United Kingdom (three).

Twenty-six investigators from 17 countries worked with scientists in the Laboratory in FY 99. This group consisted of one Special Volunteer from China; two Visiting Scientists (one from Austria and one from Georgia); one Staff Scientist from Malta; and 22 Visiting Fellows, from the following countries: Belgium (one), Bulgaria (one), Canada (three), China (four), the Czech Republic (one), Ireland (one), Israel (one), Japan (two), Korea (one), Mexico (one), Singapore (one), Spain (one), Sri Lanka (one), and the United Kingdom (three).

#### **Laboratory of Molecular and Cellular Biology**

##### *Section on Genomic Structure and Function*

The Section on Genomic Structure and Function, Laboratory of Molecular and Cellular Biology, has begun a collaboration with a scientist at Pontificia Universidad Javeriana, Bogotá, Colombia, to investigate the use of polymorphic LINE-1 (L1) inserts for studying the population genetics in South America. In addition, the Section is providing a Fogarty Fellowship for a French scientist, who is participating in the Section's studies on the evolutionary dynamics and the biological properties and effects of the L1 family of mammalian retrotransposons, which are intragenomic, autonomously replicating DNA elements. These studies include the use of L1 DNA as a genetic marker.

The Section hosted two Visiting Fellows from India. One investigator is studying the regulation of FMR1, a gene involved in brain

development that is affected in fragile X syndrome—one of the group of diseases characterized by expansion of repeated DNA sequences. The second investigator is working on the molecular basis of chromosome fragility in this disorder and on a mouse model for this group of diseases. This research team is also collaborating with an investigator at the Chiba Radiological Institute, Japan, to examine the role of the XPG endonuclease in the etiology of these diseases.

#### *Molecular Biology Section*

The Molecular Biology Section is hosting two Visiting Fellows, one from Greece and one from India. Both Visiting Fellows are part of a group that studies cell-cycle regulation in budding yeast. The Visiting Fellow from Greece is undertaking an analysis of the structure and function of the anaphase inhibitor—a key regulator in cell-cycle progression in mitosis. The function of this regulator is conserved throughout evolution, but almost nothing is known about its mode of action. The Visiting Fellow from India is using a biochemical approach to study the pathway to the checkpoint for DNA damage. This pathway is defective in many types of cancer, and the research is directed toward elucidation of key steps in the process.

#### *Nucleic Acids Biochemistry Section*

The Nucleic Acids Biochemistry Section hosted two Visiting Fellows and one Visiting Scientist, all from India. One Visiting Fellow and the Visiting Scientist have been studying protein–protein interactions among the transcriptional activator MotA for the bacteriophage T4, the coactivator AsiA, and the sigma-70 subunit of host RNA polymerase. The other Visiting Fellow is studying how the single-stranded binding protein for T4 replication and the polymerase clamp alter the nuclease activity of the RNase (ribonuclease) H.

#### *Steroid Hormones Section*

The Steroid Hormones Section hosted two Visiting Associates (one from Belgium and one from India) and one Visiting Fellow (from China). The Visiting Associate from Belgium has been studying the modulation of glucocorticoid-regulated gene expression by transcriptional cofactors. The Visiting Associate from India has been characterizing

two recently cloned, novel *trans*-acting factors that appear to be involved in the modulation of glucocorticoid-regulated expression, in this case by binding to a *cis*-acting element. The Chinese Visiting Fellow has been examining the molecular details of some of these mechanisms of modulation of glucocorticoid-regulated gene expression.

