A New Century of Science . . .
A New Era of Hope

National Institute of
Diabetes & Digestive &
Kidney Diseases

Contents

Director's Message

As we embark on a new century of scientific discovery, armed with knowledge and research tools that were unimagined even 20 years ago, we can reflect on many hard-won victories in understanding, treating, and preventing the diseases within the mission of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). These advances have saved many lives, improved quality of life, and laid a solid foundation that researchers continue to build upon and expand.

This booklet highlights research advances and directions made possible by 50 years of research in diseases such as diabetes, kidney disease, and hepatitis C. Most often, the stories of progress emerged not from dramatic breakthroughs but from steady, incremental findings and persistent investigative work. They resulted from the dedicated collaboration of physician researchers and volunteers who consented to participate in clinical trials. Sometimes they incorporated insights from studies addressing distant or seemingly unrelated research questions.

The investment in research has created scientific opportunities that have never been greater or more exciting. Every day we learn more about how genes, which direct the function of cells, interact in complex ways with other genes and environmental triggers to cause disease. We are steadily clarifying the complex molecular pathways involved in the development of diabetes, obesity, and disorders of the kidney and digestive tract. As we begin to exploit the newly available human genome sequence with the help of promising tools such as bioinformatics and microarray technology, we can expect to see major clinical advances flowing at an ever-increasing pace from new lines of discovery.

Effective treatments and true cures can only arise from a clear understanding of the molecular events that lead to cellular malfunction and disease. We are working hard to pursue every scientific avenue that brings us closer to the goal of relieving the burden of disease for patients and their families. As the new century begins, the NIDDK rededicates itself to patients and those at risk for disease in an urgent quest for answers. To researchers, grantees, and the medical community, we recommit ourselves to helping you find the answers.

Allen M. Spiegel, M.D.
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health

About NIDDK

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is one of 25 institutes and centers that make up the National Institutes of Health (NIH), part of the Public Health Service in the U.S. Department of Health and Human Services. The NIH mission is to gain knowledge that will lead to better health for everyone. NIH pursues that mission by conducting research in its own laboratories; supporting the research of scientists in universities, medical schools, hospitals, and research institutions throughout the country and overseas; helping to train investigators; and fostering communication of medical and health information.

The NIH strives to invest wisely the tax dollars entrusted to it for the support and conduct of biomedical research. About 82 percent of the NIH budget is dedicated to funding research grants, contracts, and training of more than 50,000 scientists working in about 2,000 universities, research institutions, and medical centers across the United States and abroad. About 10 percent of the NIH budget supports research conducted by federally employed scientists in NIH laboratories and in the Clinical Center, the research hospital of the NIH in Bethesda, Maryland.

Established in 1950, NIDDK shares the NIH mission of acquiring new knowledge to improve human health through high-quality biomedical research. The Institute conducts and supports basic and clinical research in some of the most serious, common, disabling, and costly conditions affecting the public's health. The diseases in NIDDK's research mission cut across the full spectrum of medicine and affect people of all ages: endocrine and metabolic diseases, such as diabetes and obesity; digestive diseases; and kidney, urologic, and blood diseases. Most arise from the complex interaction of genetic, autoimmune, neuroendocrine, metabolic, nutritional, and environmental factors. Some diseases such as diabetes, obesity, hepatitis, and kidney failure disproportionately affect minority populations.

While basic research may not play an immediate role in improving health, history has repeatedly shown that advances in basic research form critical turning points in a chain of discoveries leading to improved health.

NIDDK funds research projects that relate directly to these diseases, but it also places a high priority on fundamental, untargeted research. While basic research may not play an immediate role in improving health, history has repeatedly shown that advances in basic research form critical turning points in a chain of discoveries leading to improved health. Often, findings in one research area unexpectedly answer questions in an unrelated field of study. The NIDDK is committed to translating the findings of basic research, which are steadily improving the understanding of biologic systems and disease processes, into promising clinical studies and effective methods of prevention and treatment.

Training is critically important to continued progress in medical research. To attract and retain top-flight researchers, NIDDK supports research training and career development, with special emphasis on increasing the ranks of physician scientists and recruiting underrepresented minorities and women into biomedical research careers. For more information about research training and career development, see the NIDDK Web site at www.niddk.nih.gov under "Research Funding Opportunities."

NIDDK's Research Focus

- **Diabetes and other endocrine diseases**
- **Cystic fibrosis and other inherited diseases**
- **Digestive diseases**
- **Obesity**
- **Nutrition**
- **Diseases of the kidney, genitourinary tract, and blood**

How Research Is Funded

The NIH uses grants, contracts, and cooperative agreements to fund research and other projects performed by researchers at medical centers and universities outside of the NIH. Most applications for grant support are unsolicited by NIH and originate with an individual investigator who develops an idea and a plan for a research project or

research training. In its 50-year history of conducting and supporting biomedical research, the NIDDK has funded many of the most creative and productive minds in science. At least 25 winners of the Nobel Prize in Chemistry and in Physiology or Medicine received training or conducted research at the NIDDK, or they received NIDDK support for their research.

The Institute encourages scientific attention to a specific research problem or special concern by issuing Program Announcements (PAs) and Requests for Applications (RFAs), inviting scientists to apply for research support in areas of special interest or scientific opportunity. All NIH solicitations are published in the *NIH Guide for Grants and Contracts* at www.nih.gov under "Grants and Funding Opportunities." NIDDK's PAs and RFAs are also listed on the NIDDK home page. For instructions on how to apply for a grant or contract, see www.niddk.nih.gov under "Research Funding Opportunities."

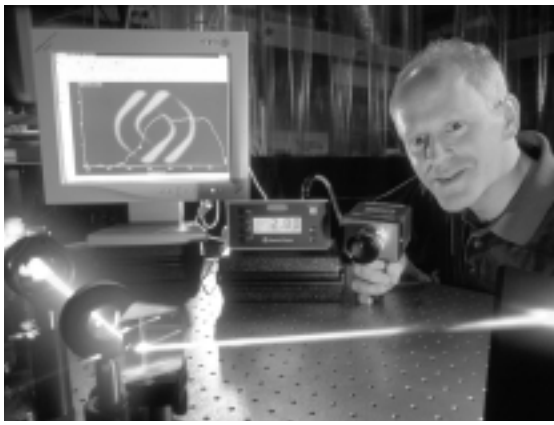
To ensure the highest scientific standards among funded projects, U.S. law requires that applications for NIH funding be subject to a two-step peer review by panels of scientific experts.

- First, a chartered scientific review group evaluates each application's scientific merit, methods, and approach and assigns a numeric priority rating to each application.
- In the second level of review, the Institute's National Advisory Council, a panel of non-government scientists and other professionals with an interest in the Institute's research, evaluates the priority scores in the context of criteria such as significance, program goals, and available funds.

Research takes time. NIH strives to fund scientists' work for a period of time sufficient for the projects to produce results.

In 1999, scientists submitted more than 26,000 applications to NIH for research support. Only the very best—the top 32 percent—were funded.

NIH awards grants for an average of 4 years, so each year most of an Institute's budget is already committed to continued funding of research projects begun in prior years.



Dr. Philip Anfinrud uses lasers to study protein action.

Cross-cutting Initiatives

Many research areas cut across the varied disciplines, programs, divisions, and specific disease interests of the NIDDK and the NIH. Often the focus of program initiatives, these areas promote multidisciplinary cooperation and the cross-fertilization of ideas, traditional strengths of the NIDDK. Examples of trans-NIDDK research areas are:

- diabetes
- prevention of obesity
- genetics of complex and single-gene diseases
- developmental biology and organ regeneration
- autoimmunity and transplantation
- gene therapy of human disease
- nutrition in disease prevention.

NIDDK and the Nobel Prize

The National Institute of Diabetes and Digestive and Kidney Diseases has supported 25 winners of the world's greatest scientific honor. Eighteen have won the Nobel Prize in Physiology or Medicine; seven have received the Nobel Prize in Chemistry.

1950s

1956

Institute grantee Dr. Dickinson W. Richards, Jr. shared the Nobel Prize in Physiology or Medicine with two other scientists. They developed heart catheterization techniques to study and diagnose circulatory disorders.

1959

Dr. Arthur Kornberg, a former Institute intramural researcher, and Institute grantee Dr. Severo Ochoa shared the Nobel Prize in Physiology or Medicine for discovering, respectively, the mechanisms of DNA and RNA synthesis.

1960s

1962

Institute grantee Dr. James D. Watson received the Nobel Prize in Physiology or Medicine along with two other scientists for discovering that DNA's structure is a double helix. This was a landmark finding of the 20th century, and it opened the field of modern genetics. Institute grantee Dr. John Kendrew shared the Nobel Prize in Chemistry. He discovered the molecular structure of myoglobin, a form of the blood protein hemoglobin found in muscle.

1965

Institute grantee Dr. Robert B. Woodward won the Nobel Prize in Chemistry for his contributions to the art of organic synthesis. Among the many compounds he synthesized were quinine, cholesterol, cortisone, and chlorophyll.

1966

Institute grantee Dr. Charles B. Huggins shared the Nobel Prize in Physiology or Medicine for discoveries concerning the hormonal treatment of prostate cancer.

1968

Dr. Marshall W. Nirenberg and two other scientists shared the Nobel Prize in Physiology or Medicine for deciphering the genetic code and explaining how it functions in protein synthesis. Nirenberg's code-cracking work was done while he was an Institute intramural scientist. He was NIH's first Nobelist.

1970s

1971

Institute grantee Dr. Earl W. Sutherland, Jr. won the Nobel Prize in Physiology or Medicine for his findings on the mechanisms of hormone action. His work greatly advanced the field of endocrinology.

1972

Dr. Christian B. Anfinsen shared the Nobel Prize in Chemistry with two other researchers. Anfinsen used the enzyme ribonuclease to show that a protein's amino acid sequence determines its three-dimensional structure, thus demonstrating a basic principle of biology. The award-winning work was done when Anfinsen was in the Institute's Laboratory of Chemical Biology. Institute grantee Dr. Gerald M. Edelman shared the Nobel Prize in Physiology or Medicine for studies of the chemical structure of antibodies that led to a better understanding of the immune system.

1970s

1976

Former Institute researcher Dr. Baruch S. Blumberg and another NIH scientist received the Nobel Prize in Physiology or Medicine. They were cited for discoveries of new mechanisms for the origin and dissemination of infectious diseases. Blumberg found the hepatitis B virus protein, or "Australia antigen," in 1963 while at the Institute. This advance was a scientific and clinical landmark in the detection and control of hepatitis.

1977

Institute grantees Dr. Roger C. L. Guillemin and Dr. Andrew V. Shally shared the Nobel Prize in Physiology or Medicine with a third scientist. Guillemin and Shally's prizes were for discoveries related to the brain's production of peptide hormones.

1980s

1980

Institute grantee Dr. Walter Gilbert shared the Nobel Prize in Chemistry for his contributions to determining base sequences in DNA.

1984

Institute grantee Dr. R. Bruce Merrifield won the Nobel Prize in Chemistry for development of solid-phase peptide synthesis.

1985

Former Institute intramural researcher Dr. Michael S. Brown shared the Nobel Prize in Physiology or Medicine with another former NIH scientist for studies on cholesterol metabolism regulation that have led to new treatments for atherosclerosis. Institute grantee Dr. Herbert A. Hauptman shared the Nobel Prize in Chemistry for creating methods to determine crystal structures. The methods advanced the development of practical instruments for learning the three-dimensional shape of molecules.

1989

Former Institute intramural researcher Dr. Harold E. Varmus shared the Nobel Prize in Physiology or Medicine with another former NIH scientist. They demonstrated that oncogenes, genes capable of converting normal cells into cancerous ones, can arise from normal cellular genes.

1990s

1992

Institute grantee Dr. E. Donnall Thomas shared the Nobel Prize in Physiology or Medicine with another NIH grantee for pioneering transplant therapy. Thomas' early advances in bone marrow transplantation have aided patients with leukemia and many other diseases.

1992

Institute grantees Dr. Edmond H. Fischer and Dr. Edwin G. Krebs received the Nobel Prize in Physiology or Medicine for their studies of the regulation of cell activities by enzymes. They discovered protein kinases, enzymes that control basic activities of the cell by adding phosphate groups to proteins.

1994

Dr. Martin Rodbell shared the Nobel Prize in Physiology or Medicine with an NIH grantee for their discovery of G proteins and their role in signal transduction in cells. Rodbell made many of his key findings in the 1970s while an Institute intramural scientist.

1997

Institute grantee Dr. Paul D. Boyer shared the Nobel Prize in Chemistry for discovering how the enzyme ATP synthase drives the formation of ATP, the carrier of energy for cells in all living things.

1998

Institute grantee Dr. Ferid Murad shared the Nobel Prize in Physiology or Medicine with two other scientists for work demonstrating that the gas nitric oxide plays a role as a signaling molecule in the cardiovascular system.

To help researchers exploit emerging scientific opportunities, such as the newly available human genome sequence, the NIDDK is also working to:

- improve research infrastructure and capacity by encouraging training, the formation of consortia, and sharing of costly resources
- broaden representation of ethnic populations in clinical trials
- increase the number of model systems for various diseases
- make data widely accessible to investigators through bioinformatics technologies.

Dr. Richard Proia develops mouse models of human diseases.

Facilitating Genetic Studies

Learning the genetic factors that contribute to disease is a critical step toward understanding a disease process and correcting it with drugs or gene therapy. Historically, NIDDK-supported researchers have excelled at the identification of single genes that cause disorders as diverse as cystic fibrosis, polycystic kidney disease, and multiple endocrine neoplasia type 1. However, identifying the many genes that contribute to polygenic, or multiple gene, diseases such as diabetes and obesity is a costly and challenging undertaking. Investigators studying such disorders need larger numbers of patients, expensive facilities, and other resources. By forming resource-sharing teams, investigators have access to information, technologies, ideas, and expertise usually beyond the scope of any one researcher or research team.

To encourage large-scale genetic studies, NIDDK supports the development of cooperative alliances such as the Type 2 Diabetes Linkage Analysis Consortium, which is searching for the genes that contribute to type 2 diabetes. The Institute is working to form similar cooperative groups to advance the search for the genes involved in type 1 diabetes, kidney disease and other diabetic complications, obesity, and other polygenic disorders.

Genomics

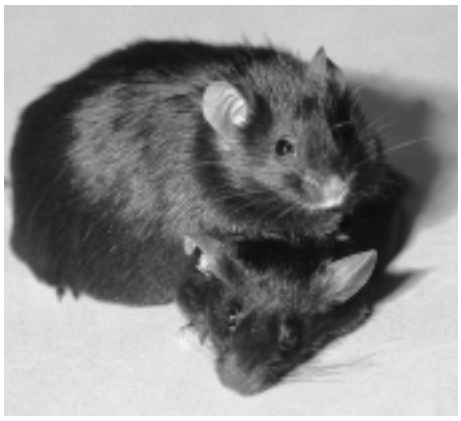
The NIDDK is encouraging promising applications of rapidly evolving technologies that are opening exciting avenues for research. One such technology is gene profiling with microarray technology, which allows researchers to understand how large numbers of genes interact and become activated or turned off in specific kinds of cells or in response to changes in the cellular environment. Researchers may soon be able to assess complex patterns of gene expression at the level of a single cell or a few cells. Applied to diseased tissue, gene expression profiles can identify candidate genes for genetic studies, determine the relevance of animal models to human disease, and identify target molecules for therapies. These methods can also identify important new genes and provide useful clues to a gene's function.

The NIDDK is committed to fostering application of these technologies to the diseases under its mission and to sharing information gleaned from these techniques among researchers through bioinformatics tools and databases.

The NIDDK supports the infrastructure needed for genomics research through initiatives such as the creation of biotechnology centers. It also supports several major projects using genomics to clarify the molecular pathways and regulatory functions of specific cells and organs. Some of the large genomics projects supported by the NIDDK include:

- The *Functional Genomics of the Endocrine Pancreas,* a collaborative effort to clarify the molecular pathways that orchestrate the development of the endocrine pancreas and to develop markers for beta cell imaging. The initiative supports the production and sequencing of complementary DNA (cDNA) libraries applying to different stages of pancreatic development. The cDNA libraries will allow creation of microarrays and a database to be used for gene discovery and functional studies of both the normal and diabetic pancreas. Once all the genes are known, scientists will gain a better understanding of how the body regulates blood glucose and will identify new targets for drug intervention. This information will also lead to a better understanding of the pathways activated in the development and regeneration of the endocrine pancreas and may assist efforts to isolate pancreatic stem cells.

- The *Diabetes Genome Anatomy Program,* which aims to identify and characterize all the genes involved in type 1 and type 2 diabetes and their complications and to identify the proteins encoded by these genes. By creating libraries of full-length cDNAs from tissues affected by diabetes, researchers will discover new genes and patterns of gene expression and will learn how the expressed genes affect energy balance, glucose metabolism, and insulin signaling. This information will shed light on how diabetes begins and progresses, helping scientists to create new approaches to diagnosis and treatment.



Mouse models help scientists understand obesity.

NIDDK is also participating in several major trans-NIH genomics efforts. Examples include:

- The *Mouse Sequencing Consortium,* an initiative to decipher the full genome, or genetic makeup, of the mouse by mapping its 21 chromosomes and sequencing full-length complementary DNAs on all of its genome. Learning the full genetic make-up of the mouse will help researchers compare the genetic material of mice, humans, and other species. This project will hasten the discovery of new genes including those that contribute to diabetes, obesity, inflammatory bowel disease, and various kidney disorders so that they can design new treatments for these diseases.

- The *Trans-NIH Brain Molecular Anatomy Project,* an interdisciplinary effort to decipher the molecular anatomy of the mammalian brain and the genes expressed in the developing mouse nervous system. NIDDK's interest in this project focuses on the hypothalamus, neuroendocrine system, and the molecular events that trigger satiety.

Animal Models

Many human genes have counterparts in simpler life forms such as yeast, roundworm, fruitfly, and zebrafish. Studies of these organisms have yielded important insights into human diseases. Animal models are an essential tool for understanding health and disease in humans. They help clarify the function of genes and provide systems for testing possible treatments that are not yet ready for human trials.

Vertebrate models such as the zebrafish and mouse, though more difficult to study than worms or flies, are genetically and organically closer to humans. The NIDDK leads a trans-NIH initiative to map the zebrafish genome. The zebrafish, because of its short reproductive cycle and its transparent and easily accessible embryos, has recently emerged as an important system for studying events during embryonic development. Zebrafish mutants with defects in appetite regulation and red blood cell formation are already improving the understanding of related human disorders.

Animal models are an essential tool for understanding health and disease in humans.

The mouse is an important model for human disease. Scientists have learned a great deal about single-gene disorders from "knock-out" mouse models in which single genes have been deleted. Mouse models also help researchers figure out how complex genetic traits interact and how one gene's action is affected by other genes or environmental factors.

NIDDK is encouraging investigators with expertise in many areas to form a consortium to develop mouse models of kidney disease, such as polycystic kidney disease and kidney disease of diabetes mellitus, and to make the models available to the research community. The NIDDK is also funding the creation of national centers to undertake detailed metabolic phenotyping of knock-out mice and other mouse models that shed light on diabetes and obesity. The centers will provide a range of standardized procedures to characterize metabolism, body composition, feeding behavior, activity, tissue pathology, and other changes that occur in these mice.

How NIDDK Works

The Institute's Division of Intramural Research conducts research and training in the Institute's laboratories and clinical facilities in Bethesda, Maryland, and Phoenix, Arizona. Its scientific focus spans the breadth of biomedical investigation, from basic science to clinical studies. Historically, NIDDK scientists have excelled at clarifying the cellular and molecular processes that contribute to health and disease.

NIDDK's other scientific divisions fund research in universities and medical centers that seeks to improve the understanding of diseases and to develop better ways to prevent, diagnose, and treat them.

- The Division of Diabetes, Endocrinology, and Metabolic Diseases supports research and research training in diabetes mellitus; endocrinology, including hormones and growth factors important in osteoporosis and breast and prostate disease; and metabolic and inherited diseases such as cystic fibrosis.

- The Division of Digestive Diseases and Nutrition supports research and research training in liver, biliary, pancreatic, and other gastrointestinal diseases; motility, immunology, neuroendocrinology, absorption, transport, and other factors affecting gastrointestinal function; nutrient metabolism; obesity; eating disorders; and energy regulation.

- The Division of Kidney, Urologic, and Hematologic Diseases supports research and research training in the physiology, pathophysiology, and diseases of the kidney, genitourinary tract, and blood.

All four of NIDDK's scientific divisions support a variety of trans-NIDDK and trans-NIH career development and training awards.

The Division of Extramural Activities, an administrative division, is responsible for grant and contract administration and review. Each year NIDDK supports about 2,200 continuing research grants and 1,000 new grants that have competed for funding. The DEA oversees the two-tiered grant review process for NIDDK and is the focus of policies and regulations for the ethical conduct of research funded by NIDDK.

The Division of Nutrition Research Coordination participates in the nutrition research efforts of all NIH institutes, agencies of the Department of Health and Human Services, and other federal agencies.

Coordination and Public Input

Close communication among the NIDDK, other NIH institutes, voluntary and professional organizations, and related Federal agencies helps to mobilize and leverage resources in vital areas of scientific investigation. The NIDDK coordinates the federal agencies that conduct or support activities in diabetes, digestive diseases, and kidney, urologic, and blood diseases. These interagency coordinating committees focus on sharing information about new, ongoing, and planned activities and identifying potential areas of collaboration.

The NIDDK benefits from public and expert input from a variety of sources, such as the National Advisory Council, a panel of non-government scientists and professionals who conduct the second level of review of grant applications. Subcommittees of Council and working groups such as the National Task Force on the Prevention and Treatment of Obesity provide advice and public input to the research process. Other *ad hoc* advisory committees such as the Diabetes Research Working Group have helped shape and define research needs and directions. Scientific workshops supported by the Institute regularly bring together renowned experts in medical specialties and research areas to solicit their ideas and advice.

NIDDK's Strategic Plan addresses the Institute's global challenges and opportunities and sets forth cross-cutting research directions for the next 5 years. The Plan is a collaborative effort of NIDDK's senior scientific management, the National Advisory Council, the scientific community, lay and professional organizations, and the public. The cross-cutting themes of the Strategic Plan help the Institute set a scientific framework to develop annual initiatives.

NIDDK's Health Disparities Strategic Plan highlights programs focusing on the major diseases that disproportionately affect minority populations. Part of a larger NIH-wide effort to address health disparities in minorities, the Plan is incorporated in the *NIH Strategic Plan to Reduce Racial and Ethnic Health Disparities*. A new NIDDK Office of Minority Research Coordination focuses efforts to increase the number of minority research investigators and to foster research that will reduce health disparities.

The NIDDK participates in the NIH Council of Public Representatives (COPR), a public forum for discussing such key NIH issues as priority setting, clinical trials and managed care, privacy and genetics, and health disparities among various populations. COPR's purpose is to

- bring the public's views to NIH activities, programs, and decision-making
- convey information about NIH processes and progress to a broader public, and
- look at NIH operations and help the agency evaluate performance.

COPR members, who serve for 3 years and represent different professional and cultural backgrounds, generally have experience with disease conditions, clinical trials, and the research process through their profession, work with advocacy groups, or personal experience. For more information about COPR, see www.nih.gov/about/publicliaison/COPR.htm.

NIDDK's strategic plans are posted on

www.niddk.nih.gov

under "Special Reports, Planning, Coordination, and Testimony."

Research Advances and Directions

Diabetes

Beginning with the discovery of insulin by Dr. Frederick Banting and Charles Best in 1921, research has provided the foundation for every advance in the understanding, treatment, and prevention of diabetes. The insights gleaned from explorations in many scientific disciplines have steadily contributed to a growing knowledge base that guides and inspires the scientists of today. Many of these gains, which have led to concrete improvements in survival and quality of life for people with diabetes, can be traced to research and research training supported by the NIH.

With the knowledge gained from NIH-supported research, doctors now use simple blood tests to diagnose diabetes and to assess long-term blood glucose control. They better understand the importance of blood glucose control in preventing complications. They can prescribe new classes of oral drugs and combinations of drugs that delay the need for insulin treatment in people with type 2 diabetes. They can also better manage the risk factors for heart disease, a major killer of people with diabetes, and to delay or prevent blindness from diabetes. People who suffer from kidney failure, another diabetes complication, are leading longer lives due to improvements in dialysis and kidney transplantation. Now more than ever before, the burden of diabetes in all its forms is giving way to understanding and hope as researchers unravel its complex mysteries and move steadily closer to improved treatments and, ultimately, cures.

Sixteen million people in the United States have diabetes mellitus, a chronic disease that lowers average life expectancy by up to 15 years and often leads to painful, debilitating complications. Diabetes is the main cause of kidney failure, adult blindness, and non-traumatic amputations in America and a major risk factor for heart disease, stroke, and birth defects.

There are several different forms of diabetes. Up to 10 percent of people with diabetes have type 1, formerly known as juvenile onset or insulin-dependent diabetes. Type 1 diabetes develops when the body's immune

Diabetes in 1950

- **The disease has been recognized for more than 2,000 years.**
- **Banting and Best discovered insulin 25 years earlier.**
- **Treatment is beef-pork insulin injections.**
- **Patients measure glucose in urine to monitor treatment.**

In 1950, a person with diabetes must wait:

- **2 years for Lente insulin**
- **6 years for oral drugs for type 2 diabetes**
- **11 years for glucose strips**
- **22 years for the glucometer**
- **26 years for the Hemoglobin A1C test**
- **31 years for human insulin**
- **43 years for the Diabetes Control and Complications Trial findings**
- **50 years for the first promising trial in islet transplantation**

And in 1950, researchers must wait:

- **3 years for Watson and Crick to describe the structure of DNA**
- **6 years for a determination of insulin's amino acid sequence**
- **10 years for a radioimmunoassay for insulin**
- **15 years for the concept of phosphorylation in hormone action**
- **17 years for a description of insulin's crystal structure**
- **20 years for a direct demonstration of insulin receptors**
- **30 years for the cloning of human insulin cDNA**
- **50 years for identification of the NIDDM1 gene**

Adapted from slides of Dr. C. Ronald Kahn, Joslin Diabetes Center

system destroys pancreatic beta cells, the only cells in the body that sense blood sugar and secrete the hormone insulin, which regulates blood sugar. This form of diabetes usually strikes children and young adults, who require daily or more frequent insulin injections or use of an insulin pump for the rest of their lives. Insulin treatment, however, is not a cure, nor can it reliably prevent the long-term complications of the disease.

Type 2 diabetes, which accounts for about 90 percent of diabetes cases in the United States, is most common in adults over age 40. Affecting about 6 percent of the U.S. population, it is strongly associated with obesity (more than 80 percent of people with type 2 diabetes are overweight), inactivity, family history of diabetes, and racial or ethnic background. With the aging of Americans and the alarming increase in obesity in all ages and ethnic groups, the incidence of type 2 diabetes has also been rising nationwide.

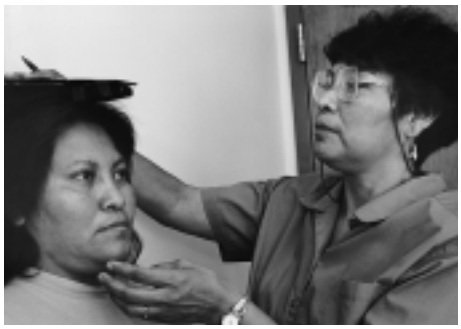
People with this form of diabetes first develop insulin resistance, a disorder in which muscle, fat, and liver cells do not use insulin properly. At first, the pancreas compensates by producing more insulin, but gradually its capacity to secrete insulin in response to meals falters, and the timing of insulin secretion is abnormal. After diabetes develops, pancreatic production of insulin continues to decline. Many people can control their blood glucose by following a careful diet and exercise program, losing excess weight, and taking oral medication. However, the longer a person has type 2 diabetes, the more likely he or she will need insulin injections, either alone or combined with oral drugs.

Once seen only as an adult disease, type 2 diabetes has been increasing in children and adolescents. National data on this form of diabetes in children are lacking, but some clinics have reported that as many as one-third of children with new-onset diabetes have type 2, and more than three-quarters of these children are minorities. High-fat, high-calorie diets and lack of exercise are probably the main reasons for the rising incidence of children with this disease. The NIDDK is urgently funding more research to prevent and treat type 2 diabetes in children, especially in minority communities.

Gestational diabetes, which occurs in about 3 to 5 percent of pregnancies, generally resolves after childbirth, though the mother has a higher risk of getting type 2 diabetes years later. Also, her child is more prone to developing obesity and type 2 diabetes in adulthood.

Diabetes in America

- **Afflicts 16 million people**
- **800,000 new cases a year**
- **One-third of cases are undiagnosed**
- **Sixth leading cause of death from disease**
- **Highest incidence in minorities**
- **Main cause of new blindness, kidney failure, and amputations**
- **Major risk factor for heart disease, stroke, and birth defects**
- **Leads to higher death rates from pneumonia, influenza, and other illnesses**
- **Shortens average lifespan by up to 15 years**
- **Costs more than $105 billion annually, including direct and indirect costs (i.e. disability, work loss, and premature death)**



NIDDK Diabetes Clinic in Arizona

Diabetes in Minorities

For reasons poorly understood, African Americans, Hispanic/Latino Americans, American Indians, and some Asian Americans and Pacific Islanders are at especially high risk for type 2 diabetes. In the United States, about 25 per cent of all adults with diabetes and most children and adolescents with type 2 diabetes are minorities. Minorities are also more likely to develop the microvascular complications of diabetes and to have more lower limb amputations than non-minorities with diabetes.

Since 1965, the NIDDK has conducted a research program in Arizona's Pima Indians, a population with a high incidence of obesity and type 2 diabetes. In recent years, the program has been expanded to include genetic studies, intervention trials, and studies to prevent and treat diabetes and its complications in this population (see page 39). NIDDK researchers have found that children born to a mother with diabetes during pregnancy have a greatly increased risk of becoming obese and developing type 2 diabetes at an early age. A girl whose mother had diabetes during pregnancy is more likely to have

diabetes when she becomes pregnant, thus setting in motion a vicious cycle that rapidly spreads diabetes from one generation to the next.

NIDDK funds an extensive portfolio of basic, clinical, epidemiologic, and behavioral research aimed at revealing the genetic and environmental factors that contribute to the disproportionate burden of diabetes in minority populations.

Advances in understanding the underlying causes of diabetes are helping to determine the factors that account for the higher rates of diabetes in minority groups. The Institute's *Strategic Plan on Minority Health Disparities* outlines research programs that comprehensively address diabetes and obesity as well as other diseases affecting minorities in greater numbers.

Blood Glucose Control

Though now an accepted tenet of diabetes treatment, the importance of intensive blood glucose control was not widely recognized before 1993, when researchers announced the results of a landmark clinical trial funded by the NIDDK. The Diabetes Control and Complications Trial (DCCT) changed conventional thinking about the management of type 1 diabetes by clearly showing that tight control prevented or delayed the eye, kidney, and other complications of diabetes. And the benefits of tight control endured for years, according to a recent follow-up study of DCCT participants. Another major study, the United Kingdom Prospective Diabetes Study, confirmed the value of intensive control for people with type 2 diabetes as well.

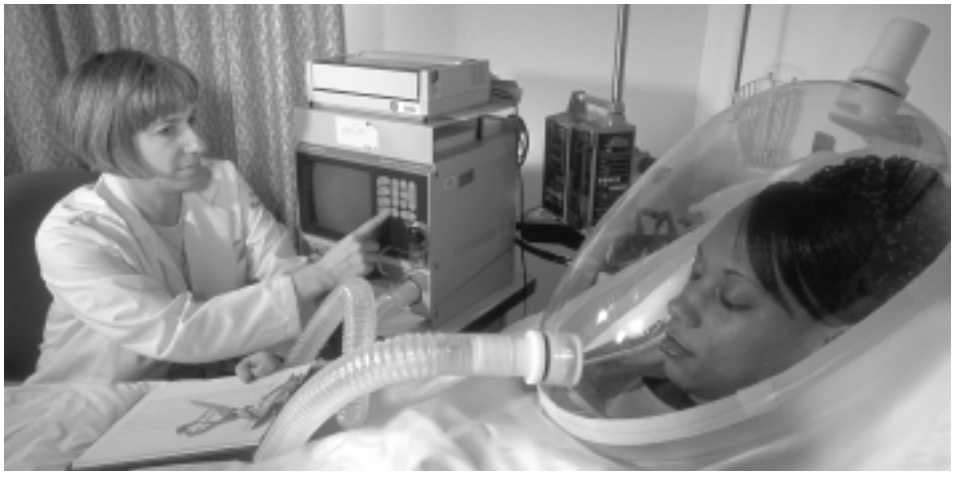
As any parent of a diabetic child knows, achieving tight control with insulin injections can be difficult and frustrating. Recent improvements in glucose-sensing devices that eliminate the need for finger sticks are helping people control their blood sugar more easily. The NIDDK is continuing to support research in noninvasive glucose monitoring and other approaches that may result in improved treatments for diabetes and its complications, while pursuing the ultimate goal of finding a true cure for this debilitating disease.

The DCCT findings made it clear that patients and health care providers alike needed to hear about the importance of intensive control. Through the National Diabetes Education Program (NDEP), the NIDDK and the Centers for Disease Control and Prevention are working hard to increase awareness of diabetes and to encourage patients and their health care team to manage diabetes more aggressively. In addressing the gap between current and desired diabetes care and practices, the NDEP strives to improve treatment and outcomes for people with diabetes, to promote early diagnosis, and ultimately, to prevent the onset of diabetes. For more information, see "Information, Education, and Outreach."

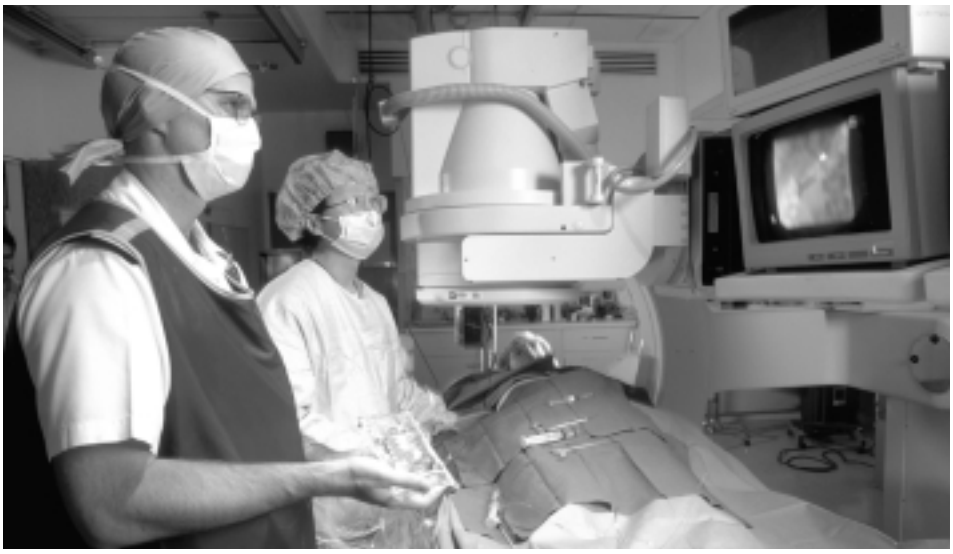
Islet Transplantation

Recently, a research team led by Dr. James Shapiro at the University of Alberta in Edmonton, Canada, announced promising results with islet transplantation in seven patients with type 1 diabetes. At the time of the report in *The New England Journal of Medicine*, all seven patients who had received the transplants remained free of insulin injections up to 14 months after the procedure.

The Immune Tolerance Network supported by the NIH and the Juvenile Diabetes Foundation is conducting a clinical trial that seeks to replicate the University of Alberta results at ten medical centers in the United States and Europe. If the results of this trial are promising, additional trials will be planned for a larger number of patients. With the insights gained from this



Dr. Anne Sumner tests volunteer's metabolic rate.



Researchers identify portal vein before islet transplantation.

research, scientists hope to further refine islet harvesting and transplantation and learn more about the immune processes that affect rejection and acceptance of transplanted islets.

To track islet transplant outcomes, the NIDDK will support the creation of a North American islet transplantation registry, which will collect and analyze data on patient and graft survival rates and other information to help researchers evaluate progress in the promising field of islet transplantation.

Research conducted and supported by the NIH, including basic discoveries in immunology and cell and transplant biology, laid the groundwork for the Edmonton advance. In 1972 NIDDK grantee Dr. Paul Lacey of Washington University first reported that islet transplantation could cure diabetes in rats. At the University of Minnesota, Dr. David Sutherland proved that a patient whose pancreas had to be surgically removed could achieve insulin independence by having his or her own islets harvested and transplanted back. Until the Edmonton advance, however, attempts at islet transplantation fared poorly: less than 5 percent of people who received transplanted islets along with immunosuppressive drugs were able to stay off insulin longer than one year.

Despite these disappointing results, several avenues of research were producing many improvements in organ transplantation and in the drugs that prevent rejection—all of which paved the way for the Edmonton advance. Dr. Thomas Starzl of the University of Pittsburgh, a longtime NIDDK grantee and premier liver transplant researcher, pioneered the use of FK-506. Now known as tacrolimus, FK-506 is an anti-rejection drug used by the Edmonton team.

Scientists also improved islet isolation techniques and ways to assess islet function. Another NIDDK grantee, Dr. Camilo Ricordi of the University of Miami, refined the method for isolating islets from pancreatic tissue and preserving them in what is now called the "Ricordi Chamber."

Despite the promise of islet transplantation, certain obstacles, such as the inadequate supply of donor islets and the need for immunomodulatory drugs, must be overcome before this technique is adopted as a standard treatment for type 1 diabetes. For information about NIDDK research initiatives that address these barriers, see "Autoimmunity and the Beta Cell."

Diabetes Research Working Group

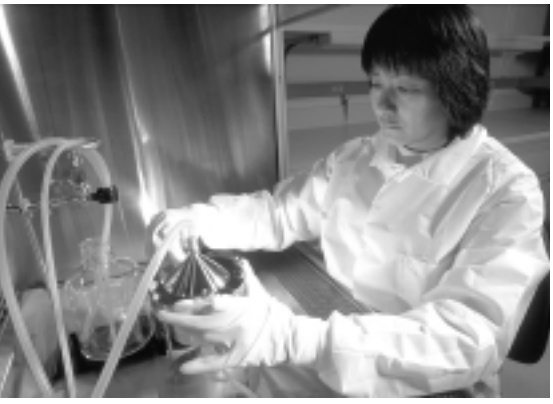
In 1999 the Congressionally-established Diabetes Research Working Group (DRWG), a panel of diabetes experts, issued its Strategic Plan and recommendations for future diabetes research. (The DRWG report is on the NIDDK Web site under "Special Reports, Planning, Coordination, and Testimony.") The NIH is pursuing the full range of the DRWG's scientific recommendations, with special emphasis on five areas of extraordinary opportunity: genetics, obesity, autoimmunity and the beta cell, cell signaling and cell regulation, and clinical research and clinical trials. Following are examples of research advances and initiatives in these areas.

Genetics of Diabetes and Its Complications

Diabetes encompasses a group of diseases that impair blood glucose regulation. Some rare forms of diabetes are caused by the mutation of a single gene. The common forms (type 1 and type 2),

however, are complex diseases that arise from genes interacting with other genes and the environment. When these susceptibility genes combine with environmental triggers—possibly viruses in type 1 diabetes or diet, obesity, and inactivity in type 2—the risk of getting diabetes rises. Still other genes may heighten the risk of developing severe complications after the onset of diabetes.

For years, scientists have known that single-gene mutations contribute to rare forms of diabetes, such as Maturity Onset Diabetes of the Young (MODY) and other rare subtypes of type 2 diabetes, which may account for up to 5 percent of diabetes cases. Five MODY genes, all involved in some aspect of regulating insulin secretion, have been identified. For example, a mutation in one copy of the gene insulin promoter factor-1 causes a rare



Islets are isolated from donor pancreas.

form of early-onset type 2 diabetes, while a mutation in both copies leads to failure of the entire pancreas to develop.

Unlike single-gene disorders, the more common forms of diabetes appear to arise from subtle defects in several genes, each contributing and probably interacting to create susceptibility. By developing new techniques and studying larger patient populations, researchers are working hard to find these genes and understand their functions. An International Type 2 Genetic Linkage Consortium has been formed to help investigators combine their individual studies and localize diabetes genes.

Recently, a team of NIDDK-supported researchers led by Drs. Graeme Bell and Nancy Cox of the University of Chicago identified a gene called NIDDM1 on chromosome 2, which interacts with a gene on chromosome 15 to increase the risk of developing type 2 diabetes. A subtle variation in the sequence of the NIDDM1 gene, which encodes a protease called calpain 10, raises the risk of type 2 diabetes in a Mexican American and two northern European populations. Further clinical studies and investigations of calpain 10 in cultured cells and transgenic animals will shed light on the gene's role in diabetes. By finding the gene, scientists have moved a step closer to understanding how type 2 diabetes arises and have identified a new target for drug development.

Research has shown that the strongest genes predisposing to type 1 diabetes are alleles, or common genetic variations, of the major histocompatibility complex (MHC), mainly HLA DR3 and DR4. These molecules play a major part in activating T cells and regulating the immune response. In addition to the HLA genes, there are environmental influences as well as other genes that influence susceptibility to type 1 diabetes. NIDDK plans to support a consortium to foster collaboration of research teams searching for these genes.

The NIDDK funds many studies on the complications of diabetes, including the search for genes that predispose people to complications such as kidney disease. Certain populations, such as the Pima Indians of Arizona, have a high incidence of kidney disease from diabetes (KDDM), and studies have shown that susceptibility to this complication runs in families. An NIDDK study of 715 Pima families recently found that one gene with a major effect can explain susceptibility to KDDM, though other genes probably play a role.

Researchers in NIDDK's Familial Investigation of Nephropathy of Diabetes program are now studying siblings of people with diabetes, some who have kidney disease and some who do not, in hopes of finding genes unique to those who have kidney disease. Once the gene or genes have been found, scientists will try to develop targeted drugs and other prevention strategies.

Autoimmunity and the Beta Cell

In the past decade, major discoveries in immunology and cell biology have helped to clarify the immunologic basis of type 1 diabetes. To capitalize on these gains, the NIH supports many initiatives that are searching for ways to block immune destruction of the beta cell and spur strategies to replace beta cell function. One initiative calls for researchers to develop methods for imaging beta cells so scientists can evaluate their mass, function, and signs of inflammation. Such imaging techniques will help doctors monitor disease progression and response to therapy in people who have diabetes or are at risk for developing it.

Major discoveries in immunology and cell biology have helped to clarify the immunologic basis of type 1 diabetes.

The University of Alberta's advance in islet transplantation has infused hope and excitement into the diabetes community. However, even if clinical trials confirm the procedure's value in treating type 1 diabetes, two obstacles may hamper its use as a standard treatment. One is the potentially harmful long-term effects of immunosuppressive drugs needed to prevent rejection of the transplanted islets. This concern especially applies to children, who would face a lifetime of immune suppression. Another issue is the limited supply of donor pancreases, from which islets are extracted. Only a few thousand become available each year, far short of the number needed.

Intensive research is yielding advances on both fronts. To circumvent the need for immunosuppressive drugs, scientists are working on immune modulators that prevent T-cell rejection of donor tissue without endangering the patient's disease-fighting ability. This work, which builds on a growing body of knowledge about the molecular signals governing immune cell communication, has exciting implications for preventing transplant rejection and treating diseases of the immune system, such as autoimmunity and immunodeficiency.

Addressing the shortage of donor pancreases, NIH-supported researchers at various medical centers are using genetic engineering methods to induce the development of functional beta cell lines. A team at the University of California at San Diego recently reported success in developing the first human beta cell line that produces insulin in response to glucose. Further studies will reveal whether these cells can retain their insulin-producing ability over the long term and safely be transplanted into people with diabetes.

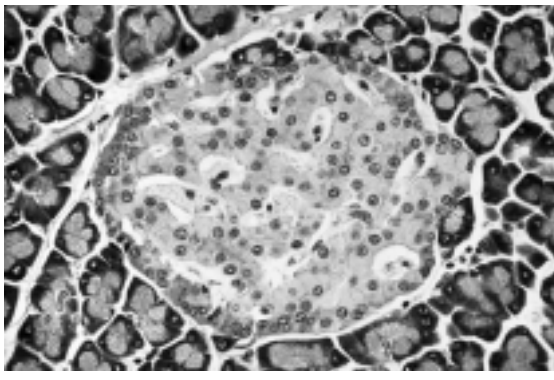
NIDDK is also encouraging researchers to develop a cell culture model of the human beta cell that responds to glucose, reflects signaling through cell surface and nuclear receptors that regulate insulin production and secretion, and responds to growth factors and cytokines active in the pancreatic beta cell.

Stem cell research holds much promise for treating many diseases such as diabetes, cancer, Parkinson's disease, and Alzheimer's disease. Pluripotent stem cells, derived from the inner cell mass of human embryos at the blastocyst stage and from fetal tissue, are capable of limitless division and self-renewal. These stem cells might be stimulated to develop into specialized cells such as beta cells. Adult stem cells that reside in the pancreatic duct and other organs may also have the capacity to differentiate into specialized cells such as beta cells, but much more research is needed to determine their potential.

Cell Signaling and Cell Regulation

Research in cell signaling and regulation holds the key to understanding the biochemical pathways that maintain normal metabolism and go awry in obesity, insulin resistance, and type 2 diabetes. The NIDDK supports a great deal of research in these processes, which underlie critical functions such as insulin action, immunity, appetite regulation, and beta cell activity. Many of the same molecules are key regulators in a variety of tissues. For example, only recently have scientists realized that fat cells are an active endocrine organ, secreting leptin, tumor necrosis factor-alpha, interleukin-6, complement C3, and other cell-signaling substances. It is critical to learn how these hormones and cytokines affect other tissues and influence the risk of diabetes.

Research in cell signaling, communication, and regulation is helping scientists understand the normal workings of the immune system and what goes wrong in autoimmune disorders such as type 1 diabetes, inflammatory bowel disease, and common thyroid disorders. Cell signaling work is also shedding light on insulin resistance, a disorder that usually accompanies, and often



A healthy pancreatic islet

Dr. Phillip Gorden conducts insulin studies.

precedes, type 2 diabetes. This condition occurs when muscle, fat, and liver cells lose the ability to respond normally to insulin, the hormone released by the glucose-sensing beta cells of the pancreas. Scientists once thought that insulin resistance arose from a defect in the insulin receptor, but they are now moving toward a deeper understanding of the intricate intracellular pathways that disrupt the balance between insulin action and insulin secretion.

Researchers at Harvard University's Joslin Diabetes Center are focusing on two proteins, IRS-1 and IRS-2, which become activated inside the cell when insulin binds to the receptor. IRS-1 and -2 then interact with other proteins in a complex signaling pathway that arouses the glucose transporter, which ferries glucose into the cell. The IRS complex also triggers a second pathway, the Ras complex, which turns on gene expression inside the cell.

In more recent studies in knock-out mice, the Joslin team found that insulin, acting through receptors in the brain, plays a key role in regulating appetite, fat accumulation, and even reproductive function. When insulin is taken up by receptors in the brain, it appears to have an appetite-suppressing effect. Without these receptors, mice became obese, insulin resistant, and abnormal in their reproductive function. These findings are reinforcing scientists' belief that a complex network of molecular signals and feedback loops involving the brain and other tissues is the key to understanding satiety, obesity, insulin resistance, and the development of type 2 diabetes. Continuing research with animal models is clarifying the specific roles of these molecules in muscle and liver cells, where insulin resistance appears to occur.

The NIDDK is also stimulating research on the role of nuclear hormone receptors in regulating gene expression in specific tissues and on growth factors that regulate beta cell growth. Several growth factors are already being tested in clinical trials for the treatment and prevention of microvascular disease, a complication of diabetes.

Clinical Research

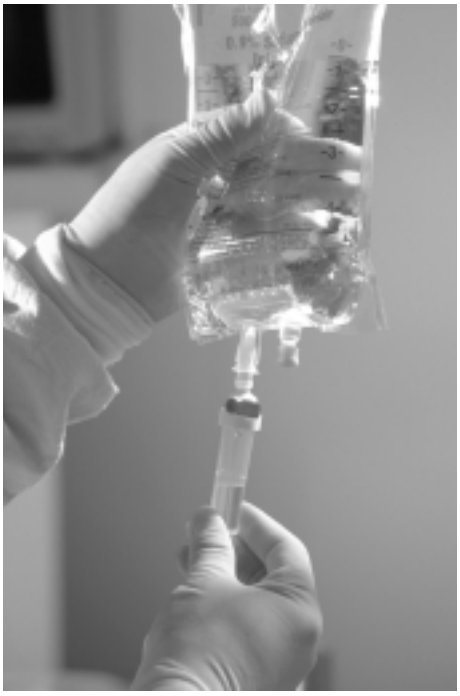
Laboratory and animal studies can answer basic biologic questions and yield potential therapies, but only clinical research, or human studies, can determine whether proposed treatments and prevention strategies are safe and effective in people.

If diabetes could be prevented or delayed, many thousands of people would enjoy improved health and freedom from the cost and burden of managing the disease. Complete prevention will only be possible when scientists have a clear understanding of the causes of diabetes. However, enough is currently known about factors that contribute to diabetes risk that two large multicenter clinical trials funded by the NIDDK are trying to prevent diabetes in high-risk groups.

The *Diabetes Prevention Program (DPP)* seeks to determine whether type 2 diabetes can be prevented or delayed in people who have impaired glucose tolerance, a condition in which blood glucose levels are higher than normal but not yet diabetic. The study compares two different approaches to prevention—an intensive regimen of diet and exercise versus treatment with metformin, a diabetes medication—to a

control group provided standard advice on diet and exercise. About 45 percent of the 3,000 DPP participants are minority group members.

In one of the most important discoveries of the past 20 years, scientists learned that type 1 diabetes results from



the slow autoimmune destruction of the pancreatic beta cells, a process that begins long before the appearance of diabetes symptoms. Most patients who develop type 1 have markers for the disease in their blood—antibodies against certain beta cell proteins, including insulin, glutamic acid decarboxylase (GAD), and the enzyme IA2. By measuring these markers of autoimmune activity and genetic markers, scientists can now gauge a person's risk for developing type 1 diabetes.

This new understanding of the immune basis for type 1 diabetes and other insights gained from immunology research opened the possibility of modulating the immune system to prevent beta cell destruction. Capitalizing on these converging discoveries, an NIDDK-supported multicenter trial called the *Diabetes Prevention Trial-Type 1* is testing whether insulin injections or oral insulin can prevent type 1 diabetes in high-risk individuals—people who have close relatives with type 1 diabetes, high levels of islet cell antibodies, and other indicators of risk. For more information about this trial, call 1-800-HALT-DM1.

Through creation of the *Type 1 Diabetes Mellitus TrialNet*, the NIDDK will expand the clinical trial infrastructure to speed studies of new agents that preserve beta cell function and prevent type 1 diabetes.

The cells lining the heart and blood vessels function abnormally in diabetes. To learn more about the factors contributing to atherosclerosis and microvascular complications, the NIDDK has an initiative to study how diabetes affects endothelial cells and how these changes lead to blood vessel damage. NIDDK also supports two multicenter clinical trials of the National Heart, Lung, and Blood Institute (NHLBI) that will try to define the factors that contribute to heart disease in diabetes and the most effective ways to treat it:

- *Action to Control Cardiovascular Risk in Diabetes (ACCORD)* compares the effects of standard versus intensive treatment of blood glucose, high blood pressure, and lipids on the development of cardiovascular disease in people with diabetes; and
- *Bypass Angioplasty Revascularization Investigations (BARI II)* examines the effects of insulin-providing and insulin-sensitizing strategies in people with type 2 diabetes who have significant coronary artery disease.

NIDDK, with NHLBI support, is launching a third national multicenter trial, the *Study of the Health Outcomes of Weight Loss* (SHOW), to assess how intentional weight loss affects cardiovascular disease and other health parameters such as blood sugar, muscle mass, bone strength, and microvascular disease in obese people with type 2 diabetes. The study will try to answer two major questions:

- ❑ Do interventions designed to produce sustained weight loss in obese people with type 2 diabetes improve health?
- ❑ How do the benefits and risks of these interventions compare with the benefits and risks of treating obesity-related conditions without weight loss?

More Information About Diabetes

Visit NIDDK at http://www.niddk.nih.gov or ask for a list of publications from the Institute's National Diabetes Information Clearinghouse, 1 Information Way, Bethesda, MD 20892-3580, 1-800-860-8747.

Obesity

An epidemic of obesity poses one of the greatest threats to the health and well-being of Americans of all age groups and ethnic backgrounds. Obesity is a major risk factor for insulin resistance and type 2 diabetes. Overweight people are also more likely to have high blood pressure, heart disease, stroke, gallbladder disease, and some cancers. More than 50 percent of adult Americans are overweight, as defined by a body mass index (BMI) greater than 25, and almost 25 percent are obese, with a BMI greater than 30. [BMI equals weight in kilograms divided by height in meters squared. (BMI = kg/m^2)] Twenty-five percent of American children are overweight or at risk of becoming overweight, and the numbers are steadily rising. For reasons that are still unknown, more than one in three Black American, Hispanic American, Native American, and Native Hawaiian American women are obese.

Although the public has long believed that obese people simply overeat, scientists now know that obesity is a disease of poorly regulated energy balance that results from a complex mix of genetic, behavioral, and environmental factors. Weight-control programs are costly, long-term lifestyle changes are difficult, and most people lose weight only to later regain the pounds they shed. Obesity costs the American economy nearly $100 billion per year in health care costs and lost productivity. This figure is bound to increase as more people develop obesity-related health problems.

Obesity in America

- **50 percent of Americans are overweight (BMI over 25).**
- **25 percent of Americans are obese (BMI over 30).**
- **Obesity is a major risk factor for diabetes, hypertension, stroke, gallbladder disease, and some cancers.**
- **Genes play a major role in determining body weight.**
- **80 percent of people with type 2 diabetes are obese.**
- **People with type 2 diabetes tend to have more body fat around the abdomen.**

The NIH supports a broad portfolio of basic and clinical research aimed at understanding the biochemical and behavioral processes that regulate weight and at improving methods to prevent and treat obesity. Such research is revealing that appetite and energy expenditure are regulated by a far more complex system of signaling and feedback loops than was previously recognized. By understanding this regulation and identifying key signaling molecules, scientists hope to find targets for therapeutic intervention and drug development.

Genes Influence Obesity Risk

While an abundance of cheap, high-fat foods and an increasingly sedentary lifestyle contribute to overweight, studies of twins, adopted children, and extended families show that an individual's susceptibility to becoming obese in such a "toxic" environment is strongly influenced by their genetic makeup. Research took a quantum leap forward with the discovery of the obesity (*ob*) gene and the gene's product, leptin, by Dr. Jeffrey Friedman, an NIDDK-funded researcher. Leptin helps regulate energy metabolism and use by influencing a complex system of signals between fat cells and the brain, within the brain, and between the brain and the rest of the body.

Appetite Regulation

Leptin research opened up exploration of the pathways that regulate food intake, energy expenditure, and fat storage. Studies of uncoupling proteins in fat cells, central nervous system melanocortin and other factors, which mediate the effects of leptin in the brain by increasing or decreasing appetite and metabolism, may lead to better therapies to prevent and control obesity.

Skin calipers measure body fat.

Mouse models of obesity syndromes have shed a great deal of light on the molecular mechanisms regulating food intake and energy balance. The identification and cloning of other appetite regulating genes such as *db*, the leptin receptor, may lead to the development of appetite-regulating drugs that may someday help in treating and preventing obesity.

Controlling appetite by modulating the synthesis of triglycerides, fatty acids that provide stored energy for metabolic functions, holds much promise for understanding and controlling obesity. A specially bred strain of mice lacking the enzyme Dgat, which aids the last step of triglyceride synthesis, are lean, active, and resistant to weight gain. Moreover, they develop normally and synthesize trigylcerides normally, suggesting that their bodies employ a different pathway for triglyceride synthesis. Further studies of triglyceride synthesis in knock-out mice will help scientists understand its role in weight control and may suggest potential new treatments.

Recently, NIDDK-supported scientists at the Johns Hopkins University discovered that an enzyme called fatty acid synthase (FAS) appears to play a major role in regulating appetite in mice. When the researchers administered a FAS inhibitor, C75, to obese mice, the animals lost a significant amount of weight with no slowing of their metabolic rate or other adverse effects.

Further studies of FAS, an enzyme that enables the production of fatty acids, will flesh out its precise role in appetite regulation and may shed light on how free fatty acids promote insulin resistance.

The researchers, who had been studying FAS's role in cancer development, initially had no idea that the enzyme played such an important role in eating behavior. Their finding is a good example of the importance of basic research in opening new, unexpected lines of discovery, often in areas unrelated to the original focus of inquiry.

Osteoporosis

The 60-year-old woman's family photos show her slow decline more vividly than any x-rays could. Once an erect, attractive newlywed in her 20s, she is now a fragile, hunched figure leaning on canes. Under her most recent picture she writes, "My daily life has changed completely. I now walk with two canes. I can't bend down, and I'm in constant pain. I can't carry or pick things up, so I can't do my own shopping. I have always been active, but after two fractures, I can't even take care of my basic needs."

Osteoporosis is a disease marked by porous, brittle bones that are prone to fracture. Treatment consists of proper nutrition, exercise, safety measures to prevent falls, and medication to slow or stop bone loss and increase bone density. Left untreated, the bones of a person with osteoporosis become weak, fragile, and more likely to fracture. All bones may become brittle with osteoporosis, but fractures in the hip, spine, and wrist occur more often and can cause prolonged or permanent disability, even death.

The long-term investment in understanding the mechanisms of bone loss and bone remodeling, and on the cellular and animal models to study these processes, has paid off in the development of new drugs to treat osteoporosis.

According to the National Osteoporosis Foundation (NOF), about 10 million Americans have osteoporosis, and 18 million more have low bone density, placing them at increased risk for developing the disease. Osteoporosis has been reported in people of all ethnic backgrounds, but it is four times more common in women. The NOF estimates national direct expenditures for osteoporosis and related fractures at $14 billion each year, and the cost is rising.

Research conducted and supported by the NIDDK on the regulation of bone development and remodeling has contributed a great deal to understanding how osteoporosis begins and progresses and is leading to effective methods of treatment and prevention. These scientific advances are opening promising avenues for research and potential new options for treatment.

Bone is a living, growing tissue. It is made mostly of collagen, a protein that provides an elastic framework, and calcium phosphate, a mineral that strengthens and hardens the framework. Present in bones are cells that respond to hormones that maintain bone, breaking it down or building it up to release or store calcium as the body's needs change. Hormones such as parathyroid hormone (PTH), produced by the parathyroid glands in the neck, play a major role in regulating bone growth and bone loss. The amount of mineral in bone at any given time reflects the net difference between these two processes.

Medical team reviews patient films.

Paradoxically, PTH's action is both anabolic and catabolic—that is, it controls the transfer of calcium into, as well as out of, bones.

NIDDK researcher Dr. Gerald Aurbach, now deceased, was the first person to isolate and purify PTH, work he began as a postdoctoral fellow in a laboratory in Boston. This achievement led to the collaborative development of the first radioimmunoassay to measure PTH in the blood. With this tool, researchers began studying the factors governing PTH secretion and found that changes in serum calcium controlled PTH production. At this point, scientists could focus on making a synthetic form of PTH to learn more about this important hormone's mechanisms of action.

A few years later, NIDDK-supported researchers announced the identification, cloning, and sequencing of the cellular receptor for PTH in rats, mice, and humans. Once the structure was known, they could analyze the hormone's molecular mechanism of action, information that was crucial to understanding PTH's effects on bones and to developing hormonal therapies for osteoporosis. Earlier studies had shown that signal transduction through the PTH receptor followed two independent paths. Different parts of the receptor activate different signaling pathways, which may explain how PTH can at times signal osteoblasts—cells involved in bone formation—to increase bone mineral, and at other times to reduce it.

Decades of basic research laid the groundwork for developing and testing synthetic PTH as a treatment for osteoporosis. Insights into the anabolic actions of hormones such as PTH led to two small NIDDK-supported clinical trials testing synthetic PTH in osteoporosis. The researchers found PTH increased spinal bone mineral density and prevented bone loss from the hip and other bones in young women. PTH treatment is well tolerated but currently requires daily injections.

Synthetic PTH may also prove helpful for severe osteoporosis caused by glucocorticoid hormones such as prednisone, which are used to treat inflammation. An NIDDK-supported clinical study is testing the effect of daily PTH injections on fracture risk in postmenopausal women who have glucocorticoid-induced osteoporosis and are currently receiving hormone replacement therapy. Early results show a greatly reduced risk of spine fractures and non-traumatic, non-spine fractures one to two years after beginning treatment.

Though calcium was known to play a role in PTH secretion, only recently have researchers figured out how the mineral signals parathyroid cells to secrete PTH. NIDDK-supported studies led to the identification and cloning of a receptor on parathyroid cells that senses blood levels of calcium and signals the parathyroid cell to release PTH. This finding offered new possibilities for designing treatments by targeting the parathyroid's calcium-sensing receptors. Animal studies of new oral drugs that act on these receptors to increase PTH secretion showed a dramatic lowering of bone turnover and prevention of bone mineral loss. Further research will determine whether the drugs prove to be safe, effective treatments for osteoporosis.

The hormone estrogen also protects against bone loss. NIH-supported research has shown that estrogen replacement therapy helps to maintain bone mass by shortening the lifespan of osteoclasts, the cells responsible for resorption of bone mineral. Estrogen does this by stimulating the production

and release of a potent growth factor in bone. Without estrogen, osteoclasts are no longer well regulated, resulting in excessive bone loss.

Despite its benefits, estrogen, when taken alone, has been shown in some studies to slightly increase a women's risk of developing breast and uterine cancer, and many questions remain about its effects on other tissues. Scientists are now testing a group of oral drugs called Selective Estrogen Receptor Modulators, or SERMs, which may turn out to protect bones more safely than estrogen. Raloxifene, the first SERM to enter clinical practice, is less potent than estrogen at preventing bone loss but does not have estrogen-like effects on the breast and uterus. Further work is under way to develop other SERMs that may prove more effective.

NIDDK's long-term investment in understanding the mechanisms of bone loss and bone remodeling, and on cellular and animal models to study these processes, has paid off in the development of new drugs to treat osteoporosis. Promising avenues of current research—for example, on nuclear hormone receptors—offer potential targets for rationally designing drugs to treat this serious, painful disease. A research imperative of the NIDDK is to capitalize on these pioneering discoveries to develop better ways to prevent bone loss, reduce the incidence of fractures, and improve the quality of life for people with osteoporosis.

Digestive Diseases

Like other areas of medical practice, treatment of gastroenterological disorders matured and became specialized in the 1950s. Major NIH conferences held in 1967 and in 1978 spotlighted digestive diseases as a national health problem, costing billions of dollars a year. The National Commission on Digestive Diseases, authorized by Congress, produced a road map to address this multi-faceted issue.

Each year, about 60 to 70 million Americans experience some digestive disorder, from heartburn or an upset stomach to more severe gastrointestinal problems such as gallstones, ulcers, or diverticulitis. Some 30 million suffer from chronic digestive diseases such as inflammatory bowel disease, hepatitis, or cirrhosis. Eleven million report acute problems. Digestive diseases cost the country $107 billion annually in direct medical expenses and lost productivity. Certain minority groups are more prone to specific digestive disorders. The risk of gallstone disease is higher among Native Americans than the general population. The prevalence of hepatitis B and C, as well as peptic ulcer from *Helicobacter pylori* infection, is higher among African Americans and Hispanic Americans. Americans over the age of 65 have more diverticular disease, peptic ulcer disease, and gallstones.

The impact of digestive diseases is significant, but NIDDK's steady effort to understand these disorders has begun to disarm some of the most debilitating: viral hepatitis, inflammatory bowel

Dr. Jay Hoofnagle discusses a young patient with her mother.

disease, and ulcers. And NIDDK research support has brought about one of the most valuable therapies of the 20th century: organ and tissue transplantation.

Hepatitis

The extraordinary advances in understanding and treating viral hepatitis during the past 40 years have led one NIDDK senior scientist to call this period "the golden age" of hepatitis research.

According to one senior scientist, the extraordinary advances of the past 40 years make this period a "golden age" of hepatitis research.

Although viral hepatitis has probably existed since ancient times, in-depth clinical study of viral hepatitis didn't really begin until World War II. High rates of blood and plasma transfusions, the use of non-disposable hypodermic needles, and reduced hygiene caused serious outbreaks of hepatitis among both soldiers and civilians. At the time, researchers believed two forms of the disease existed: "infectious" hepatitis, or hepatitis A, and "serum" hepatitis, or hepatitis B.

Separating A from B, and B from C

Former NIDDK geneticist Dr. Baruch Blumberg did the seminal work that made it possible to distinguish one form of hepatitis from the other. Working with blood samples collected from Australian aborigines and people with hemophilia, he identified a marker he called "the Australia antigen," later identified as the outer coat of the hepatitis B virus (HBV). Several years later, Blumberg was awarded the Nobel Prize in Physiology and Medicine for his discovery. This work and that of hematologist Fred Prince and others touched off an explosion of interest in this complex virus from virologists, immunologists, and others.

Among them was NIDDK's Dr. Jay Hoofnagle, who began to study and expand knowledge about the hepatitis B core antigen and its antibody. Their time and energy paid off. After developing sensitive radioimmunoassays that made it possible to identify HBV, researchers began developing a safe vaccine. The first trials in humans began in the mid-1970s.

The next critically important advance, the identification of the hepatitis A virus (HAV) and the development of diagnostic tests, also came from the NIH, aided by the emerging discipline of immune electron microscopy. As with HBV, accurate diagnostic tests for HAV, which is transmitted in contaminated food and water, led to highly effective vaccines. These tests also led scientists to recognize another form of hepatitis that was neither HAV nor HBV. First termed "non-A, non-B" hepatitis, it eventually became known as hepatitis C (HCV). In the 1970s, an Italian scientist discovered the delta, or hepatitis D virus, which attacks the liver either at the same time as hepatitis B, or when a person is already infected with HBV.

A person infected with both hepatitis B and D has a high risk of sudden onset of severe hepatitis and the worst form of cirrhosis. Following its discovery, NIH scientists accomplished much of the basic research on HDV.

The pace of progress picked up in the 1980s as scientists developed and refined new molecular biological techniques. Some of these advances grew out of the attention focused on the AIDS virus. Initially, laboratories produced hepatitis B vaccine from the blood of patients infected with chronic hepatitis B

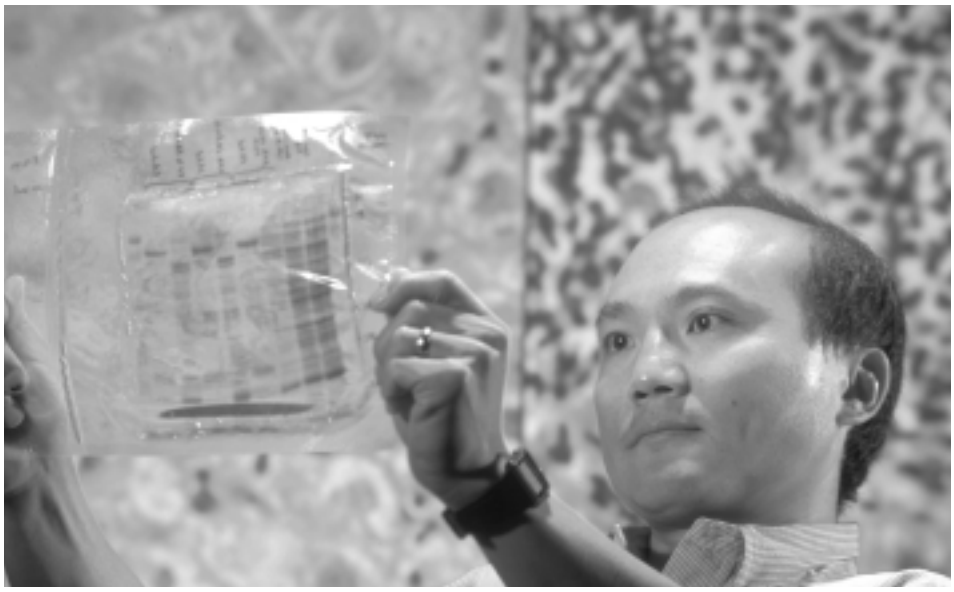
virus. Although the immunization was effective, physicians became concerned that this plasma-derived vaccine might harbor viable hepatitis or HIV. After it became possible to "clone" the hepatitis B virus, a synthetic, recombinant vaccine was produced, completely safe from any viral contamination. For the first time,

physicians could look forward to eradicating one form of hepatitis through worldwide vaccination.

From the late 1970s through the next decade, scientists worked to define the virus responsible for non-A, non-B hepatitis, a task that had become more and more critical as knowledge of the disease increased. At first, this infection seemed insignificant because it produced few symptoms of serious liver disease. Later, physicians became worried as they learned over time that many patients with no apparent symptoms suffered chronic infection that often progressed to cirrhosis and occasionally, liver cancer. "This is a very smart virus," says Dr. Leonard Seeff, an NIDDK liver expert. "It knows that if it kills its host, it kills itself. So it sits around in the lymphocytes and damages immunological function. It has a very slow evolution."

Two decades of hard work paid off in 1989, when industry biotechnologists identified the virus using blood samples developed and provided by NIH's Harvey Alter and other Federal scientists. The test for this new virus made it clear that most cases of non-A, non-B hepatitis were caused by hepatitis C and that the virus was extremely common in the United States, particularly among high-risk groups such as injection drug users. Because many of these high-risk individuals frequently sold their blood, high rates of hepatitis occurred in patients who got transfusions from donor blood procured commercially.

By 1991, a second generation test made it possible for blood banks to screen donated blood for HCV. The result has been an almost total eradication of the problem of transfusion-associated hepatitis C in the United States.



Dr. T. Jake Liang studies the hepatitis C virus.

HCV currently affects approximately 4 million Americans and is the leading cause of liver transplantation in this country. With the near elimination of post-transfusion hepatitis C, new cases have decreased, but physicians are still seeing the residue of chronic infection that occurred before the problem of transfusion-transmitted HCV was discovered. Although an important study of blood samples taken from soldiers inoculated in 1948 has shown that some patients have carried HCV for over 40 years without developing disease, physicians still expect deaths from liver disease to increase in the next 10 to 15 years because of these residual cases.

Workers in India and Russia, together with NIH scientists, have further identified a virus called hepatitis E. Endemic in some parts of the world but rare in the United States, hepatitis E simulates hepatitis A, causing especially severe illness among pregnant women. Scientists are now researching other blood-borne viral agents such as hepatitis G, and two others called TTV and SEN-V, but whether they are true hepatitis viruses is not clear.

Progress in Treatment

As understanding of these viruses has grown, NIDDK researchers have pioneered treatments for both hepatitis B and C. NIDDK's Hoofnagle has studied various nucleoside analogues and interferon therapies, and was the first to prove that interferon was effective treatment for what was then referred to as non-A, non-B hepatitis. In most of the 10 patients in his study, interferon reduced viral enzymes in the blood, and in some cases, enzymes became normal. The study further showed that normal enzyme levels could be sustained, and that HCV could no longer be detected in the blood as long as 10 years after initial treatment. Because of these preliminary studies and industry-conducted controlled trials that followed, interferon,

now combined with ribavirin, has been approved for the treatment of hepatitis C, as has interferon for the treatment of hepatitis B.

NIDDK has also led the search for new knowledge about treatment of the disease. In 1997, NIDDK sponsored a Consensus Conference on the management of hepatitis C to examine the numerous treatments then under investigation. The conference recommendations have come to represent the state of the art treatment for hepatitis C. NIDDK disseminates these guidelines in print and online and continues to modify them when controlled trials prove a therapy such as interferon combined with ribavirin is superior.

Even though curing hepatitis C is possible, at least 60 percent of patients treated with the best therapy available today remain infected with the virus. Long-term treatment trials indicate that interferon therapy may further reduce the progression of infection to chronic hepatitis C. Accordingly, NIDDK is spearheading HALT-C, a large and important clinical trial to determine whether long-term treatment of patients who do not respond to initial therapy can reduce the progression of HCV either from marked fibrosis to cirrhosis or from cirrhosis to end-stage liver disease, sometimes found to be liver cell cancer. The study will run for 7 years, treating over 1,300 patients with pegylated interferon, a new generation drug that researchers believe will prove more effective than conventional interferon.

As scientists have learned more about treating hepatitis C, they have found that African Americans don't respond as well to treatment as Caucasians do. Data from a recent NIDDK-sponsored workshop on hepatitis C in African Americans confirmed their lower response to treatment and revealed that hepatitis C is a major health concern, especially for the African American community. As a result, NIDDK is issuing a proposal for additional study of hepatitis C in African Americans.

Because treatment of hepatitis C is difficult and can cause adverse side effects, patients often turn to other treatment options, such as herbal remedies typical of Chinese traditional medicine. To explore these options, NIDDK and the National Center for Complementary and Alternative Medicine (NCCAM) sponsored a meeting to discuss alternative medicine for chronic liver disease. NCCAM plans to examine products such as silymarin, or extract of milk thistle, for future controlled trials.

Finally, intramural NIDDK scientists are studying the immunologic components of HCV infection and are working to develop a vaccine that will make it possible to prevent hepatitis C in future generations.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is the collective name for two of the most damaging gastrointestinal diseases after cancers of the gut: ulcerative colitis and Crohn's disease. These chronic, intractable diseases cause inflammation of the gastrointestinal tract, resulting in severe abdominal pain, diarrhea, intestinal bleeding, bowel ulcers, fever, and many potential serious complications. Although ulcerative colitis can be cured by surgical removal of the whole colon, a relatively drastic operation, neither form of IBD can be cured with medication. NIDDK-funded basic and clinical research in recent decades has significantly clarified understanding of disorders that were undescribed at the turn of the last century. Today, these diseases are well recognized diseases that are estimated to affect as many as a million Americans, many of them adolescents and young adults in the prime of life.

NIDDK funding of research in IBD has increased six-fold in the last decade, and strategic planning has brought new investigators to this research field. Although causes remain unknown, the best current theory is that in IBD, the immune system reacts to a virus or a bacterium, producing intestinal inflammation. Research goals focus on understanding how the immune system turns against the body it is meant to protect in diseases such as Crohn's and colitis. Scientists are searching for the causes of these diseases, specific targets for intervention, and of course, a cure. Advances in the last few years have been dramatic and are raising hope among the men and women suffering their debilitating effects.

That was hardly true in 1950. People suffering with IBD were often thought to be the victims of poor parenting and were referred for psychiatric evaluation. All that began to change when NIDDK-funded researchers first recognized the complex nature of these diseases. There is now a much better understanding of how the immune system interacts with the gut and the environment to cause disease. There is increasing interest in how the gut naturally repairs itself, and the role of normal and abnormal bacteria in the body. Although it has been known for many years that IBD is strongly influenced by

genetics, it is only since the Human Genome Project provided powerful genetic tools to IBD research that scientists have begun to hope to understand the genes contributing to the disease. As animal models have become more sophisticated, research has begun to provide a more detailed picture of the molecular and biochemical steps that lead to IBD.

Nurse and patient review medication schedule.

This knowledge has presented several opportunities for new therapies, particularly in Crohn's disease. Prior to the 1990s, treatment focused on aminosalicylates such as sulfasalazine for mild to moderate Crohn's, and anti-inflammatory agents, such as corticosteroids for more severe disease. Basic and clinical research funded by NIDDK established the effectiveness of these treatments for IBD. With these therapies and related drugs, some patients achieved remission, but many didn't maintain it. By the mid 1990s, a new generation of these conventional treatments came into use. Researchers have identified a new corticosteriod called budesonide that appears to be as effective as other corticosteroids, but causes fewer side effects. Immunosuppressants such as azathioprine, 6-mercaptopurine and methotrexate, proved effective in many patients. Although there is no clear evidence that infection causes IBD, antibiotics such as metronidazole and ciprofloxacin have also proved useful when combined with other therapies.

Even more exciting are clinical findings that have resulted from basic research in immune cells and cytokines, the proteins that promote or inhibit inflammation. NIDDK-supported researchers have had significant success treating patients with antibodies that block the action of pro-inflammatory cytokines such as Interleukin 10 and tumor necrosis factor alpha (TNF-alpha). NIDDK supported research on animal models of IBD resulted in the development of infliximab (Remicade™), an antibody against TNF and the first drug approved by the Food and Drug Administration for Crohn's.

NIDDK-funded researcher Dr. Stephan Targan found that cA2, another antibody to TNF-, is effective in treating moderate to severe Crohn's as well as the abnormal channels in the gut called fistulas that are a complication of Crohn's. Drs. Rummele, Targan, and colleagues also developed a new, highly specific diagnostic blood test that allows physicians to diagnose IBD in children, who can't always be specific about their complaints.

NIDDK-supported scientists are also learning more about factors that protect the lining of the gut and encourage healing. Trefoil peptides are a family of proteins that appears to protect against gastric injury by reinforcing the mucus gel that defends intestinal tissue. Researchers studying these proteins in mice have found that trefoil proteins increase after gastrointestinal injury, and that when added to wounded intestinal tissue, they promote healing. Understanding the action of these proteins may suggest other solutions to IBD injury. Other factors, such as growth hormone, may also help promote healing in IBD-damaged tissue.

NIDDK-supported geneticists are trying to identify the genes that make a person susceptible to IBD and its environmental triggers, as well as molecules that influence the initiation and progress of disease. The PPAR gamma gene, for example, helps regulate which genes are

turned on and off in a cell. In the large intestine, researchers have found that PPAR gamma can inhibit proinflammatory cytokines, and experiments in mice suggest that PPAR gamma may be another component of better therapy. An NIDDK pilot clinical trial is pursuing this possibility.

Researchers studying trefoil proteins in mice have found that when added to wounded intestinal tissue, they promote healing.

Because researchers have identified multiple genetic locations apparently linked to development of IBD, they believe they will eventually find there is more than one underlying defect responsible for disease development.

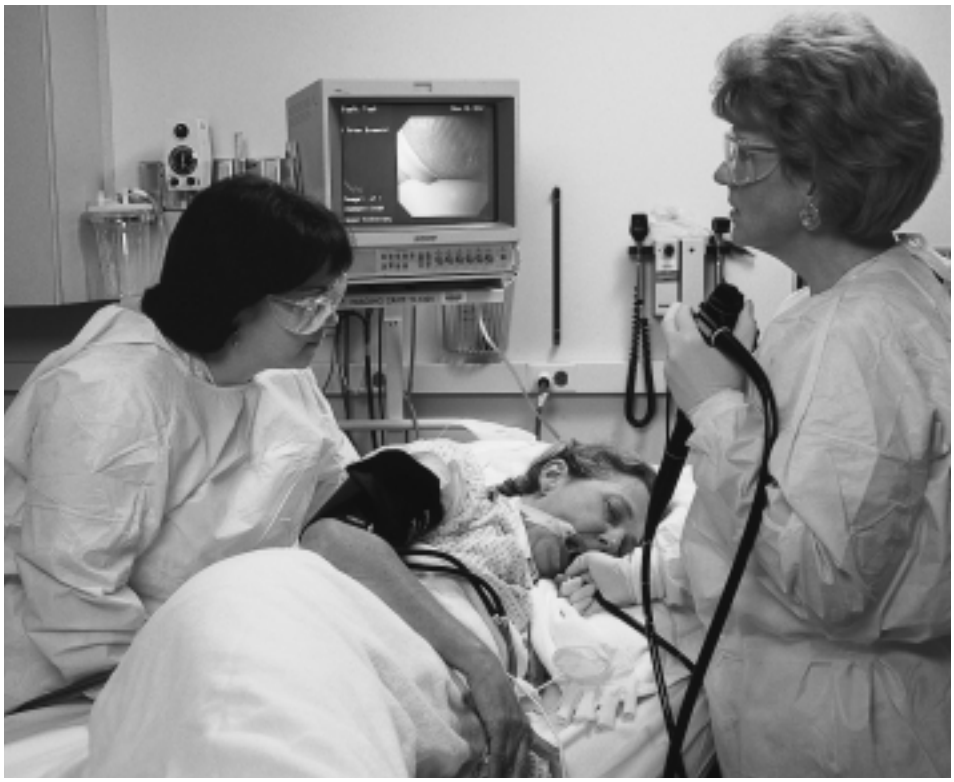
At the leading edge of the 21st century, NIDDK supports three Digestive Diseases Centers devoted to IBD, and is moving forward with plans for a clinical trial network for IBD, and a consortium on the genetics of Crohn's and colitis. The Institute will also invest new millions in coming years to understand the mechanisms of autoimmune diseases, such as IBD.

Helicobacter pylori and Ulcer Disease

For decades, the medical community believed that acid, stress, alcohol, and diet caused ulcers. In 1982, however, that changed, when researchers J. Robin Warren and Barry Marshall isolated a new bacterium named *Helicobacter pylori* (*H. pylori*) and showed that it was the culprit behind gastritis and stomach ulcers. Before and since this discovery, NIDDK has played a pivotal role in the basic and clinical advancements made in how we understand and treat *H. pylori.*

H. pylori infection is common in the United States, affecting about 20 percent of people under 40 and half of people over 60. However, only 15 percent of those infected will develop ulcers. Researchers are not certain how people become infected with *H. pylori* but they think it may be through food or water. Pain is the most common symptom of *H. pylori* and ulcer disease. Other symptoms of ulcer disease include weight loss, bloating, burping, nausea, and vomiting.

Prior to NIDDK's Consensus Conference on *H. pylori* in 1994, drugs called H-2 blockers, which disrupt the release of histamine in the body and prevent acid production in the stomach, were the standard ulcer treatment. However, the body of knowledge presented at the 1994 Consensus Conference moved the NIH panel to conclude that the link was strong enough between *H. pylori* and ulcers to recommend that ulcer patients infected with the bug receive antibiotics. *H. pylori* was initially associated with 80 percent of stomach ulcers and about 90 percent of duodenal ulcers, but antibiotic treatment has reduced peptic ulcers associated with *H. pylori* infection to 75 percent. In addition to antibiotics, doctors treat ulcers caused by *H. pylori* with acid blockers, proton pump inhibitors, and bismuth subsalicylate.



Patient undergoes endoscopy to evaluate abdominal pain.

Infection with *H. pylori* appears to cluster in families. To understand *H. pylori*'s physiology and how it becomes active, researchers have completely sequenced the genes for *H. pylori*. The complete genome sequence will also help researchers develop vaccines that may prevent *H. pylori* infection.

Researchers are trying to determine whether a person's genes or exposure to an infectious agent contributes to the spread of *H. pylori*. Cigarette smoking, alcohol abuse, and emotional stress are also considered risk factors for *H. pylori* and peptic ulcer disease. Researchers supported by NIDDK are also exploring the role urease, an enzyme produced by *H. pylori*, plays in creating an environment in the stomach that allows the organism to persist and alter the protective qualities of stomach acid.

NIDDK will continue to develop animal models for ulcer disease, and to support research that clearly identifies protective immune responses to infection and what determines the virulence of the infection, as well as work in bacterial genetics and antibiotic resistance.

NIDDK-supported researcher Thomas Starzl discovered that a combination of prednisone and azathioprine prevented rejection. He was able to perform the first successful human liver transplant in 1967.

Transplantation

The advent of organ and tissue transplantation is one of the most remarkable medical innovations of the last century. Researchers who first attempted organ transplantation in dogs in the mid-1950s ran head on into one of the main obstacles to a successful outcome: the immune system's unequivocal rejection of tissue it identifies as foreign.

Researchers performed the first kidney transplant in 1954 in identical twins whose shared physiology made it unnecessary to match antigens in the blood and suppress any immune response.

NIDDK-supported researcher Dr. Thomas Starzl, at the forefront of many of the landmark advances of the last 50 years, discovered that a combination of prednisone and azathioprine prevented rejection, and further, that corticosteroids could reverse acute rejection. With this knowledge, he was able to perform the first successful human liver transplant in 1967. Over the next dozen years, physicians sought improvements in preservation of donated organs, surgical techniques, and immunosuppressive agents, but transplantation remained an experimental procedure.

Immunosuppressive Therapy

In the late 1970s, British researchers led by Sir Roy Calne demonstrated the effectiveness of cyclosporine. U.S. clinical trials began in 1979, and FDA approval followed in 1983. The success of cyclosporine, used in combination with steroids, produced a tidal wave of organ transplants. The 1983 NIH Consensus Conference sponsored by NIDDK was a watershed event that confirmed liver transplantation as accepted and standard care for patients

with liver failure. This confirmation not only increased physicians' recognition of the need for liver transplants, but for the supporting infrastructure to identify, retrieve, and distribute organs to waiting patients as well.

The National Organ Transplant Act of 1984 created the Organ Procurement and Transplantation Network. Soon after, the Federal Government contracted with a private, nonprofit group, the United Network for Organ Sharing (UNOS), to develop and implement national policies for transplantation.

By 1988, 16,000 people in the United States had received solid organ transplants. Although the percentage of patients surviving transplantation and the median duration of survival increased following the introduction of cyclosporin, it also had drawbacks, notably potential kidney failure. Nevertheless, by 1994, there were some 277 transplant centers around the country, and despite a shortage of donated organs, more recipients were being given extra years of life.

Other immunosuppressive drugs came into use as alternatives to cyclosporine, and surgical procedures improved. Starzl pioneered the use of tacrolimus (FK506) in 1989, which gave physicians new options, particularly combined with other drugs. The FDA recently approved mycophenolate mofetil (MMF) as a combination therapy in kidney transplantation. Monoclonal antibodies such as nuromonab-CD3, OKT3, anti-CD4 MAb, rapamycin, and sirolimus began to be used in patients who couldn't tolerate steroids.

Immune Modulation

Now, as the 21st century dawns, many feel that immunosuppression has reached its useful limits. In its current form, immunosuppressive therapy is not selective and must be used chronically. Researchers have begun to focus instead on selectively blocking immune system action in transplantation using novel agents.

Options for transplantation took a major leap when scientists discovered the co-stimulatory pathway in T cell regulation. T cells are the part of the immune system designed to protect the body from foreign invaders. The T cell first recognizes the invader, and then a second step, called a co-stimulation, initiates the production of killer T cells that attack the invader. NIDDK's Dr. David Harlan and Naval surgeon Dr. Allan Kirk showed in nonhuman primates that it might be possible to re-educate the immune system to accept new organs instead of suppressing it. Harlan and Kirk showed that two proteins, CTLA4-IG and 5C8, that block the co-stimulatory pathway preserve a transplanted kidney. With colleagues at the University of Miami, Harlan and Kirk subsequently confirmed their work on kidney transplants using an islet transplant model.

These breakthroughs led to collaboration among NIDDK, the Navy Medical Research Center, Walter Reed Army Medical Center, and the University of Miami to achieve immune tolerance for kidney transplants and for transplantation of pancreatic islets, which could potentially cure people with type 1 diabetes. The NIDDK/Navy Transplantation and Autoimmunity Branch, under Harlan and Kirk, began transplanting patients in 1999. (See Edmonton Protocol, page 13.)

A Shortage of Organs

The Federal Government initiated a nation-wide effort to increase organ donation in 1997. The NIH and other Federal agencies convened concerned experts in 1998 to develop ways of meeting the critical shortage. In 2000, there are three patients waiting for every liver donated from cadavers in the United States. Because hepatitis C is a major reason for liver transplantation in the U.S., one NIDDK-supported effort aims at developing pre-emptive therapy for HCV and careful identification of patients who have a good chance to survive following transplantation.

Until recently, surgeons had no alternatives for patients waiting for organs, especially livers. But in the early 1990s, surgeons in Japan and Hong Kong performed living-donor transplants of the liver in adults. Prior to this discovery, NIDDK-supported surgeons performed partial liver transplants from adults to children, which requires removing a much smaller piece of the donor's left lobe. With the adult-to-adult transplant, the right lobe of the donor's liver, which makes up 60 percent of the organ, is surgically removed and transplanted in the recipient. Living donor livers are more difficult to transplant than a liver from a cadaver because blood vessels and bile ducts must be divided between the donor and recipient, instead of all being given to the recipient.

Disparities Among Minorities

As transplantation therapy has become more viable, it is increasingly apparent that, along with other disparities in health care, minorities have even fewer chances of getting a good match for a kidney or a liver than Caucasians do. NIDDK began the Minority Organ Tissue Transplant Education Program several years ago to raise awareness among minorities of the great need and to encourage them to donate.

Challenges for the Future

The challenges of the new century include finding more organs or alternatives through transplantation biology, which NIDDK will emphasize in coming years. Besides increasing use of living donor organs and exploring split liver transplantation, researchers are exploring the use of animal organs, or xenotransplantation. In addition to overcoming the species transplant barrier, serious concerns regarding endogenous animal viruses must be addressed before xenotransplantation can be considered in humans.

Other avenues to consider include the transplantation of stem cells, the bioengineering of cells, and gene therapy.

A second major challenge is to find ways to sustain healthy function of the transplanted organ in the long term. Despite the availability of a wider array of immunosuppressive drugs, chronic rejection is a significant cause of later loss of heart, kidney, and lung grafts. Recurrence of an underlying disease such as hepatitis C can compromise the success of liver transplantation. As solid organ transplantation has moved from an experimental procedure to standard care throughout the United States, some patients have lived for as many as 20 years with a transplanted organ.

Nevertheless, the drugs that suppress the immune system so that a transplanted organ can survive can contribute to additional illness, such as kidney failure, high blood pressure, heart disease, new or recurrent cancer, and other complications. NIH research on immune tolerance will maximize the success of organ transplantation. It holds the promise of overall health for transplant recipients that is the goal of every researcher involved in this pursuit.

More Information About Digestive Diseases

Visit NIDDK at http://www.niddk.nih.gov or ask for a list of publications from the Institute's National Digestive Diseases Information Clearinghouse, 2 Information Way, Bethesda, MD 20892-3580, 1-800-891-5389.

Kidney Diseases

To keep blood clean and chemically balanced, the kidneys process 200 quarts of blood every day. From that, the kidneys return to the blood precise amounts of sodium, phosphorus, and potassium to ensure the right chemical balance and send about 2 quarts of waste and extra water as urine to the bladder. The kidneys also produce two important hormones: erythropoietin, which tells the bone marrow to make red blood cells; and renin, which regulates blood pressure. The kidneys also convert vitamin D to an active form. Vitamin D promotes intestinal absorption of calcium for bones and the body's chemical balance.

When a person has kidney disease, wastes and fluid build up in the body, causing nausea and fatigue; blood pressure soars; bones become thin and brittle; and anemia and malnutrition develop. Children who have kidney disease don't grow or develop normally, and they have greater risks from treatments to prevent rejection of transplanted kidneys.

Most kidney damage happens slowly and silently over years or even decades before the disease becomes obvious. People with a family history of any kind of kidney problem, the elderly, and

Kidney Disease in America

- **20,000 babies born each year with kidney problems**
- **Highest incidence and prevalence in minorities**
- **Major risk factor for heart disease and stroke**

minorities are at higher risk for kidney disease. For example, in 1996 African Americans represented 29.8 percent of people treated for kidney failure but only 12.6 percent of the total U.S. population.

Poisons, trauma, and over-the-counter pain medicines may cause kidney disease, but the most common causes are diabetes, high blood pressure, glomerulonephritis, and polycystic kidney disease.

Diabetes and the Kidneys

Diabetes keeps the body from properly using sugar, effectively transforming this normally sweet substance into poison to the kidneys. Kidney disease of diabetes (KDDM), or diabetic nephropathy, was responsible for roughly 35,000 new cases in 1998. Keeping blood sugar levels down, controlling blood pressure, and taking medicines called ACE inhibitors (ACEi) can protect the kidneys, but some people get kidney disease anyway.

The use of ACEi is a relatively new and significant treatment advance resulting from research funded by NIDDK. Because some families appear prone to kidney disease from both type 1 and type 2 diabetes, NIDDK's Family Investigation of Nephropathy and Diabetes study is investigating genetic factors that may increase susceptibility to or severity of KDDM. Caucasian, Hispanic American, Native American and African American families are involved, including families from NIDDK's African American Study of Kidney Disease and Hypertension Trial (AASK). Read more about diabetes and its complications on page 13.

Dialysis and Transplantation Treatments for Kidney Failure

- **400,000 people were treated in 1998; 6,000 were children and young adults.**
- **85,500 new cases in 1998**
- **20 pecent annual mortality**
- **Direct health costs $17 billion a year.**

High Blood Pressure and the Kidneys

High blood pressure is both a symptom and a cause of kidney disease. Medication and dietary changes can help control blood pressure and prevent complications, and yet hypertension is the second leading cause of kidney disease in the United States and the leading cause in African Americans. High blood pressure was responsible for about 19,000 new patients and 25 percent of all people treated for kidney failure in 1998.

Research by an NIDDK grantee has suggested that lower than usual blood pressure may curb kidney disease, especially in African Americans who have protein in the urine. With support from the NIH Office of Research on Minority Health, NIDDK's African American Study of Kidney Disease and Hypertension Trial (AASK) is testing whether lower than usual blood pressure and specific classes of medicines, such as ACE inhibitors, beta blockers, and calcium channel blockers are better guardians for the kidneys. The calcium channel blocker arm of the study was halted early after a data and safety monitoring board found that participants with at least one gram of protein in their urine benefited more from the other two drugs.

Glomerulonephritis

Glomerulonephritis is actually a group of diseases in which the glomeruli, or filtering units of the kidneys, become inflamed, scarred, and damaged. Glomerular diseases such as lupus, IgA nephropathy, focal segmental glomerulosclerosis, and idiopathic nephrotic syndrome were responsible for about 9,500 new cases of kidney failure in 1998. NIDDK-supported research has found that P-selectin, a protein on cells lining blood vessels in the kidneys, may help protect these organs from inflammation. If studies confirm these early results in animals, P-selectin could be used to develop anti-inflammatory treatments for use in people. NIDDK's Chronic Renal Insufficiency Cohort Study is characterizing people with vari-

A healthy glomerulus

ous kidney diseases to identify factors that promote disease progression, cardiovascular disease, and other problems, information expected to benefit people with glomerulonephritis.

Polycystic Kidney Disease

Polycystic Kidney Disease (PKD) is a genetic disease characterized by the growth of many cysts in the kidneys. The disease is the fourth leading cause of kidney failure. There are two types of PKD. The most common, autosomal dominant PKD (ADPKD), is usually silent until adulthood, while the rare autosomal recessive form is found in newborn babies or those still in the womb. Complications include fatal brain aneurysms, mitral valve prolapse, enlarged heart, and cysts on the ovaries or testes. High blood pressure, urinary tract infections, and chronic flank or back pain are common.

In the 1990s, NIDDK grantees helped pinpoint two genes causing ADPKD and identified proteins produced by the genes. These discoveries are being used to search for effective therapies for PKD. Today, four NIDDK centers are working to identify and study animal models of human PKD; to locate the gene for ARPKD; and to understand how proteins, including those from the PKD1 and PKD2 genes, direct cell formation and misdirect it in PKD. The Institute is also funding centers to develop innovative imaging techniques to assess progression of PKD and to test potential interventions.

Kidney Failure

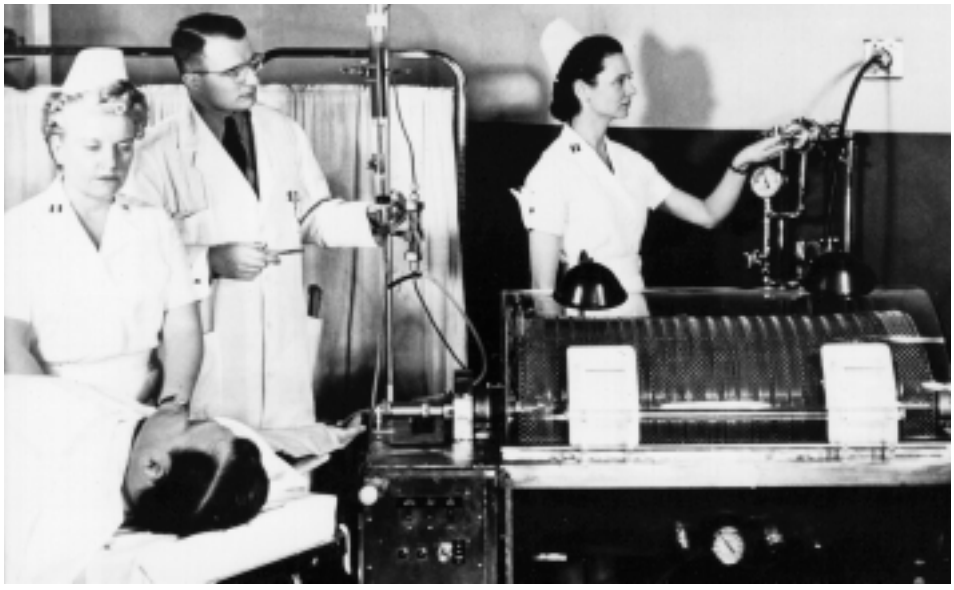
While the exact point at which permanent kidney failure occurs varies among individuals, the general rule of thumb is that a transplant or dialysis is needed once levels of the metabolic waste, creatinine, reach 8 to 10 mg/dl in the blood. Untreated kidney failure may lead to seizures or coma and ultimately results in death. In 1998, 398,000 people experienced kidney failure and needed dialysis or a transplant to stay alive, more than double the number requiring such treatment 10 years before. The average annual per-patient cost of treating kidney failure is $43,000, or more than $16.7 billion a year, which is borne largely by the Federal Government.

Leading Causes of Kidney Failure

- **Diabetes**
- **Hypertension**
- **Glomerulonephritis**
- **Polycystic Kidney Disease**

Dialysis. When NIDDK was established in 1950, the number of people living with kidney failure was small—dialysis was not available, so death for most was imminent and certain. In contrast, in 1998 more than 246,000 people relied on dialysis at least three times a week to clean waste and excess fluid from their blood. Since dialysis has become available, the kidney is, in fact, the only vital organ for which there is a life-sustaining alternative other than organ transplantation. There are no long-term substitutes for failed hearts or livers.

Hemodialysis was the first and is still the most common form of dialysis. It uses a machine to remove blood from the body, filter out wastes and extra fluid, and return the newly cleaned blood to the body. Early dialysis and "artificial kidneys" or filters were crude and little



Early dialysis

more than washing machines. Some were round, some were flat. They contained sheets of cellophane (originally used as sausage casing) over which the patient's blood passed and through which waste passed out. Surgery was required for each treatment, which could last 12 hours, and removed so much blood (about 25 percent) at one time that patients needed blood transfusions.

NIDDK played a major role in developing and improving dialysis, a modern miracle to those whose lives it has saved. In 1960 an NIDDK grantee developed a "no-stick" Teflon shunt, the first effective device allowing repeated access to a patient's veins and arteries. This simple but revolutionary "vascular access" also reduced blood clots during dialysis. NIDDK also funded much of the basic research by nephrologists, hematologists, bioengineers, and biochemists that led to the development of smaller dialysis filters, reducing both the amount of blood withdrawn and the need for blood transfusions. Filters are now about the size of a can of tennis balls, and hemodialysis takes about 4 hours.

The development of a Teflon shunt by an NIDDK grantee allowed access to a patient's veins and arteries and reduced blood clots during dialysis.

Today, doctors participating in NIDDK's Hemodialysis Study are trying to improve on the treatment by testing whether giving more dialysis and using special filters to remove more potentially harmful substances will improve patient health and survival. Despite improvements in medical management, problems with infection and clotting of vascular accesses continue to complicate the delivery of dialysis, accounting for an estimated 25 percent of hospital visits and about 20 percent of the expense for hemodialysis patients. Consequently, NIDDK has initiated the Hemodialysis Vascular Access Clinical Trials Consortium to investigate ways to reduce complications associated with hemodialysis access sites. The Institute is also planning a new study to identify what suppresses appetite in people on dialysis and whether nutritional supplements, including protein, will reverse malnutrition.

Contemporary dialysis

Transplantation. Few people know that the kidney was the first organ to be experimentally transplanted in humans and laid the early groundwork for making other organ transplants possible. The kidney was an ideal subject for early studies of transplantation because patients could resort to dialysis if needed, and no similar life-saving option was available to people with disease in other major organs. NIDDK-funded research on kidney transplantation led to the development of the first successful treatment regimen—azathioprine combined with steroids—to protect against immune system attacks on the transplanted organ. This drug combination produced significant leaps in patient survival, and is still used today. Read more about transplantation beginning on page 28.

More Information About Kidney Disease

Visit NIDDK at http://www.niddk.nih.gov or ask for a list of publications from the Institute's National Kidney and Urologic Diseases Information Clearinghouse, 3 Information Way, Bethesda, MD 20892-3580, 1-800-891-5390.

Urologic Diseases

Benign Prostatic Hyperplasia

As men age, it is common for the prostate gland to enlarge, a condition called benign prostatic hyperplasia (BPH). It is not cancer, but can cause incontinence, or urine leakage; inability to urinate; infections; bladder stones, and, rarely, kidney damage. In the United States alone, 375,000 hospital stays each year involve BPH.

NIDDK supported much of the basic research that led to understanding testosterone's role in prostate growth and the ultimate development of finasteride (Proscar™), the first drug approved to treat the symptoms of BPH. NIDDK extended that early work to the Medical Therapy of Prostatic Symptoms Trial, a clinical study testing whether finasteride and another drug, doxazosin (Cardura™), can stop prostate growth and reduce the need for surgery. The answer is expected in the spring of 2002.

More recently, the increasing use of plant-based products such as saw palmetto for prostate problems has skyrocketed, heightening doctors' concerns about the lack of data on effectiveness and potential risks to the men taking these products. An estimated 20 percent of men who finally go to urologists are already using plant therapies. NIDDK and NIH's National Center for Complementary and Alternative Medicine will be collaborating on an initiative to study over-the-counter products that are touted for prostate health.

Kidney Stones

Kidney stones, one of the most common and most painful problems of the urinary tract, are hard masses of crystals that have separated from the urine and built up on the inner surfaces of the kidney or bladder. More than 1 million cases were diagnosed in 1996. About 10 percent of people in the United States will have a kidney stone at some point in their lives. Whites, men, and people between the ages of 20 and 40 are more likely to get a kidney stone, and once one develops, others are likely to follow.

NIDDK funding has led to a better understanding of many factors that promote stone formation and improved treatments. In the 1970s and 1980s, an especially productive NIDDK-funded investigator, C. Y. Pak, identified 16 separate metabolic causes of stones, developed selective treatments for each, and set a record for developing orphan drugs approved by FDA. Among the endocrinologist's credits are potassium citrate for calcium oxalate stones, approved by FDA in 1984; and alpha-MPG (Thiola™) for cystine stones, approved in 1988. In 1993, the same researcher again provided new insights when he reported that consuming too much salt increased stone formation and that drinking orange juice had the opposite effect.

NIDDK grantees have helped identify genes for type I and type II primary hyperoxaluria, metabolic diseases that cause kidney stones, and found that a defect in the regulation of oxalate synthesis in the liver results in excessive oxalate levels in the urine. Other research has revealed a gene and its product responsible for the stone-forming disorder called X-linked hypercalciuric nephrolithiasis.

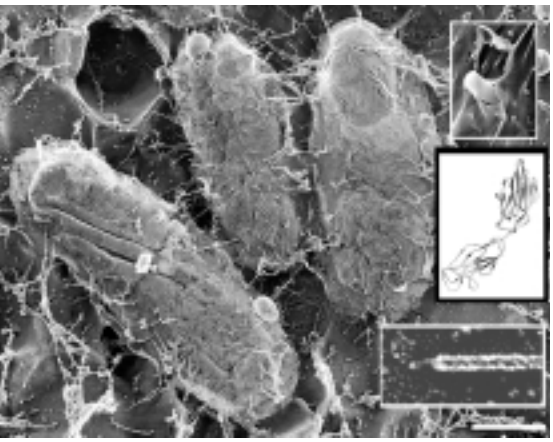
These significant insights into genetic susceptibility to kidney stones has prompted the Institute to encourage new research on hereditary calcium oxalate stone disease and the regulation of oxalate.

Chronic Pelvic Pain, Prostatitis and Interstitial Cystitis

Interstitial cystitis (IC) and prostatitis are syndromes characterized by chronic pelvic pain. The causes and optimal treatments for these persistent problems are unknown. Symptoms such as recurring discomfort or pain in the bladder and surrounding pelvic region, frequent urination, and painful sexual intercourse resemble a bacterial infection, but medical tests usually reveal no organisms in the urine and antibiotics are broadly ineffective.

About 90 percent of the people who have IC are women, whereas abacterial prostatitis is the most common genitourinary ailment in men, especially among those younger than age 50. Prostatitis accounts for an estimated 2 million visits to doctors each year.

NIDDK is supporting clinical studies for both IC and prostatitis. The IC Clinical Trials Group is doing a series of treatment studies that ultimately will help doctors recommend therapies most likely to relieve symptoms, while the Chronic Prostatitis Cohort Study is gathering detailed information about medical histories, possible risk factors, symptoms, treatments, and the results of blood, prostate fluid, semen, and urine tests. The cohort study is also focused



A specialized protein on *E. coli*'s thread-like arms, or pili (insets), helps these bacteria invade the bladder wall.

on recruiting minorities; exploring possible relationships between chronic prostatitis, urethral and bladder inflammation, and other chronic pelvic pain disorders such as IC; and planning studies to assess whether potential treatments actually reduce symptoms.

Previous epidemiologic studies have focused on highly specific populations and have not yielded reliable estimates of the impact of these disorders. NIDDK has initiated a study to better estimate the prevalence, health impact, quality of life, and use of health resources for chronic pelvic pain of the bladder, including IC and chronic prostatitis.

Bladder Infections in Adults

Infections of the bladder are responsible for about 9.6 million doctor visits each year. Women, men who have an enlarged prostate, and anyone with diabetes, a urinary catheter, a kidney stone or blockage are especially prone to this problem. Left untended, infections can be difficult to treat and can spread to and damage the kidneys.

Work by NIDDK grantees is identifying factors that increase the likelihood of getting infections. At least some women may get infections over and over when harmful bacteria sneak past a special protein called uroplakin, which is designed to ward off microbes such as *E. coli*. Uroplakin coats the top layer of bladder cells and helps them die and drop off when under attack. Researchers have found that infection-causing *E. coli* use thread-like arms to reach past the uroplakins for a more secure hold on the bladder wall beneath. In addition, spermicides have been found to increase the risk of bladder infections, at least in part by decreasing the number of good bacteria.

Other investigations are focusing on preventing infections. Researchers funded by NIDDK are testing oral, vaginal, and injected forms of an experimental vaccine that may help patients mount their own defense against bladder infections by producing antibodies that can later fight bacteria.

While bladder infections are usually easily treated with antibiotics, in women with diabetes these infections are not so easily resolved and can alter metabolism and glucose control. Studies suggest that bladder infections are more common, more severe, more difficult to treat, and more often lead to kidney infections in women with diabetes compared to nondiabetic women. NIDDK is initiating a network of centers that would test treatment regimens and determine whether these measures improve glycemic control.

Blood Research

Iron Overload

Too much iron, or iron overload, is a problem for an estimated 50,000 people in the United States who need regular blood transfusions to treat two genetic disorders: Cooley's anemia and sickle cell disease. The small intestine usually controls the amount of iron absorbed into the blood, but transfusions bypass this safety mechanism and route iron-rich blood straight into the bloodstream. To rid the body of extra iron, which damages the liver, heart, and pancreas, patients use a drug that attaches to iron called a chelator, helping it escape from body cells and pass into stool and urine. Unfortunately, the standard chelator, desferoxamine (DFO), is expensive and has effects patients don't like.

For years researchers have been searching for an oral alternative to DFO. NIDDK-funded researchers may have found a better treatment and are performing the necessary preliminary studies in a small number of people before considering wider use. Studies in animals have shown that the drug, HBED or hydroxybenzylethylenediamine diacetic acid, removes up to 3 times more iron than DFO. HBED still must be injected under the skin, but the researchers are hopeful that its increased effectiveness over DFO will mean that one quick injection two or three times a week will do the job. Because HBED is man-made, it will not only be cheaper to produce but also less likely to provoke allergic responses, increasing patients' willingness to use it.

Although iron is required for the function of hemoglobin, DNA synthesis, and a host of other processes, scientists still do not fully understand exactly how iron passes through cell membranes or how cell levels of iron are determined. However, recent genetic discoveries by NIDDK-supported researchers have shed light on the regulation of iron metabolism.

Iron is taken up and transferred to the blood mainly by the duodenum, a part of the small intestine. The duodenum can increase iron absorption when the body iron levels are depleted, or reduce iron uptake when the body has excess iron. Thus, release of iron from the duodenum into general circulation is tightly controlled. In 1997, NIDDK-funded investigators studying a mouse mutation found *DMT1*, a gene that codes for the transport protein responsible for absorbing iron from the food passing down the intestinal tract into the cells of the intestine. In 1998, another NIDDK-supported investigator found SFT, or Stimulator of Fe Transport. This protein seems to increase the ability of the cells to take up circulating iron. The next year, an NIDDK-supported investigator discovered the protein Hephaestin, which appears to help the movement of iron from the intestinal cells into the blood circulating through the intestine, but does not actually transport the iron. Finally, early in 2000, researchers found the gene for the transporter protein by studying mutations in zebrafish, an important model animal. The protein produced by the gene called *ferroportin1* actively transports iron from the intestinal cells to iron-binding proteins in the blood.

From these findings, scientists hope to develop therapeutic strategies to manipulate iron uptake and iron concentrations at the cellular level. Such interventions could help patients with hemochromatosis and other iron overload disorders.

NIDDK-funded researchers were the first to try manmade erythropoetin in people. The FDA later approved its use for the severe anemia of kidney disease.

Anemia

A condition in which the blood does not contain enough oxygen-carrying red blood cells, anemia is common and serious in people with kidney disease. In the 19th century, scientists suspected an association between kidney disease and anemia, but researchers did not discover the connection until modern times. Doctors now know that the kidneys not only filter waste from the blood but also produce erythropoietin (EPO), a crucial hormonal switch that triggers the bone marrow to produce red blood cells.

EPO's story began back in the 1950s when scientists discovered the hormone and its function. But researchers didn't learn where EPO came from or why people with kidney disease were anemic until 1957, when a blood specialist, Dr. Leon Jacobson, and colleagues at the University of Chicago traced EPO to its origin—the kidneys—cementing the connection between anemia and kidney disease.

The next leap came in the 1970s from work funded by NIDDK at the University of Washington in Seattle. Blood diseases specialist Dr. John Adamson and kidney specialist Dr. Joseph Eschbach developed a process to separate EPO-rich plasma from the blood of healthy sheep. By 1980, they and another group showed that EPO raised hematocrit levels, a measure of anemia, in sheep with kidney disease. Eschbach and Adamson predicted that EPO would be an effective treatment for people who were anemic because of kidney disease, but there was no source for the hormone.

Help arrived from two groups of researchers. One determined EPO's amino acid composition, key information that allowed a second team to identify the EPO gene and produce larger quantities of the hormone.

With the shortage solved, the pace of research accelerated. In 1985, Eschbach and Adamson were the first to try the manmade hormone in people. They treated only 25 dialysis patients, but 12 no longer needed transfusions to replace oxygen-deprived blood, results that supported their earlier optimistic predictions. Larger studies followed and confirmed EPO's effectiveness, and in 1989 FDA approved the hormone's use for the severe anemia of kidney disease.

Anemia that once required high-risk blood transfusions now can be treated much more effectively and easily, significantly improving the lives of people on dialysis. And while NIDDK-supported scientists and others worked for decades to discover and understand EPO and to apply that knowledge to people with kidney disease, the fruits of these labors have extended to people with other diseases. Erythropoietin is also now used to treat anemia in premature infants and in people with cancer, rheumatoid arthritis, and AIDS.

Division of Intramural Research

Since its inception in 1950, NIDDK has put the focus on basic research—with stellar results. The Institute has supported 25 Nobel laureates, 7 of whom have worked in the Division of Intramural Research (DIR). Eight current intramural investigators are elected members of the National Academy of Sciences, one of the highest honors given to those who take innovative approaches to research. NIDDK's cadre of young scientists includes several who are already world leaders in their fields.

DIR's biologists, physicians, chemists, physicists, and mathematicians tackle fundamental questions about the workings of organisms and run clinical studies in the diseases covered in NIDDK's mandate. They also train scientists and physicians in the Institute's laboratories and at NIH's clinical facilities in Bethesda, Maryland, and at the Phoenix Epidemiology and Clinical Research Branch in Arizona. Many scientists who received their first training at NIDDK now lead prominent programs in the United States, Europe, and Japan.

Nearly 100 principal investigators direct studies in the 11 branches and 10 labs that make up DIR. Most branches focus on basic and patient research in diabetes, bone metabolism, endocrinology, hematology, digestive diseases, kidney and urological conditions, and genetics. One branch specializes in mathematically modeling biological functions such as the production of insulin by cells in the pancreas.

At the Phoenix Epidemiology and Clinical Research Branch, scientists use genetic and clinical methods to identify the causes of type 2 diabetes and obesity and their complications. The researchers work closely with the Pima Indian community, which has the highest rate of type 2 diabetes in the world.

"When you have people of this quality, you give them freedom and you let them go where their research will lead them….We simply let minds explore."

—Dr. DeWitt Stetten, Jr.

The Institute's newest branch is an unusual collaboration of scientists from NIDDK, NIH's Clinical Center, the Navy Medical Research Center, Walter Reed Army Medical Center, and the University of Miami's Diabetes Research Institute. Established in 1999, the Transplantation and Autoimmunity Branch seeks ways to blunt or prevent autoimmune diseases like type 1 diabetes, improve the transplantation of insulin-making pancreatic islet cells into patients with type 1 diabetes, and increase the long-term survival rate for recipients of transplanted kidneys and pancreases.

Researchers from the branches transfer their knowledge and technologies to practicing physicians. Many standard methods for the diagnosis and treatment of certain tumors of the pituitary, parathyroid, and thyroid glands and of pancreatic islets are based on the work of intramural scientists. NIDDK intramural researchers also developed the current regimen for the treatment of kidney disease associated with lupus.

The NIDDK labs focus more on projects that are further removed from the clinic. Several researchers in the labs create innovative methods of x-ray crystallography and nuclear magnetic resonance spectroscopy, techniques that use x-rays and radio waves, respectively, to study the structures of proteins and other biologically significant molecules.

Other investigators learn what makes living things tick. They ask broad questions: What do organisms do to repair their own DNA? How do humans and animals produce millions of infection-fighting antibodies from a handful of gene segments? Which paths do molecules take to get from point A to point B in a cell? Are there similarities between faulty proteins in yeast and those associated with "mad cow" disease? What can fruit flies tell us about sex determination in people? Knowing such fundamentals can explain the abnormalities that underlie human diseases and make it possible to develop effective treatments when biological processes go wrong.

NIDDK intramural scientists have expertise in molecular, developmental, cell, and structural biology as well as biophysics, biochemistry, genetics, neuroscience, toxicology, and pharmacology. Many collaborate with scientists at other NIH institutes and in academia and industry.

Each year, new scientists join the Institute temporarily as visiting scientists or postdoctoral fellows. A few enter a tenure track that is similar to that found in universities. As in academia, NIDDK intramural scientists are tenured only after proving themselves capable of creating an effective, independent research program, a process that can take six to eight years. During this time,

The Pima Contribution to Medicine

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) began working with Pima Indian volunteers in the Gila River Indian Community in Arizona in the mid 1960s after a health survey to track rheumatoid arthritis revealed an astonishing rate of type 2 diabetes. Since that accidental discovery, NIDDK has shown that the Pimas have the highest prevalence of type 2 diabetes in the world. Over half of Pima Indians who are 35 and older have the disease. Pimas also develop diabetes much younger than people in other populations, and the number of Pima children with the disease is increasing.

With the support of many Pima volunteers and the Indian Health Service, NIDDK's Phoenix Epidemiology and Clinical Research Branch has studied the origin, development, and natural history of diabetes, its complications, and obesity for more than 35 years. NIDDK operates a diabetes clinical research center at the Hu Hu Kam Memorial Hospital in Sacaton, Arizona, and in Phoenix.

The Pimas' help has been invaluable in the study of diabetes, obesity, and kidney disease because the community is unique. Research conducted in Phoenix and Sacaton established that the Pima Indians have 10 times the prevalence of type 2 diabetes found in Caucasian populations, and the rate of kidney failure is 20 times greater than in the general U.S. population. Diabetes is the cause of the kidney failure in over 90 percent of the cases.

Studies in the Pima Indians made it possible to develop a formal diagnostic criterion for diabetes now used by the World Health Organization. Long-term studies showed that obesity and high levels of insulin in the blood were strong risk factors for diabetes. The high levels of insulin resulted from resistance of the body to insulin's effect on sugar uptake (insulin resistance), a hallmark of type 2 diabetes.

These long-term studies also demonstrated that diabetes and obesity run in families, developing from genetic, prenatal, and environmental influences. NIDDK research highlighted obesity's genetic complexity and clarified how metabolic rate and fat metabolism contribute to weight gain, a risk factor for type 2 diabetes.

Other work among the Pimas has helped scientists understand the role of insulin secretion and insulin resistance in the development of diabetes. In addition, doctors now recognize that high blood pressure predicts the complications of diabetes such as eye and kidney disease. Lowering blood pressure slows the onset and progression of diabetic kidney disease.

NIDDK scientists showed that children of mothers who are diabetic during pregnancy are at higher risk for obesity and diabetes than the children of nondiabetic mothers. Female children are then more likely to have diabetes by the time they reach their childbearing years. Because of these findings, pregnant women in the community and elsewhere are routinely given glucose tolerance tests so that any increases in blood sugar can be controlled before a baby is born. Within the Pima community, there has already been a 50 percent reduction in the death rates among newborns of diabetic mothers.

By eating well, Pima Indian children may lower their diabetes risk.

More recently, NIDDK researchers have shown that breast-feeding exclusively for the first two months of life significantly lowered the rate of type 2 diabetes among Pimas.

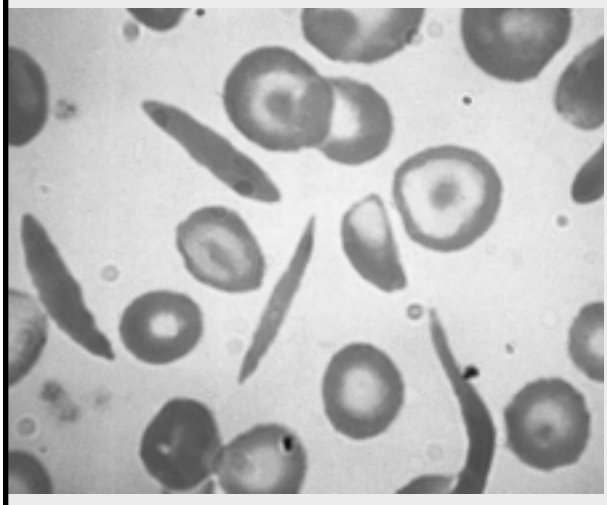
Current research focuses on studies of food intake and the search for genes that predispose a person to obesity, insulin resistance, and diabetes. The Pima, Zuni, and Navaho are also participating in the ongoing Diabetes Prevention Program. This multi-center clinical trial is testing the effectiveness of two approaches to preventing type 2 diabetes: intensive lifestyle changes in diet and exercise, or use of metformin, an oral medication. Nearly half of the 3,200 participants are members of U.S. minority groups. Minorities are more predisposed to type 2 diabetes.

Researchers hope their collaboration with the Pima community at Gila River will continue to produce insights into diabetes. The Pima Indians' generosity in volunteering year after year for research studies has already contributed to better health for all people. Meanwhile, NIDDK continues in its commitment to use the most current knowledge to prevent and treat diabetes within the Pima community. ■

From Lab to Clinic

Dr. Griffin P. Rodgers, chief of NIDDK's Clinical and Molecular Hematology Branch, became acquainted with sickle cell anemia in high school when two friends died from the disease. Today, he studies genetic diseases of blood and develops therapies to treat people suffering from them. He focuses on sickle cell disease and the various types of thalassemias, which are most common in people of African, Mediterranean, Middle Eastern, and Southeast Asian ancestry. These diseases are caused by different genetic defects in hemoglobin, the oxygen-carrying part of red blood cells.

For more than a decade, Rodgers has been evaluating the anticancer drug hydroxyurea in sickle cell patients, always looking for ways to make it more effective. In 1990, Rodgers and his colleagues demonstrated that the drug increases levels of fetal hemoglobin and relieves patients' anemia. With extended use, hydroxyurea also reduced pain and other debilitating symptoms of sickle cell disease for most people who took it. And in 1993, Rodgers led a joint effort by NIDDK, the National Heart, Lung, and Blood Institute, and the Johns Hopkins Medical School that showed how another drug called recombinant erythropoietin and an iron supplement could boost the effect of hydroxyurea in some patients who received all three. Now, Rodgers and his associates are conducting research to learn at what minimal dose erythropoietin, a costly medicine, can be effective. The U.S. Food and Drug Administration, in 1998, approved hydroxyurea's use for the treatment of adults with sickle cell disease.



Sickled red blood cells lose flexibility.

Adult hemoglobin is made of alpha and beta chains. The hemoglobin of people with sickle cell disease is flawed in the beta globin chain. The inherited defect causes hemoglobin molecules to polymerize, or clump up, in the red blood cells after oxygen is released. The clumping deforms red blood cells into sickle shapes that are too rigid to squeeze easily through blood vessels, thereby depriving cells of oxygen and leading to acute pain, organ failure, and death.

Hydroxyurea benefits sickle cell patients particularly by allowing them to sharply increase production of fetal hemoglobin, which interferes with the polymerization of the faulty hemoglobin. Fetal hemoglobin, the predominant form in early development, continues to be made but at quite low levels after birth. It does not contain beta chains and does not polymerize, and it carries oxygen more efficiently than adult hemoglobin.

Some thalassemia patients likewise have improved with hydroxyurea treatment. Rodgers is now leading a collaboration of researchers from the United States, China, Thailand, Israel, Greece, and Italy to learn whether people whose thalassemia is caused by different genetic mutations will respond differently to hydroxyurea treatment.

To help people with alpha thalassemia, Rodgers' lab developed a technique to recognize the most common forms of the disease. Normally, people inherit four alpha-chain genes—two from each parent. Anywhere from one to all of them may not be passed on. Individuals missing one to three of their alpha-chain genes are frequently misdiagnosed with iron deficiency anemia and are inappropriately treated with iron supplementation for extended periods. When all four genes are missing, fetuses die in the womb, and their mothers can develop potentially fatal preeclampsia. Rodgers hopes the test will lead to earlier identification of women of Southeast Asian descent who are most at risk for this complication. The Mayo Clinic is now evaluating this new diagnostic approach.

Rodgers' ultimate goal: gene therapy targets for sickle cell disease and beta thalassemia, a disease where people produce insufficient levels of beta chains. He works with Dr. John Tisdale, a new tenure-track scientist who studies stem cells that give rise to all kinds of blood cells. They and their colleagues remove stem cells

Dr. Griffin P. Rodgers

from animals with sickle cell disease, introduce a gene that corrects the condition, and return the altered stem cells to animals. They hope that the altered stem cells will then produce sufficient levels of blood cells with the corrected gene.

To eventually bring the treatment to people, the Hematology Branch will collaborate with Memorial Sloan-Kettering Cancer Center in New York and other sites. Initially, they will define the optimal approach for carrying healthy human globin genes into the cells of animal models of sickle cell anemia and beta thalassemia. ■

NIDDK and NIH senior researchers and the Board of Scientific Counselors, an external group of experts, review the tenure-track scientists' progress. To maintain the Institute's high standards for research, the Board of Scientific Counselors also evaluates the accomplishments of previously tenured investigators every four years.

Pursuing the Unknown

Traditionally, intramural scientists have been free to pursue lines of promising research, often in areas where little was known. The premise is that unexplored avenues are the most likely to be fruitful.

Dr. DeWitt Stetten, Jr., the Institute's first director of intramural research, believed that "The greatest return will be secured if the mature scientist is allowed and encouraged to select the problems on which he will work."

Over the years, the returns have been abundant. NIDDK intramural investigators have discovered enzymes that control DNA's behavior and cellular receptors that bind hormones such as insulin and insulin-like growth factors. They've synthesized thousands of chemicals, some of which are used to understand the brain's response to addictive drugs and others that might someday treat people with diabetes, retinopathy, and asthma. National Academy member Dr. John Daly has long been a world leader in the pharmacological study of natural substances from animals and plants. Epibatidine, one of his more famous finds from the skin of a poisonous South American frog, is 200 times more potent than morphine.

Dr. Kenner Rice, chief of the Laboratory of Medicinal Chemistry, invented the NIH Opiate Total Synthesis, a technology capable of large-scale production of synthetic opiates critical for patient care and research. The process makes it possible to use petrochemicals as the starting material for morphine derivatives normally produced from opium poppies.

Recent inventions include a novel imaging device to look at cell samples that was created by Dr. Ira Levin and colleagues. Dr. T. Jake Liang and coworkers developed a method to produce hepatitis C virus-like particles that could be useful in diagnostic kits and vaccines against the disease.

National Academy member Dr. Gary Felsenfeld found the first vertebrate insulators, elements that buffer genes from other regulators. An insulator he derived from chickens is used by biotechnology companies to produce transgenic animals. When a gene that produces a desirable protein is introduced into a transgenic animal, the gene stays active if it has the insulator on each side. An element of this insulator also has been identified as critical in controlling certain genes that are "imprinted." Imprinting is a necessary biological function in which either the maternally or paternally inherited copy of some genes is switched off.

In keeping with government policy, many NIDDK discoveries have been patented to encourage industry to develop them for the medical and research communities. Since 1970, NIDDK scientists have been named as inventors on 131 patents issued to the U.S. Department of Health and Human

Services. Many of the patents have been licensed to various companies, and royalties have been paid to the NIH.

On the genetic front, the scientific community has traveled light years since the establishment of NIDDK's predecessor, the National Institute of Arthritis and Metabolic Diseases, in 1950. Long before scientists could link a disease to a specific gene, intramural scientists and physicians were developing screening techniques to identify inherited metabolic disorders like phenylketonuria, which can lead to

Computers speed molecular studies.

mental retardation if untreated, and Lesch-Nyhan syndrome, which causes brain damage and early death.

Today, NIDDK scientists hunt genes and make animal models to better understand a wide variety of conditions caused by defects in DNA or proteins. Institute director Dr. Allen Spiegel, Dr. Stephen J. Marx, and colleagues in NIDDK, the National Human Genome Research Institute, and the National Cancer Institute co-discovered the gene for multiple endocrine neoplasia type 1. People with mutations in the MEN1 gene develop benign tumors in the parathyroid and pituitary glands or tumors in islet cells that can lead to pancreatic cancer.

Genes help scientists get to proteins.

Researchers in the Diabetes Branch have linked specific genetic defects to particular kinds of insulin resistance. To learn more about lipoatrophic diabetes, a rare condition where people lack fat deposits and develop the symptoms of type 2 diabetes, Dr. Marc Reitman studies genetically altered mice that lack fat cells. Lipoatrophy is paradoxical: The lack of fat cells causes diabetes. This trait makes the mice a good model for comparison to animals whose diabetes is a result of obesity.

Dr. Lothar Hennighausen, chief of the Laboratory of Genetics and Physiology, has identified genes that control development of the mammary gland during pregnancy. He and NIDDK scientist Dr. Chuxia Deng collaborated to breed mice that are missing BRCA1, a gene that increases human susceptibility to breast and ovarian cancer, and Hennighausen has created the Mammary Genome Anatomy Project. MGAP (http:mammary.nih.gov/mgap/) aims to discover and isolate genes that operate during normal breast development and tumor progression. In Dr. Richard Proia's lab, the researchers have developed mice that have Tay-Sachs disease, a fatal disorder caused by an enzyme that works inefficiently. Children who have the inherited disease often die by age 5. Lipids accumulate in the brains of people with the faulty enzyme. Proia uses the mice to test an agent that inhibits the lipid's production and accumulation with the aim of developing a treatment for people with Tay-Sachs disease.

The World of Proteins

As the sequencing of the human genome reaches completion, NIDDK and the broader scientific community are putting greater emphasis on proteins. Genes help scientists get to proteins, but the ability of researchers to create therapies for any number of medical conditions would be enhanced if they could more easily make proteins and manipulate defective ones. Drug design has been somewhat hindered because scientists haven't yet learned the shapes or functions of most human proteins. Because protein structure and function are related, knowing more about both will speed the creation of substances capable of binding to particular proteins to turn them on or off.

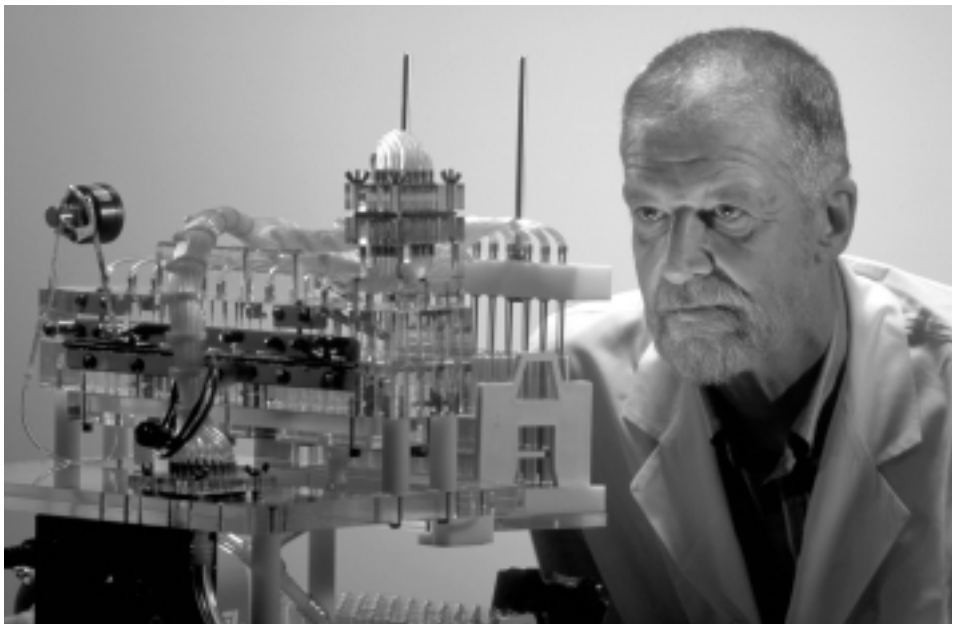
Critical in virtually all biological functions, proteins vary tremendously in type, size, shape, and function, and their conformations change depending on whether or not they are active. Some are enzymes that break down other proteins, others are hormones, and still others shuttle smaller molecules through the body. Antibodies are proteins that

animals produce in response to foreign substances such as invading viruses, bacteria, and cells from other organisms, and collagens are proteins that give the body structure.

There are proteins to control gene activation, the stages of cell division, and the differentiation of embryonic cells into the various tissues that make up an organism. Receptors on cell surfaces are proteins that bind other proteins and molecules and receive signals from other cells. Within cells are yet another group called G proteins, which take signals from receptors on the exterior surface of the cell and transmit them inward so the cells can take appropriate action. The late Dr. Martin Rodbell proved the existence of such signal transducers in the 1970s while he worked at NIDDK.

By the time Rodbell's discoveries won the Nobel Prize in Physiology or Medicine in 1994, G proteins had been shown to be ubiquitous. They play a role in many areas, including human growth and the detection of light, color, taste, and smell, and in diseases ranging from cholera, which is caused by a bacterium, to McCune-Albright syndrome, a crippling endocrine and skeletal disorder. Scientists estimate that more than 1,000 different kinds of G protein-coupled receptors (the cell surface receptors that control intracellular G proteins) exist in mammals, and nearly half of all drugs in clinical use act on specific receptors.

NIDDK scientists continue to study these proteins and their receptors. Dr. Lee Weinstein, recently tenured in the Metabolic Diseases Branch, studies the pattern of inheritance in Albright's hereditary osteodystrophy, an inherited G protein disorder. Patients with the disease develop different hormonal problems depending on whether the disease is inherited from the mother or the father.



Dr. John Daly investigates binding of bioactive compounds.

Dr. Jürgen Wess, meanwhile, works on G protein-coupled receptors to understand how they assemble in cell membranes and to determine which portion of the receptors binds hormones or neurotransmitters on the outside and which part couples with the G protein. Understanding these mechanisms could pave the way for the development of novel drugs to activate or inhibit certain receptors. Wess also has been able to mimic a rare human kidney disease in mice by mutating the gene for a particular G protein-coupled receptor. This mouse model will allow testing of gene therapy for the human disease.

Several tenure-track and recently tenured scientists study other diseases caused by problems with proteins. Dr. Jeffrey B. Kopp in the Kidney Disease Section works on focal segmental glomerulosclerosis, a syndrome characterized by the accumulation of collagen and related proteins in the kidney. An estimated 5,000 to 10,000 people develop the condition each year, and 80 percent of the cases are of unknown cause. Sometimes the condition occurs in people infected with HIV-1, and it appears to be more likely in people who have already lost a kidney or who are morbidly obese. Because the disease affects African Americans disproportionately, Kopp has begun a study to learn whether genetics plays a role in the disease. He also will look at the roles played by HIV and other viruses.

Dr. Chuxia Deng from the Genetics of Development and Disease Branch is interested in receptors that are flawed in their ability to bind fibroblast growth factors that are important in skeletal development. At least 10 human diseases are caused by mutations in the genes for these receptors. The diseases cause dwarfism, craniofacial distortions, and other skeletal problems. Deng has

developed genetically altered mice with mutations in three different receptors. Now, he has models that make it possible to study five disorders in detail.

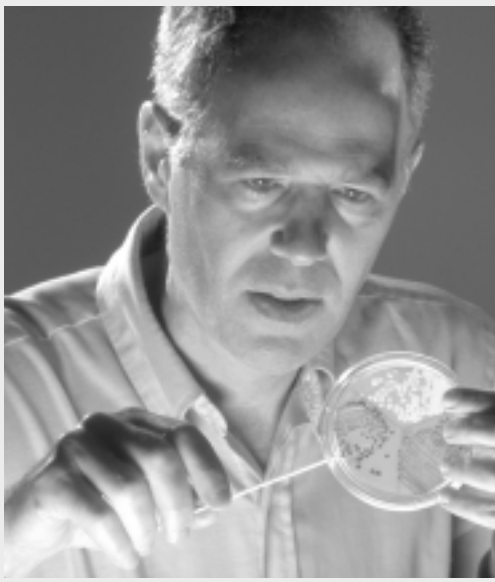
Dr. Roland A. Owens in the Laboratory of Molecular and Cellular Biology studies proteins that may someday be helpful in human gene therapy. For gene therapy to be more successful, scientists need better ways to deliver and fully incorporate healthy genes into sick people. One possible carrier, or vector, for genes is a virus called adeno-associated virus type 2. Owens studies proteins in the virus that allow it to cut into human chromosome 19 and insert its DNA.

Dr. Barbara Rehermann, who works in the Liver Diseases Section, is interested in the immune responses of patients with hepatitis B and C. She wants to know how and why some people are able to clear hepatitis viruses from their systems after they have been infected while others develop persistent infection with long-term risks of irreversible liver injury and liver tumors. She and colleagues from NIDDK and institutions in Germany and Belgium showed that in a group of women accidentally infected 20 years ago with hepatitis C, 30 percent cleared the virus. While that rate was high, it was even more surprising that half of those who cleared the virus also lost their antibodies to the virus. Antibodies form at the time of an infection and usually persist for decades. The presence of these proteins tells doctors that a person has been exposed to the infectious agent. Because antibodies to hepatitis C disappeared in a significant number of patients, Rehermann suspects that the exposure to and recovery from hepatitis C virus infection in the general population is much higher than currently estimated.

The study of proteins in other organisms enhances scientists' understanding of those in us. Dr. Orna Cohen-Fix studies a protein called Pds1 from the yeast *Saccharomyces cerevisiae* to learn more about the control of cell division. When cells divide, a process called mitosis, chromosomes are segregated equally between daughter cells. The protein Cohen-Fix studies prevents cells from going through mitosis if one or more chromosomes are broken, and it also ensures that the daughter cells receive the right number of chromosomes. What she learns has implications for the study of certain cancers, where cells sometimes have the wrong number of chromosomes.

During development, proteins direct cells to specialize and become nerve, muscle, skin, or some other cell type. This differentiation is often difficult to study in complicated organisms like humans, so Dr. Michael Krause in the Laboratory of Molecular Biology studies the process in a tiny worm named *Caenorhabditis elegans*, which is hatched with only 558 cells and consists of only a few different cell types. He's identified several proteins that influence whether cells become nerve or muscle in the worm. These proteins are closely related to proteins in humans that function in similar processes. Sorting out the principles that govern cell specialization may provide insights into developmental disorders and some cancers.

Dr. Jenny E. Hinshaw in the Laboratory of Cell Biochemistry and Biology explores the ways of a protein, dynamin, found in most species. Dynamin's work



Dr. Reed Wickner and Dr. Orna Cohen-Fix

A yeast key to making bread and beer is proving to be as useful in understanding prions, infectious proteins implicated in diseases fatal to humans and animals. In experiments with *Saccharomyces cerevisiae*, NIDDK's Dr. Reed B. Wickner studies how seemingly normal proteins transform themselves into pathogens. By unraveling the process in a one-celled organism, he and other yeast geneticists could help scientists working on prions in complex mammals.

The prion diseases—among them Creutzfeldt-Jakob disease in humans, scrapie in sheep, and "mad cow disease"—are spongiform encephalopathies, neurodegenerative disorders characterized by formation of holes in the brain and the development of clumps of protein called amyloids.

All other known pathogens, such as bacteria and viruses, need the nucleic acids DNA or RNA to reproduce. But current evidence suggests prions can arise spontaneously and multiply, they can be inherited, or they can be transmitted—surprisingly, with no help from nucleic acids.

How prions work is unclear, but

Proteins Gone Bad?

many scientists think that, once a protein is converted to a prion, the prions multiply by converting additional normal proteins into aberrant shapes. That a protein could become so altered itself and then pass on the alterations to other molecules challenges expectations about protein behavior.

Significant research supports this protein-only hypothesis; however, no one has found the components in mammals that are directly responsible for generating and then propagating prions. Researchers also don't know how the misshapen proteins contribute to the degeneration of brain tissue.

Evidence for the triggering of prions is stronger in yeast than in humans, according to Wickner, chief of the Laboratory of Biochemistry and Genetics. Herman Edskes, a postdoctoral fellow in Wickner's group, recently showed that a protein called Mks1p is required for prion generation, but not propagation, in *Saccharomyces cerevisiae.*

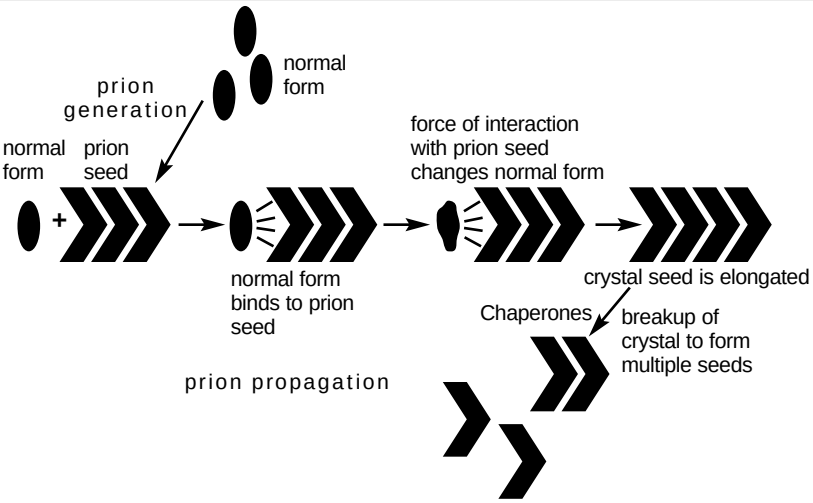
A member of the National Academy of Sciences, Wickner first proposed in the journal *Science* in 1994 that some transmissible elements in yeast were prions that passed from cell to cell when they mated. Unlike genes on chromosomes, these elements float in the cytoplasm. In his paper, Wickner described three ways to identify which transmissible elements are prions, and then successfully applied the tests to two elements. A key test is that even when prions are destroyed in a cell, they will arise again so long as the gene that produces the normal starting protein continues to function.

Since his initial hypothesis, Wickner and his colleagues have proved that prions sometimes form from a protein called Ure2p, which the yeast needs to use nitrogen. Cells that develop the misshapen proteins have problems in metabolizing nitrogen, thus causing a yeast "disease" that serves as a model for other prion disorders.

Wickner's lab also found that URE3, the prion version of Ure2p, forms aggregates and cannot be dissolved with an enzyme called protease, two characteristics of amyloids. When Wickner and his colleagues tested a synthetic version of Ure2p, they found it likewise could form amyloid filaments. And another experiment showed that if a segment called the prion domain was missing from Ure2p, the protein could not form amyloid filaments.

"We know that URE3 is a prion, but we're still not ready to say definitively that it is an amyloid. Next, we want to purify Ure2p from cells with prions to prove that it is in amyloid form," says Wickner. If amyloids are present, it adds credence to the idea that prions have a role in diseases characterized by amyloid formation. Solving the amyloid question in prion diseases may provide insight into more common illnesses like Alzheimer's, Parkinson's, and type 2 diabetes, where amyloids also arise.

"It's also possible that prions aren't all bad," says Wickner. He notes that another lab demonstrated that prions are mandatory for one species of fungus to recognize its own kind, suggesting that the altered proteins sometimes may provide necessary functions. For this yeast geneticist, prions add a little "biological spice" to infection and inheritance. ■

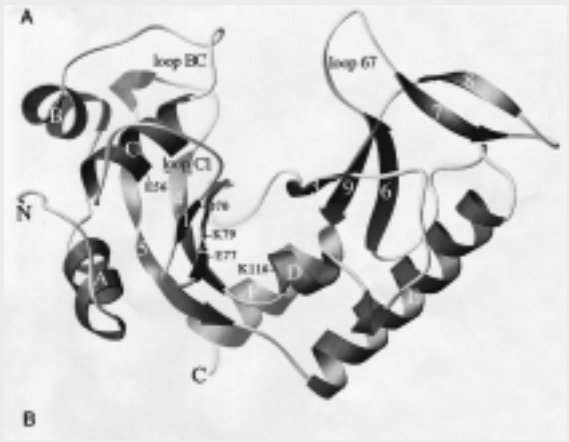


Generation and propagation of prions. A normal protein can spontaneously change to form a prion seed which then propagates by adding molecules of the normal form. The prion form is (in the known cases) an amyloid form of the protein. Chaperones are proteins that then break up large amyloid aggregates into smaller aggregates which can then seed further prion propagation.

DNA's Repair Kit

When DNA makes a copy of itself, there are countless opportunities for tiny typos to appear in the genetic code. If uncorrected, these little errors go a long way: They destabilize DNA, boost mutation rates, and sometimes lead to cancers. To clean up such mistakes, all living things have built-in editors. Dr. Wei Yang, recently tenured in NIDDK's Laboratory of Molecular Biology, studies the structures of three proteins that routinely correct errors that arise during DNA replication.

DNA contains four bases that form pairs—guanine (G) with cytosine (C) and adenine (A) with thymine (T)—along the helix. At the beginning of replication, the two DNA template strands separate and daughter strands are made to complement each half. For instance, if a string of bases on the parent strand reads GGATTC, the corresponding stretch on the daughter strand should read CCTAAG. If the wrong nucleotide slips in on the daughter strand, mismatch repair begins. Yang, who is a biochemist and x-ray crystallographer, focuses on MutS, MutL, and MutH, proteins that fix mismatches between nucleotide bases in *Escherichia coli*. DNA replication is well-studied in this bacterium, making it a good model to better understand the principles governing repair.



Ribbon diagram of MutH, a DNA repair protein

Scientists have a general model for the steps involved, but they aren't sure how the three proteins coordinate their activities. They think that MutS finds mismatches between the template and daughter strand and works with MutL to activate MutH, which then snips the daughter strand up to a thousand base pairs away from the error. MutH's cut allows another protein called exonuclease to come in to take out bases, including the faulty one, much like a backspace key on a computer.

Until recently, scientists had no crystal structures of the repair proteins, making it impossible to visualize how the proteins work at the molecular level. Crystal structures give scientists a three-dimensional view of the curves, twists, and indentations on a protein that indicates how and where it might bind to another protein or DNA.

Since her arrival at NIDDK in 1995, Yang's group has determined crystal structures of MutH and MutL. In collaboration with Dr. Peggy Hsieh's group in the Genetics and Biochemistry Branch, they have also crystallized MutS from *Thermus aquaticus*, a bacterium, and MutS combined with DNA. The process was difficult—it took nearly five years just to work out one MutS structure—because sections, or domains, of the proteins are quite mobile.

"A crystal structure is just a snapshot of a moving object," says Yang. But from the snapshots, Yang has a better idea how the three proteins


Dr. Wei Yang

change shape as they interact with each other and with the DNA to initiate repair. She's learned that MutH is shaped like a clamp, with a cleft dividing the molecule into two domains. The site where MutH binds to DNA is in the cleft. Pivoting of the domains relative to each other allows the space where DNA could bind to be opened or closed. Yang thinks a different site on MutH interacts with MutS and MutL to regulate the opening of the domains so that binding of DNA can occur.

MutL has two domains, one box-shaped, the other barrel-shaped. DNA binds at the groove formed when two MutL molecules combine. MutH attaches in a crevice between the box and barrel domains. But before MutH can be activated, Yang and her colleagues found that MutL must bind another molecule called ATP mainly in the box domain. But for both MutS and MutL to work on MutH, the ATP has to be broken

down. Yang speculates that the way MutL uses ATP somehow coordinates the repair steps.

MutS, the most complex protein, has five domains and is usually shaped like a comma. One of its features is a junction of three domains that appears to be an area where a signal could go from MutS, which would let MutL know when an error was found.

Homologs of MutS and MutL occur in humans. People who inherit faulty genes for mismatch repair proteins are susceptible to cancers in various organs, including colorectum, ovary, stomach, small intestine, and kidney.

The crystals of the microbial repair proteins have given researchers a starting point toward learning the structure of human mismatch repair proteins, which have not been crystallized to date. Yang will use what she's learned from the microbial repair proteins to create models of human proteins and solve their structures. ■

takes place during endocytosis, a method a cell uses to bring substances across the cell membrane into the cytoplasm. The substances come in vesicles that have to be detached from the cell membrane. Dynamin does the job by wrapping around the necks of vesicles and pinching them off. Although the protein does not appear to cause any medical problems and no commercial applications have been proposed for it, Hinshaw says it is too soon to predict how the protein could be used. In the tradition of basic research, she'll keep studying the protein to develop further leads.

Today, scientists learn the genetic sequences for tens of thousands of proteins yearly, but they still struggle with a fundamental question that puzzled the late Dr. Christian B. Anfinsen: How does a string of amino acids form a shape? Anfinsen, who headed the Laboratory of Chemical Biology from 1963–1981, hypothesized in the early 1960s that all the information a protein needed to get into its characteristic shape resided in its sequence of amino acids. To prove his hypothesis, he unfolded the enzyme ribonuclease and found that the protein would refold into its correct conformation on its own. In 1972, Anfinsen won the Nobel Prize in Chemistry for demonstrating that amino acid order determines three-dimensional structure.

Today, Dr. William Eaton and Dr. James Hofrichter attack the protein-folding problem with lasers. The lasers trigger the unfolding and refolding process, allowing the researchers to study the mechanism of formation of helices and sheets, the basic structural elements in proteins.

Solving the protein-folding puzzle will aid in predicting protein structures from amino-acid sequences and could help in the design of new molecules. It will also improve scientists' understanding of the pathology of illnesses (such as Alzheimer's and the prion diseases) that are caused by the aggregation of unfolded or misfolded proteins.

Other labs take different approaches to learning about protein structure, drawing on NIDDK's five decades of leadership in the field. Since the 1950s, intramural scientists have been figuring out the three-dimensional structures of infection-fighting antibodies, disease-causing agents, and proteins that regulate a living thing's most basic functions using x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

In structural biology's early days everything was unknown. NIDDK scientists like Dr. David Davies, a leading x-ray crystallographer who now studies the structure of the HIV enzyme that helps the virus integrate into its host, remembers that he and colleagues had to "make up all the techniques as we went along." Over the years, they have perfected methods of crystallizing proteins and then bombarding them with x-rays to reveal their shapes.

Davies used these methods to learn the shapes of antibodies alone and combined with other molecules. The findings helped scientists better understand how antibodies recognize foreign substances.

X-ray crystallography also enabled Davies and Dr. Edith Miles, in the Laboratory of Biochemistry and Genetics, to study the two-enzyme complex that makes tryptophan, an amino acid. They found that the two enzymes have a tunnel between them. After the reaction producing a tryptophan precursor occurs at the first enzyme, the precursor travels

down the tunnel to the second enzyme where the final reaction to produce trytophan takes place. Initially, scientists thought that the tunnel—the first to be discovered—was a biological rarity, but in recent years such channels have been seen in other enzyme combinations.

It is too soon to predict how knowledge of these tunnels could be useful in medicine. Often, it takes decades for potential applications of basic research findings to become apparent. In the 1970s, for instance, Davies studied the shape of a fungal enzyme that could cut protein. His group's experiments revealed how this protease's action could be stopped by inhibitors, a kind of small molecule. Many years later, when scientists learned that the fungal proteases were similar to the HIV protease, the early work by the Davies group helped speed the development of inhibitors that act as HIV antiviral agents.

Now, there are many inhibitors for protease and reverse transcriptase, another HIV enzyme, which are used in the so-called antiviral cocktail used to treat AIDS. The current studies by Davies, Dr. Robert Craigie in the Laboratory of Molecular Biology, and their colleagues focus on HIV-1 integrase, the third enzyme in HIV. There are no therapeutically available inhibitors for it. But researchers consider integrase an attractive target for drug design because it has no mammalian counterparts that inhibitors could act upon to create a negative reaction. The NIDDK researchers have figured out the structure of a portion of integrase and have found a place on the enzyme where an inhibitor could bind, knowledge that may help in future inhibitor design.

To get to protein structures that have remained elusive, NIDDK scientists continue to tweak established methods and create new ones. Dr. Adriaan Bax, chief of the Laboratory of Chemical Physics, seeks to improve nuclear magnetic resonance, which can be used to determine structure even when scientists cannot produce a protein crystal.

Investigators using NMR generally work with their samples in liquid, usually water. The samples go into a magnetic field and are showered with radio waves that are absorbed and emitted in patterns. Typical proteins emit thousands of signals, one for every hydrogen atom in the molecule. The intensity and interrelationships of the signals contain information about the distances between the various hydrogens in the molecule, provided they are close. From that information scientists reconstruct the protein's shape.

The idea is straightforward, but, in practice, identification of the individual signals is so complex that only the smallest proteins can be studied this way. Bax and his colleagues have solved this problem by replacing the standard carbon and nitrogen atoms in the protein with forms that provide NMR signals. These changes simplify the analysis. The improvement can be compared to the difference between putting together a jigsaw puzzle relying just on the shape of the pieces and putting it together using the shapes and colors of pieces.

NMR structures of proteins determined simply from distances between hydrogens also tend to be of limited resolution, somewhat like a photograph that is slightly out of focus. To produce sharper images, Bax developed another method that measures both the distance between chemical bonds and their orientation. Bax's new method was used to create a better picture of the protein cyanovirin-N, which inhibits the HIV virus from entering human cells.

A third advance by Bax's group allows researchers to quantify the flexibility of proteins using NMR. The methods were used in a recent collaboration between the National Institute of Dental and Craniofacial Research and NIDDK. The study of HIV protease highlighted how the enzyme changes its mobility upon binding various types of drugs. Understanding that mobility may aid in creating more powerful inhibitors.

Dr. Robert Tycko, recently tenured in the Laboratory of Chemical Physics, tackles other NMR problems. He develops solid-state nuclear magnetic resonance methods in order to study proteins without using liquid. He's trying the technique on malformed proteins called amyloids, found in the brains of people with Alzheimer's. Amyloids of all sorts are medically important, but scientists have had difficulty determining their structure with available technologies. The solid-state NMR technology is so new that only a handful of labs in the world can do it.

Proteomics

Identifying all the different kinds of proteins that humans can make during a lifetime and learning how they work with each other is one of the great challenges for researchers in the 21st century. The challenge so excites scientists, they've dubbed the new field proteomics.

Researchers at NIDDK are moving into this area, particularly the subfield known as structural genomics. In structural genomics, scientists look for systematic ways to determine the three-dimensional structures of unknown or poorly understood proteins. They work on the premise that, no matter how varied proteins are, many share similar components, or domains. Increasingly, scientists use bioinformatics, a field that combines biology and information science, with other tools to look for such matches.

Science and NIDDK have come a long way.

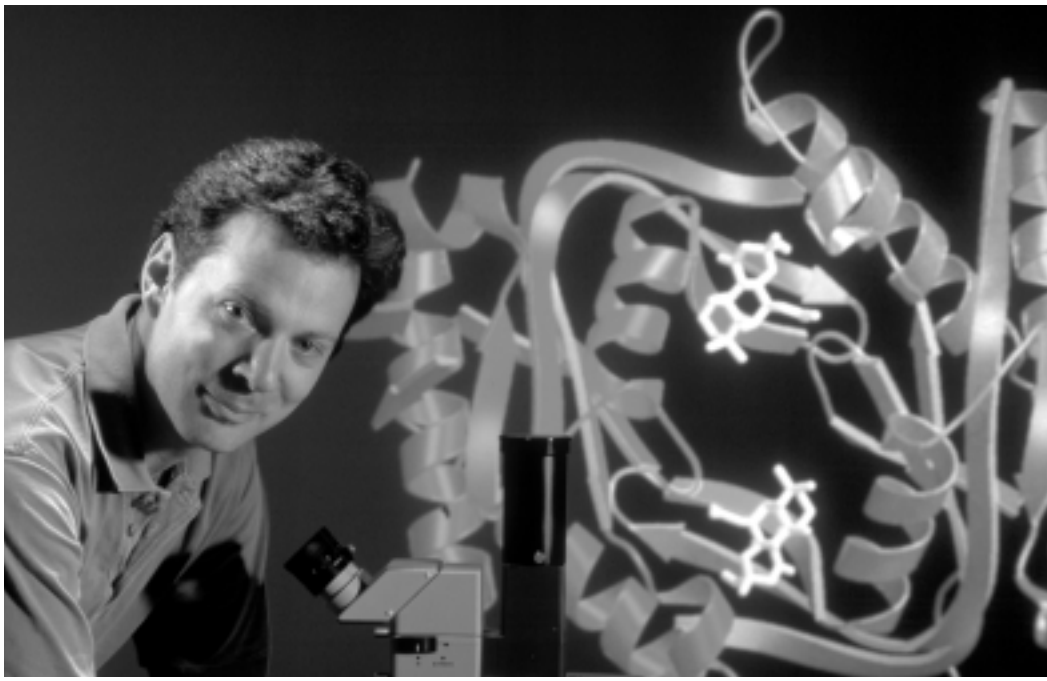
In bioinformatics, researchers create and organize huge biological databases that include gene and protein sequences and protein structures that can be compared with each other in a variety of ways. A match between something known in the database and a mystery protein can give hints about the unknown's function and structure. A program is under way at NIDDK to look at the structure of proteins that share components involved in signaling in cells.

Dr. James Hurley, who has worked out the shape of key cell-signaling molecules, recently used bioinformatics and other techniques to determine a domain structure in a protein called MLN64, which is found in some breast carcinomas. MLN64, which triggers the formation of steroid hormones, is being used as a model for a similar, but more-difficult-to-study protein called StAR. Steroidogenic acute regulatory protein plays a role in triggering normal hormone production. Finding that the domain of MLN64 binds cholesterol, the starting material for many hormones, suggests that StAR could also be shuttling cholesterol to the enzyme that converts it to a steroid.

Other scientists like Dr. Jeffrey Lynn Miller use genomics to solve different problems. Miller compares the sequences of activated genes in red blood cells grown in the lab with all the sequences from the human genome to learn which genes control the development of red blood cells from their progenitors. The gene sequences from red blood cells are then used to determine which proteins are being produced, and to identify specific proteins of clinical importance in hematology, malarial research, and transfusion medicine. Using this approach, Miller and his colleagues were able to identify Dombrock, a red cell protein that causes transfusion reactions. Dombrock has eluded scientists since the 1960s, but its identification will make it possible for blood banks to screen for it in the future. To help scientists worldwide who are interested in red blood cells, Miller has created Hembase (http://hembase.niddk.nih.gov), an online database.

When NIDDK was established, there was no molecular proof for the existence of genes. Computers were clunky, slow, and rare in the lab, and a database might have been a notebook. There were no transplants of kidneys for people with kidney failure, or of livers for people with hepatitis, or islets for people with type 1 diabetes. Science and NIDDK have come a long way.

As NIDDK's Division of Intramural Research enters a new century, its scientists will continue to pursue promising lines of research. Who knows where their explorations will take science and medicine in this new era of hope?



Dr. James Hurley seeks ways to compare protein structures.

Information, Education, and Outreach

NIDDK supports the NIH mission by translating biomedical research advances into accurate, understandable health and science information for diverse audiences. The Institute disseminates health information and research findings to patients and their families through

- fact sheets and other publications
- information for the news media and voluntary and professional organizations
- responses to individual inquiries
- information clearinghouses
- outreach campaigns
- national education programs
- the NIDDK Web site.

The public, patients, and their families can obtain information about all NIDDK programs through the Institute's Office of Communications and Public Liaison, Bldg. 31, Room 9A04, 31 Center Drive MSC 2560, Bethesda, MD 20892-2560. (Tel: 301-496-3583; e-mail address: NIDDK_Inquiries@ nih.gov)

NIDDK's four information clearinghouses answer inquiries, refer people to patient-support and professional organizations, and provide printed and online health education materials. All of NIDDK's health information materials, which are reviewed for scientific accuracy by NIDDK scientific staff and outside experts, are posted on the NIDDK Web site (www.niddk.nih.gov).

National Diabetes Information Clearinghouse

1 Information Way
Bethesda, MD 20892-3560
ndic@info.niddk.nih.gov
1-800-860-8747

The National Diabetes Information Clearinghouse (NDIC) disseminates printed materials on many diabetes-related topics, including easy-to-read and Spanish-language publications. Certified diabetes educators answer inquiries about diabetes and keep abreast of materials from other organizations and individuals for the diabetes section of the Combined Health Information Database (http://chid.nih.gov). CHID indexes diabetes publications, videos, book chapters, articles, and foreign-language diabetes health education materials. A current NDIC feature is a new easy-to-read library on diabetes control, nutrition, medications, and complications. The NDIC actively supports the National Diabetes Education Program, a joint effort of NIDDK and the Centers for Disease Control and Prevention.

National Digestive Diseases Information Clearinghouse

2 Information Way
Bethesda, MD 20892-3570
nddic@info.niddk.nih.gov
1-800-891-5389

The National Digestive Diseases Information Clearinghouse (NDDIC) disseminates information on a wide range of topics in digestive diseases. Information specialists at NDDIC can help people find the information they seek. Easy-to-read and Spanish formats are available on some topics. NDDIC is currently featuring "what to expect" information sheets on various diagnostic tests and an information set on the symptoms, prevention, and management of viral hepatitis A, B, and C. The Clearinghouse is also working to raise awareness of the increased incidence of hepatitis C in the African American population.

National Kidney and Urologic Diseases Information Clearinghouse

3 Information Way
Bethesda, MD 20892-3580
nkudic@info.niddk.nih.gov
1-800-891-5390

The National Kidney and Urologic Diseases Information Clearinghouse disseminates information on kidney and urologic diseases. From polycystic kidney disease to prostate enlargement, the range of renal and urologic health concerns can touch every aspect of day-to-day life. Clearinghouse fact sheets and booklets help patients feel better equipped to discuss their personal health questions with their doctors. Easy-to-read and Spanish formats are available for some topics. Coming soon: expanded and improved materials on kidney failure for people facing treatment choices and long-term self-

care issues. The Clearinghouse currently features a women's bladder control awareness program, a campaign that encourages women to discuss their concerns about bladder control with their doctors.

Weight-control Information Network

1 WIN Way
Bethesda, MD 20892-3665
WIN@info.niddk.nih.gov
1-877-946-4627

Overweight people are more likely to have type 2 diabetes, high blood pressure, heart disease, stroke, gallbladder disease, and some cancers. Now more than ever before, a national epidemic of obesity poses one of the greatest threats to the health and well-being of Americans. The Weight-control Information Network (WIN), in conjunction with NIDDK's National Task Force on Prevention of Obesity, provides science-based health information on many obesity-related topics of consumer interest including gastric surgery, obesity in children, very-low-calorie diets, weight cycling, and more. WIN currently features a program, "Sisters Together: Move More, Eat Better," to encourage African American women, who are at high risk of obesity, to get active and to take charge of their nutrition.

Find NIDDK's health education materials at www.niddk.nih.gov by clicking on "Health Information."

Education

National Diabetes Education Program

The National Diabetes Education Program (NDEP)—a joint program of the National Institute of Diabetes and Digestive and Kidney Diseases, the Centers for Disease Control and Prevention, and more than 200 public and private partners—seeks to improve the treatment and outcomes for people with diabetes.

Guided by state-of-the-art science and expert advice, the program aims to improve the lives of people with diabetes through a variety of strategies, including diabetes awareness campaigns, community-based interventions, changes to the health care system, and an inclusive partnership network.

The NDEP has launched campaigns for people with diabetes and health care providers to increase awareness of the importance of controlling diabetes. Working with minority groups, NDEP has tailored campaigns for populations disproportionately affected by diabetes--namely, African Americans, Hispanic/Latinos, Asian Americans and Pacific Islanders, American Indians, and senior citizens.

NDEP's *Feet Can Last A Lifetime* campaign, launched in 1995, is raising awareness among health care providers that diabetes-related amputations can be prevented through early detection of diabetic foot disease. In the last 2 years, the campaign has distributed 20,000 kits that include a monofilament for sensory testing, a foot screening form and instructions, forms to facilitate Medicare coverage of therapeutic footwear, a literature review of current research, and patient education materials. The kit is being marketed through the communication channels of 12 partner organizations and other professional and medical organizations, and through the health care media.

The NDEP provides educational materials and reference tools in English and Spanish for people with diabetes and health professionals. In addition, the program assists individuals and organizations to plan, develop, and implement diabetes activities in their communities through useful media materials, handbooks, resource guides, videos, and slide presentations.

To deliver diabetes messages in thousands of communities across the country, the NDEP has created a Partnership Network of more than 200 public and private organizations at the national, state, and community level. These partners promote program materials and messages and integrate them into their existing education programs and activities.

To learn more about the National Diabetes Education Program or to order publications, call 1-800-438-5383 or visit http://ndep. nih.gov, the NDEP Web site.

Acknowledgements

PHOTOGRAPHY:
Richard Nowitz

Nobel Prize Committee and the National Library of Medicine: Nobel Prize winners

Marc Reitman, M.D., Ph.D.: Mouse models

William L. Branson: Pima Indian photos;
Jay Hoofnagle with parent and patient

Walter Reed Hospital: Early dialysis

Scott J. Hultgren, Ph.D.: *E. coli* invades bladder.

DESIGN:
Janet Minker, Minker Design

EDITORIAL:
Joan Chamberlain, Leslie Curtis, Jane DeMouy,
Anna Maria Gillis, Mary Harris, Elizabeth Singer

National Institute of
Diabetes & Digestive &
Kidney Diseases

NIH Publication No. 01-4636