XI.
National Institute of Dental and Craniofacial Research

INTRODUCTION

The National Institute of Dental and Craniofacial Research (NIDCR) continues to serve as a leader in the support of dental, oral, and craniofacial research and research training in the United States. At the beginning of the 21st century and the new millennium, this research effort has increasingly become global. As the U.S. population becomes more diverse, the health needs of our residents reflect the health needs of the countries that provide the sources of immigrant flow to our shores. Issues of disease etiology and preventive strategies, control of infectious and chronic disease and disability, and assurance of optimum oral health–related quality of life span both domestic and international concerns.

In the new, interactive, information age, the research to address these needs and issues knows no geographic boundaries. More rapidly than ever before, researchers can collect data, exchange information, share insights, compare findings, acquire and apply new technologies, and probe pertinent questions, but efficiency is not the only, or the most valuable, benefit of this new era. Perhaps more important is the expansion of horizons, the greater appreciation of nuances and differences that may hold the key for unlocking unknown causes of disease and the disparities in their effects. The prevalence and patterns of disease expression related to each oral health issue vary throughout the world and among populations, whether the issue is infectious periodontal diseases, caries, HIV (human immunodeficiency virus), AIDS (acquired immunodeficiency syndrome), or oral gangrene or a chronic condition such as Sjögren's syndrome, orofacial pain, a temporomandibular disorder, or oral cancer. By exploring the similarities and differences in the prevalence and patterns, researchers can more easily identify the nuances that are relevant for U.S. residents and potentially beneficial to others.

Science is global, and especially in recent years, international collaborative research teams have led the way in identifying genes causing or associated with disease; clarifying the role of environmental factors; collecting and organizing epidemiologic data; developing improved methods; testing new treatments; and establishing health outcomes. NIDCR has supported many of these efforts. International collaborative research has been a mainstay at NIDCR since its inception in 1948.

In 1998, NIDCR established an Office of International Health (OIH) to enhance international collaboration in oral health and to target strategies for advancing dental, oral, and craniofacial health worldwide. OIH coordinates and represents NIDCR's international efforts within the National Institutes of Health (NIH) and the U.S. Government and serves as NIDCR's liaison with international agencies and foreign organizations involved in global research on oral health. The Office coordinates and facilitates NIDCR bilateral and multilateral agreements, fosters and sponsors international workshops and associated research agendas, and disseminates information about opportunities for international collaborative research and research training.

In fiscal year 2000 (FY 00), OIH emphasized the development and support of international collaborative networks, to take advantage of the increasing globalization of research and to maximize the scientific community's approach to the research opportunities presented. Such networks will enhance oral health research by stimulating and strengthening research communications in three directions—among U.S. scientists, between U.S. and foreign scientists, and among foreign scientists.

To foster the development of international networks, NIDCR awarded six International Collaborative Oral Health Research Planning Grants in six areas in FY 00:

- reduction of disparities in dental caries among children;
- oral infections and risk of vascular disease;
- genetic epidemiology of oral clefts;
- oral cancer;
- temporomandibular disorders; and
- oral health–related quality of life in children.

The 2-year planning grants are awarded to U.S. investigators to support the planning of research protocols for biomedical, epidemiologic, and behavioral studies in priority areas of international collaborative research, as identified in NIDCR's strategic plan. The awardees are organizing international collaborations and consortia involving investigators and research institutions in many countries, to develop and propose large, international research studies in the six areas.

The topics represent major scientific and oral health issues in the United States and around the globe, and they will be explored in both industrial and developing countries. The benefits to be gained are substantial, not only in increased knowledge and improved health, but also in the development of research relationships and infrastructures that will sustain continued research for many years to come. U.S. interest in these collaborative networks is high, as indicated by the volume of such proposals for NIDCR grant support. (For details on each planning grant study, see the section on "Extramural Programs.")

In FY 00, OIH initiated support for another major effort. NIDCR awarded a contract to the World Health Organization (WHO) to support research planning and support services for international collaborations related to craniofacial anomalies. The funds provided by NIDCR through WHO will support planning and development of research protocols, databases, and network infrastructure to sustain and coordinate collaborative biomedical, epidemiologic, and behavioral studies. Specific networks will in-

clude epidemiology and surveillance experts and scientists who will design and conduct clinical trials for prevention and treatment.

Craniofacial anomalies continues to be an important area of international research for NIDCR. In May 2000, NIDCR cosponsored an international workshop on Quality of Life and Oral Craniofacial Issues, in Ann Arbor, Michigan. The workshop was attended by investigators from Argentina, Brazil, Canada, and the United States. Staff participated in this and other international meetings on craniofacial anomalies, and the Division of Extramural Research supported a number of international research studies. (For details, see the section on "Extramural Programs.")

Also in FY 00, NIDCR continued support for international research and communication in other priority areas of the international oral health agenda. These include noma (orofacial gangrene), a devastating infectious disease that primarily afflicts malnourished children; other infectious and emerging infectious diseases (e.g., HIV/AIDS); fluoride use; oral cancer; biomaterials and biomimetics; and reduction of disparities in health through health promotion efforts. NIDCR efforts in these areas include support of intramural and extramural investigators, organization and sponsorship of international meetings, support of scientist exchanges, presentation of research data and findings, and dissemination of scientific and public information. To stimulate research on fluoride, for example, the summary and recommendations of an international meeting on fluoride, hosted by NIDCR at the NIH, Bethesda, Maryland, in May 1999, were published in the *Journal of Dental Research* (April 2000), and NIDCR is preparing a draft program announcement to solicit grant applications for research on fluoride.

To secure continued support for these research activities, NIDCR continued in FY 00 to seek and encourage partnerships in funding. NIDCR promotes partnerships as a way of leveraging its own research dollars to accomplish research goals for the benefit of all. During FY 00, OIH continued discussions with public, private, and nonprofit organizations in the United States and worldwide to expand the network of potential funding partners. NIDCR's efforts to foster globalization of research, research networks, and partnerships in funding are detailed here. Additional information on the full range of NIDCR's international activities can be accessed through the NIDCR home page (http://www.nidcr.nih.gov).

HIGHLIGHTS OF RECENT SCIENTIFIC ADVANCES RESULTING FROM INTERNATIONAL ACTIVITIES

Receptors for Bitter Taste

Scientists in the Taste and Smell Unit of NIDCR's Oral Infection and Immunity Branch authored two outstanding reports published in the journal *Cell* (March 2000), which included on the cover a photomicrograph of receptors for bitter taste. The articles, entitled "T2Rs function as bitter taste receptors" and "A novel family of mammalian taste receptors," describe the discovery, sequencing, and characterization of genes that encode taste receptors for detecting bitter substances that may be toxic to mammals. This significant work was guided by two Visiting Scientists from the United Kingdom. Experiments with mice genomes indicate that the family of receptors may contain as many as 40–80 genes that encode receptors with differing specificities. These discoveries present opportunities for better understanding of human taste responses and modulation of taste perception. (See also the section on "Intramural Programs and Activities.")

Pain in Newborns and Adults

Researchers in the Cellular Neuroscience Section of NIDCR's Pain and Neurosensory Mechanisms Branch authored a provocative research report entitled "Altered nociceptive neuronal circuits after neonatal peripheral inflammation," which appeared in the journal *Science* (July 2000). The article, coauthored by an investigator from Japan, received significant national and international attention for its implications about acquired pain responses. The scientists showed that peripheral pain induced by injection of an inflammatory agent in the hind paw of newborn rat pups altered the developing neuronal circuitry, subsequently increasing the sensory input into primary spinal axons in the animals as adults. The experimental group of adult animals exhibited significantly exacerbated pain response to standardized touch and pinch tests. These findings present new questions about medical interventions in newborn infants. (See also the section on "Intramural Programs and Activities.")

Gene for Papillon-Lefèvre Syndrome

An international research team led by an NIDCR-supported investigator at the University of Pittsburgh, Pennsylvania, discovered the gene responsible for Papillon-Lefèvre syndrome. Individuals with this rare, devastating condition have thick, cracked skin and lose all their teeth by young adulthood. The research team, which included investigators from Wake Forest University, Winston-Salem, North Carolina, and the University of Istanbul School of Dentistry, Turkey, examined the DNA from Turkish families having members with or without the syndrome. The gene was located on a small region of chromosome 11. Detailed analysis of the region showed gene mutations that coded for the enzyme cathepsin C. Identification of the gene for Papillon-Lefèvre syndrome may provide clues to the loss of teeth accompanying periodontal disease, which is widely found in the general population. This significant discovery was reported in the *Journal of Medical Genetics* (December 1999).

SUMMARY OF INTERNATIONAL PROGRAMS AND ACTIVITIES

Country-to-Country Activities and Bilateral Agreements

In FY 00, OIH staff continued to collaborate with the Centers for Disease Control and Prevention on the U.S.-Russian Micronutrient Malnutrition initiative of the U.S.-Russia Joint Commission on Economic and Technological Cooperation. The Associate Director for International Health, NIDCR, continued to serve as the U.S. lead for the Fluoride Group within the initiative, and OIH staff participated in efforts to implement community water fluoridation in Russia. Staff coordinated the training of Russian water engineers and public health officials at a demonstration site in Novomoskovsk, in March 2000. In addition, OIH hosted a visit by the chief executive officer of Carelift International, which may help to locate and acquire water fluoridation equipment and transport it to Russia. Within NIH's partnership with the U.S. Civilian Research and Development Foundation, NIDCR provided support for a collaborative genetic research

project involving investigators at the University of Washington, Seattle, and St. Petersburg State University, Russia.

Also in FY 00, NIDCR scientists and research administrators collaborated with colleagues in Germany to plan for a U.S.-German workshop on chronic pain research. NIDCR and the NIH Pain Consortium staff will continue to develop these plans, involving both intramural and extramurally supported scientists.

Activities With International and Multinational Organizations

World Health Organization

The Associate Director for International Health serves as Director of the WHO Collaborating Center for Dental, Oral, and Craniofacial Research, which was reauthorized by WHO during FY 00 for extension through 2004. On April 10–11, 2000, NIDCR hosted the second meeting of all WHO Collaborating Centers for Oral Health, at the NIH, in Bethesda, Maryland. The center's views on strategic support, integration of activities, and enhanced management among the centers were submitted formally to WHO, Geneva, Switzerland. Subsequently, NIDCR worked closely with other national and international oral health organizations to sustain permanent positions for oral health within WHO, and staff briefed WHO and U.S. officials on the importance of this visibility within WHO.

NIDCR continued to work closely with WHO to stimulate collaborative research on noma, craniofacial anomalies, fluoride, and other global oral health issues. In collaboration with WHO and investigators at the University of Maryland, Baltimore, NIDCR staff will visit Niger in November 2000 to explore the availability of data for the study of noma, an effort that would involve four countries. Building on previous WHO and NIDCR projects, NIDCR cosponsored a workshop on Noma: Developing an International Infrastructure for Research, held at the NIH, in Bethesda, in April 2000, to stimulate research on noma and to identify resources to support this research.

As noted previously, NIDCR awarded a contract to WHO to facilitate planning of international collaborative research on craniofacial anomalies. To guide this effort, NIDCR organized network meetings in Manchester, England; Geneva, Switzerland; and Bethesda, Maryland.

In FY 00, the Associate Director for International Health continued to serve on the Technical Advisory Group for the Regional Oral Health program at the Pan American Health Organization and spearheaded the creation of a network of dental organizations in Washington, D.C., which have international programs and activities. This network is intended to strengthen each organization's global objectives by sharing of information on a regular basis, coordination of efforts and responses, and eventually, leveraging of resources as appropriate.

European Union

Through the European Union-U.S. Science Technology Agreement, NIDCR collaborated with the John E. Fogarty International Center for Advanced Studies in the Health Sciences (FIC), NIH, to foster relations with the European Union as a potential funding partner for international research on oral health, particularly in the area of craniofacial anomalies.

Fédération Dentaire Internationale

NIDCR staff gave scientific presentations at the 1999 World Dental Congress of Fédération Dentaire Internationale (FDI), in Mexico City, Mexico, in October–November 1999. The Associate Director for International Health helped to plan the scientific program, organized a session on women's health, and contributed planning ideas for an FDI Developing Countries Fund to enhance international collaborations with these countries and to leverage funds to support these activities. She continues to serve as a consultant to FDI's Congress and Education Committee and contributed substantially to the planning of future World Dental Congresses, in Paris, France, in 2000, and in Kuala Lumpur, Malaysia, in 2001. The Associate Director also served as the representative to the FDI General Assembly, from the Behavioral Scientists and Health Services Research Group of the International Association for Dental Research (IADR).

International Association for Dental Research

The 78th annual meeting of IADR was held in Washington, D.C., in April 2000. NIDCR staff attended the meeting to present research findings; discuss opportunities for collaborative research and research training; coordinate ongoing projects and activities; meet with representatives of international organizations; staff an NIDCR exhibit booth and consultant areas; and convey NIDCR opportunities and mechanisms of support to interested researchers.

To promote extramural and intramural collaborative research, OIH staff developed and tested the first exhibit and publications for international audiences at the joint meeting of IADR's Continental European and Scandinavian Divisions, in Warsaw, Poland, in August 2000. NIDCR has been invited to continue this outreach at IADR's annual meeting in Rome, Italy, in 2001, and at IADR's all-Europe meeting in Cardiff, Wales, in 2002. In October 1999, the Associate Director for International Health described NIDCR's international collaborative research on noma for IADR's East and Southern Africa Division, in Nairobi, Kenya.

American Dental Association

The Associate Director continued to serve as consultant to the American Dental Association (ADA) Council on Annual Sessions and International Relations. In FY 00, she also served as coauthor for the ADA Future of Dentistry report on global oral health, to be released in the fall of 2001. The Associate Director served on a subcommittee of ADA's board of trustees as a consultant for international health. In collaboration with ADA, NIDCR hosted the 36th Annual ADA Dental Students Conference on Research, which was held at the NIH and drew a record number of 60 participants from U.S. and Canadian dental schools.

John E. Fogarty International Center for Advanced Studies in the Health Sciences

The Associate Director represented NIDCR at the NIH international representative meetings coordinated by FIC, and OIH staff worked closely with FIC to advance research training related to HIV/AIDS and other emerging infectious diseases. In April 2000, the Director, NIDCR, presented invited comments at an FIC network meeting of participants in two FIC programs on emerging infectious diseases. In FY 00, NIDCR collaborated with FIC on various initiatives, including the planning for FIC's Global Forum for Bioethics in Research, to be held in

Bangkok, Thailand, in October 2000, and on the development of a request for applications for research on health and economic development.

In addition, NIDCR provided cofunding support for an FIC training award in bioethics and for multiple awards under two FIC Programs—the AIDS International Training and Research Program and the International Training and Research Program in Emerging Infectious Diseases.

Other Activities

International AIDS Activities

Oral manifestations of HIV/AIDS present many problems for patients and many opportunities for researchers. To address these problems and research opportunities, NIDCR staff contributed to two major international conferences on HIV/AIDS during FY 00.

At the 4th International Conference on the Oral Manifestations of HIV Infection, in Skukuza, South Africa, on July 4–7, 2000, the Deputy Director, NIDCR, presented opening comments, and NIDCR staff chaired two plenary workshops and presented research reports. In her remarks, the Deputy Director noted the importance of research on HIV/AIDS, as highlighted in *Oral Health in America: A Report of the Surgeon General,* which was published in FY 00. The workshop was organized to assess all aspects of the science base—basic research, population-based studies, and clinical issues. Information discussed at the meeting highlighted the importance of the oral cavity in HIV/AIDS.

Oral transmission of HIV infection is a continuing problem and was accorded high priority at the Skukuza conference and at the subsequent XIIIth International AIDS Conference, in Durban, South Africa, on July 9–14, 2000. Several NIDCR staff also participated in the Durban meeting, which extended and underscored similar topics (i.e., oral HIV transmission, microbicides, AIDS-associated viral and fungal infections, and social and epidemiologic issues).

NIDCR staff also participated in the International AIDS Malignancy Conference, sponsored by the National Cancer Institute, NIH, in Bethesda, Maryland, in May 2000, to share information on opportunistic viral infections in HIV/AIDS.

Tobacco Control Initiatives

Use of tobacco in various forms is a major risk factor for oral cancer. NIDCR is actively engaged in efforts to confront this problem nationally and internationally.

In June 2000, NIDCR supported and staff participated in an international workshop on Tobacco and Oral Diseases: Strategies for Dental Professional Interventions, in Iowa City, Iowa. Investigators from Canada, China, Hungary, Iceland, and the United Kingdom attended the meeting, to discuss tobacco-related oral diseases; prevention, control, and cessation of tobacco use; and research needs and opportunities.

In August 2000, NIDCR staff participated in several related international meetings to promote research on oral health and disease related to tobacco use. At the 2nd International Conference on Smokeless/Spit Tobacco, in Chicago, Illinois, the Deputy Director, NIDCR, presented opening remarks and an NIDCR staff member chaired a panel on patterns of use of spit tobacco around the world. Staff also participated in the subsequent 11th World Conference on Tobacco OR Health, also in Chicago, and the attached Symposium for International Tobacco Researchers.

At the NIH, NIDCR participated in the planning of the Trans-NIH International Tobacco Control Research and Training Initiative and collaborated with FIC on the development of a Request for Applications to support an international program on tobacco control research and research training.

Health Promotion

Many aspects of dental, oral, and craniofacial health and disease relate to health promotion—to improve individuals' behaviors (e.g., oral hygiene, cessation of smoking, and better nutrition and diet); to promote oral health and encourage health professionals to adopt more effective techniques and methods (e.g., sealants and diagnostic examinations); and to increase the public's awareness of oral health and disease. Health promotion is an important part of NIDCR's activities, and Institute staff provide leadership in this area both domestically and internationally.

The Deputy Director, the Associate Director for International Health, and other NIDCR staff participated in a major meeting of the International Forum on Oral Health Promotion and the European Association of Dental Public Health, in Cork, Ireland, in September 2000. The Associate Director and the International Health Officer also participated in the annual meeting of the Global Health Council, in Arlington, Virginia, in June 2000. Also in June 2000, the Associate Director presented the keynote address at the Colgate-Palmolive Symposium 2000 on Women in Dentistry, in Piscataway, New Jersey. In addition, the International Health Officer made a presentation at the dental symposium of the 1st International Conference on Rural Aging, in Morgantown, West Virginia.

A health promotion specialist in NIDCR's Office of Science Policy and Analysis was the invited keynote speaker at the 4th Congress of the Asian Academy of Preventive Dentistry, in Beijing, China, in September 2000. She spoke on planning and evaluating programs for improving oral health. In April 2000, she described the process for the NIH Consensus Conference on Diagnosis and Management of Dental Caries, for the Evidence-based Dentistry-International Collaborative Group, which met in Washington, D.C. In addition, she made a poster presentation on oral cancer, at the 78th annual meeting of IADR in Washington, D.C., in April. The health promotion specialist also worked with several foreign researchers and research administrators from Japan, Korea, and Thailand, while they were at the NIH. Another staff member from the Office of Science Policy and Analysis continued her doctoral studies at University College Medical School, London, England, where she focused on research in health promotion and health disparities.

Dental Education

Education is the "middle person" in the triad of dental research, education, and practice. Without education of health professionals in particular, the benefits of research are not translated into clinical practice, and practice is less able to inform research. NIDCR is a major link in this triad and collaborates with both researchers and practitioners to improve dental education, which, like research, has "gone global."

In FY 00, OIH staff met with representatives of the American Dental Education Association (formerly the American Association of Dental Schools) and the Interna-

tional Federation of Dental Education Associations to formulate plans for incorporating research issues into the format of a global conference on dental education, to be held in Prague, Czech Republic, on March 29–April 2, 2001. The purpose of the conference will be to identify core priorities in dental education. In addition, the Associate Director for International Health served on the planning committee for the 2nd International Women's Leadership Conference in dental education, practice, and research, which will take place in Vancouver, British Columbia, in October 2001.

NIDCR's Special Expert for International Health participated in the meeting on the Thematic Network Project Achieving Convergence in Standards of Output of European Dental Education, in Stockholm, Sweden, in September 2000. Also, NIDCR staff led a 1-week seminar on clinical trials methods at the School of Dental Public Health, University of Washington, Seattle, in August 2000. The meeting was attended by 25 health professionals and researchers from Brazil, France, Germany, Japan, Portugal, Thailand, and the United States.

Other Global Opportunities

NIDCR collaborates with other Federal agencies to promote oral health globally. For example, in FY 00, the Institute cooperated with other NIH Institutes and in partnership with the Bill and Melinda Gates Foundation, to cosponsor a request for applications developed by the National Institute of Child Health and Human Development, NIH, for research in support of a global network for studies of women and children's health. The request was issued and awards are to be made in FY 01.

In November 1999, NIDCR sponsored an NIH lecture on the Natural History of the Face, presented by a clinical neuroscientist at Southampton University, England, who authored the book *About Face*. In addition, the Deputy Director, NIDCR, met with the Croatian Minister of Health and delegates during a visit to the NIH in September 2000 to give an overview of NIDCR activities. OIH staff also provided orientation for visiting dental faculty from the Medical University of Lodz, Poland, and arranged for an NIDCR Special Volunteer to work with the Vaccine Action Initiative in Korea.

Within the U.S. Department of Health and Human Services, NIDCR staff participated in the first Global Health Workshop to develop a department-wide strategy for international health. With the U.S. Geological Survey, staff facilitated development of a joint research project with an NIH researcher investigating fluorosis in China. In FY 00, as in previous years, NIDCR contributed funds to NIH support of the multinational Human Frontier Science Program, which funds research grants and fellowships in the neurosciences and cellular biology.

Internationally, NIDCR advised on the development of the program for the World Congress on Medicine and Health, held in Hannover, Germany, in July–August 2000, during World Expo 2000. At the congress, NIDCR's Scientific Director was able to communicate the importance of oral health in several venues. Staff also participated in the Hellenic Dental Association meeting, in Crete, Greece, in October 1999.

In addition, the Associate Director for International Health continued to serve as a member of the international advisory board of the D. W. Cohen Middle East Center for Dental Education, Jerusalem, Israel. The regional center is affiliated with Hebrew University and Hadassah School of Dental Medicine, Jerusalem. In FY 00, the Associate Director sought to advance international collaborative research on fluoride for the region. Building bridges for peace through collaborative science is a major theme of the center.

Extramural Programs

In FY 00, extramural activities included 13 foreign grants, 29 domestic grants with a foreign component, and 1 domestic contract with a foreign component. Of these, 4 foreign grants and 9 domestic grants with a foreign component were new awards in FY 00. In addition, NIDCR cofunded 16 awards with FIC and provided supplemental grants for 3 FIC awards. This extramural international activity involved research institutions and scientists in 37 countries and Taiwan. The research conducted was integral to the six scientific programs in NIDCR's Division of Extramural Research, as described here.

Craniofacial Anomalies and Injuries

NIDCR's international activities in the Craniofacial Anomalies and Injuries Branch included 2 foreign grants and 11 domestic grants with a foreign component. Among the six scientific programs, this international activity was the second largest in number of awards, reflecting the Institute's emphasis on craniofacial diseases and disorders. One-fourth of all birth defects include craniofacial malformations, and frequently, the persons affected require multiple surgeries and other substantial treatments, beginning in early infancy. NIDCR's objective in this program area is to promote research that advances understanding of the underlying causes of these defects and, thereby, to enhance prevention, diagnosis, and treatment.

Cleft Lip and Cleft Palate

Investigators are analyzing the contribution(s) of genetic or environmental factors, or both, to cleft lip with or without cleft palate. During FY 00, an investigator at Johns Hopkins University, Baltimore, Maryland, was awarded a new International Collaborative Oral Health Research Planning Grant. This 2-year grant will support development of the infrastructure needed to perform an international collaborative study of the genetic epidemiology of oral clefts at five centers in Hong Kong (China), Japan, Singapore, the United States, and Taiwan. The investigator will examine the interaction of genetic and environmental risk factors for nonsyndromic oral clefts in populations with elevated rates of oral clefts. Investigators from each site will meet at Johns Hopkins University in October 2000 to discuss the standardization of protocols for collecting DNA and data on environmental exposure. A second meeting will be held in Singapore in April 2001.

Although 70% of cases of oral clefts occur as isolated abnormalities, the remaining 30% occur as part of more complex syndromes. In a second new award in FY 00, NIDCR funded a scientist at the University of Colorado Health Sciences Center, Denver, to identify the gene responsible for the autosomal-recessive syndrome CLPED1. This syndrome is characterized clinically by cleft lip and cleft palate, hidrotic ectodermal dysplasia, developmental defects of the teeth and hands, and in some cases, mental retardation. CLPED1 is highly prevalent among the population of Margarita Island, Venezuela. By comparing unaffected islanders with affected islanders, this research team was able to identify the gene for the syn-

drome as PVRL1, on chromosome 11. PVRL1 encodes nectin 1, an immunoglobulin-related, transmembrane cell–cell adhesion molecule. PVRL1 was originally isolated as a gene for poliovirus receptor, which is now known to be the principal receptor for α-herpesviruses. Mutations in the PVRL1 gene produce truncated proteins that prevent the function of cell–cell adhesion properties. Future studies will focus on the role of this gene and its interactions with other genes associated with syndromic and nonsyndromic cleft lip and cleft palate. The purpose of this research is to increase understanding of the mechanisms involved in orofacial development.

In some single-gene craniofacial disorders, clefting malformations are associated with mutations of the gene for transforming growth factor α (TGF-α). During FY 00, a researcher at the University of Iowa, Iowa City, continued to document associations between these malformations and TGF-β_3, as well as interactive effects between TGF-α and smoking. The NIDCR-supported research was conducted initially in the Philippines and then extended to Denmark, Japan, and Vietnam. Research to identify other genes linked to clefting malformations is under way.

Also with NIDCR support, an investigator at the University of Aarhus, Denmark, continued research to analyze samples collected in Denmark from 300 cases of cleft lip and palate and from 600 control subjects. The investigator previously found that smoking was associated with a moderate increase in risk of cleft lip with or without cleft palate. He found no association in cases of cleft palate only. The investigator also updated the Danish Facial Cleft Database, which will be used for genetic studies.

Another investigator, at the University of Pittsburgh, Pennsylvania, continued collaborations with investigators at the University of British Columbia, Vancouver, and Hacettepe University, Ankara, Turkey, to study an inbred Turkish population with inherited nonsyndromic cleft lip with or without cleft palate. So far, 22 families have been studied for genetic markers that may be related to cleft formation. In related work, this investigator continued a long-term study to identify genetic loci for these conditions in Asian populations and in white European populations. This research effort has been expanded to include both Beijing and Shanghai, China, and has been enlarged to allow comparison with a white Asian population in West Bengal, India.

Another study was conducted by a researcher at Ohio State University, Columbus, working with a researcher at Jordan University of Science and Technology, Irbid. The researchers focused on families in Jordan, where cultural practices of consanguineous marriages and high birth rates, combined with an expected high incidence of cleft lip with or without cleft palate, are advantageous for identifying genetic loci for these conditions. They have recruited eight families with nonsyndromic clefts and one family with unidentified syndromic clefts. The researchers are focusing on genetic markers on chromosome 4.

These and other researchers aim to clarify the genetic and environmental aspects of cleft lip with or without cleft palate, and still other researchers are exploring ways to improve surgical treatment for these malformations. Cleft lip and palate is one of the most common congenital malformations, occurring in about 1 in 750 births. Surgical repair, which typically is performed within a child's 1st 2 years, generally is associated with retrusion of the upper jaw, possibly related to surgical repair of the lip. In FY 00, NIDCR concluded support for a prospective, randomized, controlled study to assess outcomes of two surgical techniques for repairing cleft lip. This study involved collaboration between investigators at the University of Florida, Gainesville, who used one technique, and investigators at the University of São Paulo, Brazil, who used another method. The study included 200 infants with cleft lip and 200 infants without cleft lip.

Papillon-Lefèvre Syndrome

During FY 00, a research team led by a scientist at the University of Pittsburgh, Pennsylvania, announced discovery of the gene responsible for Papillon-Lefèvre syndrome, a rare condition that results in total loss of teeth by young adulthood. The finding of this gene may yield clues to the process of periodontal disease, which also results in loss of teeth and affects many individuals. This discovery was made possible by the use of DNA samples from Turkish families. (See also the section on "Highlights of Recent Scientific Advances Resulting From International Activities.")

Comprehensive Research Centers

In FY 00, NIDCR supported two Comprehensive Oral Health Research Centers of Discovery to study craniofacial development and disease. One of the Centers of Discovery is located at the University of Iowa, Iowa City. The work at this center encompasses a number of projects, including a study conducted in collaboration with investigators at Zhabei Eye Hospital, Shanghai, China. The scientists are searching for genetic causes of cleft lip and cleft palate in 150 families from the Shanghai region and in 145 families at sites in Columbus, Ohio, and Pittsburgh, Pennsylvania. The families have multiple members affected with cleft lip and cleft palate. The scientists will perform analysis to detect genetic linkages in an additional 100 patients and their parents in Shanghai. The population in Shanghai is relatively homogeneous, which will enhance investigators' ability to identify and map genetic loci for this condition. Information from the study will increase understanding of normal and abnormal craniofacial development and may lead to therapies to prevent cleft palate.

The second center, the Center for Craniofacial Development and Disorders, is located at Johns Hopkins University, Baltimore, Maryland. Researchers are studying normal craniofacial development and the pathogenesis of craniosynostosis, oral clefting, and other craniofacial disorders in zebra fish, mice, rabbits, and humans, from the genetic to the molecular level, by using a multidisciplinary, interactive approach. One project involves collaboration with researchers at clinical sites in Buenos Aires, Argentina; Sofia, Bulgaria; Prague, Czech Republic; and Mexico City, Mexico. Multiple families from these four sites, in addition to families from Maryland, are being used in a genome-wide search to identify genes contributing to the risk of oral clefts. Preliminary findings indicate a region on chromosome 2 that shows evidence of a susceptibility gene. Further fine-mapping studies are under way. The researcher is collaborating with a researcher at the National Human Genome Research Institute to compare and confirm the findings with samples from multiplex Syrian families. The information gained from this linkage

study may help to identify novel genes controlling risk of oral clefts, which would enhance understanding of the cause(s) of this common craniofacial malformation and possibly help to identify individuals at highest risk.

Biomineralization

Investigators at three institutions collaborated on a multidisciplinary program project to obtain fundamental information on the development of mineralized tissues, such as tooth enamel. Investigators at Forsyth Dental Center, Boston, Massachusetts, McGill University, Montreal, Quebec, and the University of Texas Health Science Center, San Antonio, used state-of-the-art techniques to pursue a hypothesis that the formation of highly organized enamel tissue results from a set of genetically controlled processes. The Canadian investigators contributed their specific expertise to the integrated project by focusing on cellular processes that control the composition, transport, and deposition of mineral ions. The information gained from the entire project will ultimately be useful for designing new methods for preparing biomaterials, preventing and diagnosing diseased mineralized tissues, and developing procedures for regenerating damaged and diseased mineralized tissues.

A scientist at the Weizmann Institute of Science, Rehovot, Israel, continued to define the structure of proteins involved in the formation of teeth and bones. Information gained from these studies will enhance understanding of how teeth and bones are formed and how they function mechanically. Studies carried out in FY 00 focused on the structural properties of the tooth and used new techniques to examine strength and structural integrity.

Infectious Diseases and Immunity

International activities in NIDCR's Infectious Diseases and Immunity Branch included support for six foreign grants and six domestic grants with foreign components. In collaboration with FIC, NIDCR also cofunded 3 awards under FIC's AIDS International Training and Research Program (AITRP) and 13 awards under FIC's International Training and Research Program in Emerging Infectious Diseases (ITREID). In addition, NIDCR supported two supplemental grants for AITRP awards and one supplemental grant for an ITREID award. The Infectious Diseases and Immunity Branch supported the largest amount of international activity, in both number of awards and dollars, in the extramural program. This activity reflects the global importance of dental caries, periodontal diseases, and HIV infection and AIDS, as well as other emerging infectious diseases. The complex environment of the oral cavity presents a formidable challenge for selectively controlling pathogenic microbes that infect and reinfect hard and soft oral tissues of both healthy persons and those with compromised immunity.

Oral Biofilms, Dental Caries, and Periodontal Disease

In FY 00, NIDCR made three new awards to two foreign investigators. One investigator, at the University of Western Ontario, London, Ontario, is studying bacteria-associated antigens important for the immune and inflammatory response in human periodontitis. Human periodontal diseases are heterogeneous and result from specific bacteria–host immune interactions. Periodontitis is the major cause of tooth loss in adults and is a significant risk factor associated with coronary heart disease, stroke, and bacterial pneumonia. In collaboration with an investigator at the University of Toronto, Ontario, the NIDCR awardee is exploring the periodontal immune response to *Actinobacillus actinomycetemcomitans,* a cause of juvenile periodontitis, in an animal model of immunodeficient mice engrafted with human peripheral blood leukocytes. It is expected that the study will elucidate critical immune functions in periodontitis and may suggest ways to enhance or interfere with the immune activity.

NIDCR also provided research training support for a scientist at the University of Leeds, England, who is studying the effects of dentin matrix proteins on mineralization. The long-term objective is to understand the role of the extracellular matrix protein, phosphoprotein, in biomineralization, especially of dentin. Dentin phosphoprotein has a great affinity for both calcium and collagen and appears to be the nucleator of mineral and the controller of crystal growth in dentin. These possible roles will be explored with use of recombinant technology and an in vitro system. With better understanding of the role of this protein, scientists may be able to develop preventive or therapeutic methods to ameliorate tooth aplasia and malformations and to correct faulty repair of cavities.

In addition, NIDCR funded two new domestic grants with foreign components. One grantee received a 2-year International Collaborative Oral Health Research Planning Grant to develop the infrastructure for an international, multisite collaboration to evaluate and quantify the independent contribution of oral infections and inflammation to the onset and progression of cardiovascular and cerebrovascular diseases. The investigator at the University of Minnesota, Minneapolis, is establishing collaborations with investigators at the University of Texas, San Antonio; five major foreign sites, in Australia, Finland, Germany, Ireland, and Scotland; and two additional foreign sites, in Australia and Haiti. The collaboration would enable the testing of hypotheses in populations that have varying degrees of periodontal and cardiovascular diseases. Epidemiologic, human, and animal studies would be included.

The second grantee, at the University of Florida, Gainesville, is working with a researcher at the University of Otago, Dunedin, New Zealand, to develop an improved method for vaccinating against dental caries. The New Zealand researcher maintains a special established colony of microbiologically defined mice that will be used for anticaries vaccination experiments. The researcher has considerable experience in using this model to study oral colonization by streptococci.

In FY 00, NIDCR also continued support for two grants to foreign scientists to accelerate basic research on the environmental conditions, physiology, and genetics of oral biofilms (plaque), a major cause of dental caries and periodontal disease. Applying their specific expertise, these scientists are improving understanding of (a) the formation of biofilms and (b) the possibilities for designing therapeutic strategies based on establishing nonpathogenic dental plaque.

In one study, researchers at the University of Toronto collaborated with other researchers at the University of Florida, Gainesville, to understand streptococcal communication in oral biofilms associated with dental caries. Looking at the exchange of genetic material in situ by the bacteria

Streptococcus mutans, a primary cause of dental caries, they found that the exchange of material is greater in biofilm than when the bacteria are growing in culture medium. The biofilm bacteria may be using a unique peptide to communicate and regulate gene expression under high-density situations.

The second study was conducted by a team of scientists in Sydney, Australia, at the Institute of Dental Research, the University of New South Wales, and Macquarie University. These scientists used a unique method, biofilms expression technology, to detect bacterial genes involved in enhancing gene expression associated with high-density colonization and growth of bacteria on biofilm. The scientists found differences between protein expression in growth under planktonic (free-growing) and biofilm conditions.

An investigator at the University of Tennessee, Knoxville, collaborated with investigators at the University of Otago, Wellington, New Zealand, on a complementary study, to develop and use advanced technology to analyze the structure and function of microbial communities on biofilm. These investigators found differences in the fatty-acid composition of streptococci grown in anaerobic planktonic conditions and in biofilm conditions.

NIDCR also supported a joint study by scientists at Forsyth Dental Center, Boston, and the University of Göteborg, Sweden. The aim of this study is to control periodontal infections by suppressing or eliminating the organisms that cause them. In FY 00, the scientists focused on recruitment of patients and on standardization and calibration of evaluation procedures and clinicians at both the U.S. and Swedish sites. They also initiated analysis of clinical specimens obtained during 12 years after therapy. Such longitudinal history data have not been available in the United States.

Comprehensive Research Center

NIDCR continued support for a Comprehensive Oral Health Research Center of Discovery, at the University of Washington, Seattle, where researchers are addressing the basis of oral and craniofacial health and susceptibility to disease in children, as a key to lifelong oral health. During FY 00, the center also provided research training in a summer institute of clinical dental research for students from Brazil, Germany, Japan, Portugal, Thailand, and the United Kingdom.

Two of the center's research projects involved foreign investigators and sites. In one project, a 5-year clinical trial, the effectiveness of a motivational interviewing approach in preventing early childhood caries is being evaluated among more than 200 healthy infants (12- to 18-months of age) and their mothers, from a Punjabi community in Surrey, British Columbia. In FY 00, the investigator, who is from the University of British Columbia, Vancouver, conducted focus groups to learn more about the participants and how best to motivate them. Early childhood caries is a severe disease of infants and toddlers that has a lasting effect on dentition and is especially prevalent among disadvantaged and immigrant populations.

In the second project, a researcher at the University of Washington, Seattle, planned for the recruitment of patients for a 3-year, cross-sectional study to assess the correlates of and specific risk factors for early-onset periodontitis among more than 1,000 children in Senegal. This project complements additional ongoing studies of women's oral health and disease that are being conducted by the investigator in Senegal. Early-onset periodontitis includes a number of periodontal diseases in children and adolescents that result in rapid destruction of periodontal attachment, leading to pronounced tooth loss.

HIV Infection and AIDS

The same researcher at the University of Washington collaborated with researchers at the University of Dakar, Senegal, on another project, to evaluate the association between oral sex and HIV infection among female commercial sex workers in Senegal. The researchers will assess the conversion to HIV seropositivity after oral sex, the amount of viral load in the saliva of seropositive women, and reductions in the HIV load resulting from treatment of oral inflammatory diseases. The study will expand understanding of the spread of HIV in Africa and of the biology of oral transmission of HIV. During FY 00, the researcher recruited participants for the study.

In addition, NIDCR continued cofunding of three AITRP awards to train oral health professionals from developing countries to address the AIDS epidemic more effectively through research. The awards are made to investigators at the University of California School of Public Health, Berkeley; the University of Maryland, Baltimore; and the University of Washington, Seattle. In FY 00, NIDCR also funded two new supplemental AITRP awards to support international research training. One award was made to an investigator at Baylor College of Medicine, Houston, Texas, to support long-term U.S. training and the development of collaborative research relationships with investigators from African countries, Mexico, and Romania. The second award was made to an investigator at Harvard University, Boston, Massachusetts.

Herpesvirus

Researchers at Eastman Dental Institute, University of London, England, continued to examine the factors influencing oral expression of human herpesvirus 8 (HHV-8) in patients with HIV. Applying a highly sensitive and technically difficult technique to identify cell types in the oral mucosa, the researchers found HHV-8 in oral tissues of persons with non-Kaposi's sarcoma and noted that the genetic material of virus isolated from the oral cavity had areas of high mutation. No particular genotype of HHV-8 was prevalent in the oral lesions. The researchers have a substantial number of archival samples of oral tissue.

Emerging Infectious Diseases

During FY 00, NIDCR continued to cofund research and training for participants from developing countries in 13 ITREID centers at universities in the United States. These centers provide a focus for strengthening the research infrastructure and training personnel to address emerging and reemerging infectious diseases worldwide.

In collaboration with FIC, NIDCR also continued to support a supplemental award for an ITREID grant to study the devastating disease, noma, among children in Niger and Nigeria. Working with investigators in these countries, the grantee from the University of Maryland, Baltimore, continued to try to identify the etiology and pathogenesis of noma. Although the disease is associated with nutrition, it appears to be multifactorial. Poverty and compromised immunity are contributing factors, and noma has been as-

sociated with preceding cases of measles and exposure to microbes from farm animals. In conjunction with researchers at the University of North Carolina, Chapel Hill, the investigators are exploring a possible molecular basis for children's susceptibility to this disease, and additional efforts are under way to examine bacteria found in the earliest stages of the lesion.

To stimulate scientific interest in noma, NIDCR cosponsored a workshop entitled Noma: Building a Research Infrastructure for Developing Countries, at the NIH, in Bethesda, Maryland, in April 2000. The other sponsors were the NIH's Office of Rare Disorders and Office of Dietary Supplements. The participants emphasized the need for global support and collaboration, nutrition research, and development of infrastructure to facilitate disease prevention and health promotion, and they recommended organization and maintenance of databases and coordination and standardization of epidemiologic methods.

Neoplastic Diseases

International activities in NIDCR's Neoplastic Diseases Branch included one foreign grant and three domestic grants with foreign components, one of which was a new award. The researchers are addressing three areas: prediction of risk for developing head and neck cancer, prediction of clinical outcomes, and selection of appropriate therapies for these malignant diseases. The Chief of the Branch represented NIDCR at the 5th International Conference on Head and Neck Cancer, in San Francisco, California, in July–August 2000, and at the XVth Congress of the European Association for Cranio-Maxillofacial Surgery, in Edinburgh, Scotland, in September 2000.

International Centers Against Oral Cancer

In FY 00, NIDCR awarded a 2-year International Collaborative Oral Health Research Planning Grant to an investigator at Strang Cancer Prevention Center, New York, New York. The grant will support the organization of a multi-institutional collaboration of seven international centers against oral cancer, which are affiliated with existing research institutions in São Paulo, Brazil; Bombay, India; Amsterdam, the Netherlands; Singapore; and New York, New York, which has three centers. The centers would conduct an international epidemiologic trial to investigate factors responsible for the changing trends in the incidence of oral cancer, including genetic susceptibility; to determine the effect of environmental factors on the pattern of genetic mutations; and to develop a strategy for medically preventing oral cancer in high-risk populations. Overall, the centers evaluate more than 3,000 patients with oral cancer each year and have tremendous capacity for addressing critical issues in head and neck cancer.

Genetic Markers of Oral Cancer

Investigators in British Columbia continued to evaluate the temporal patterns of clonal changes in oral lesions of patients at high risk of oral cancer. Two hypotheses are being tested: (a) that loss of alleles in cells scraped from these lesions can independently predict risk of lesions progressing abnormally in patients who do not have cancer and (b) that information obtained from cells scraped from former cancer sites can predict the clinical outcome during follow-up for patients with oral cancer. The research team, consisting of investigators from Simon Fraser University, Burnaby, the British Columbia Cancer Agency, the University of British Columbia, and Vancouver General Hospital, is building on previous work with archival tissue samples. Samples are being obtained over a 2-year period from 50 patients with oral dysplasia and 100 patients with squamous cell cancer. The samples will be assayed for allelic loss at eight specific chromosome arms. If the pattern of allelic loss is predictive of clinical outcome, this noninvasive approach could be used by clinicians to identify patients requiring more aggressive treatment and to monitor the success of treatments.

In another study, scientists from the University of Michigan, Ann Arbor, led a research team seeking to determine predictive markers for selection of appropriate therapies for patients with oral cancer. They will collaborate with investigators from Loyola University, Chicago, Illinois, and Institut Curie, Paris, France, in efforts (a) to determine whether overexpression and mutation of the p53 gene predicts response to chemotherapy, organ preservation, or survival and (b) to discover how such an effect is influenced by expression of the Bcl-2 and Bcl-x proteins, which block cell death. These investigators are testing the hypotheses in in vitro experiments with known cell lines and in tumor samples obtained from patients before and after organ-sparing therapy and from patients undergoing conventional therapy. If shown to be predictive, these potential markers could be evaluated further in clinical trials. Predictive markers for identifying patients with oral cancer who are likely to respond to various treatments are a critical need.

Genes for Gingival Fibromatosis

In addition, an investigator at the University of Pittsburgh, Pennsylvania, collaborated with other investigators to identify and characterize genes that cause hereditary gingival fibromatosis—a condition involving overgrowth of the keratinized tissues surrounding the teeth. The other investigators in this study are from the Center for Human Genetics, Louvain, Belgium, and the State University of Campinas, Piracicaba, and the University of Taubate, São Paulo, Brazil. Tissue samples were obtained from Brazilian families affected by this disease, and the Belgian investigator, who is an expert on the disease, consulted on the delineation of genetic aberrations. The investigators are using molecular and cytogenetic approaches to map the loci of candidate genes and to identify specific gene mutations.

Chronic Diseases

NIDCR's international activities in the Chronic Diseases Branch included two foreign grants and one new domestic grant with a foreign component. These grants focus on two major chronic conditions affecting the dental, oral, and craniofacial complex: dental and orofacial pain and temporomandibular disorders (TMD). Other conditions addressed in the Branch include osteoporosis and related bone disorders; neuropathies and neurodegenerative diseases; autoimmune diseases (e.g., Sjögren's syndrome); and oral conditions (e.g., periodontitis) related to systemic diseases (e.g., diabetes mellitus and cardiovascular disease).

International Research Consortium on Temporomandibular Disorders

In FY 00, NIDCR awarded a new, 2-year International Collaborative Oral Health Research Planning Grant to an investigator at the University of Washington, Seattle, to

conduct multinational research into the cause, prevention, and management of TMD. The university has been the center for developing standardized examination and diagnostic approaches, such as the Research Diagnostic Criteria for TMD. The investigator will organize a consortium of researchers at institutions in Sydney, Australia; Helsinki, Finland; Halleand Leipzig, Germany; Tel Aviv, Israel; Singapore; and Linköping, Sweden. Other U.S. participants include the State University of New York, Buffalo, the University of Michigan, Ann Arbor, and the University of Minnesota. Minneapolis. For subsequent cross-national studies of TMD, the consortium will train international researchers to administer the standardized test for TMD (Research Diagnostic Criteria for TMD).

Pain Mechanisms

Two investigators at the University of Toronto, Ontario, continued long-term studies to clarify brain stem mechanisms involved in dental and orofacial pain. During FY 00, one investigator addressed the relationship of specific brain stem nuclei to central sensitization of thalamic neurons. The findings demonstrate that thalamic nociceptive neurons can be sensitized by orofacial nociceptive afferent input, induced by the inflammatory irritant to dental pulp (mustard oil). The brain stem nuclei mediating this effect include the subnucleus caudalis of the trigeminal brain stem complex, which appears to play a role in changes in neuroplasticity in the neurons in other structures of the central nervous system. Another investigator completed studies of the reflex pathways in the trigeminal subnucleus caudalis. His research, which has enhanced understanding of the relationship between nerve fibers in the temporomandibular joint and pain reception in the central nervous system, supports the importance of the central nervous system in pain reception.

Biomaterials, Biomimetics, and Tissue Engineering

NIDCR's international activities in the Biomaterials, Biomimetics, and Tissue Engineering Branch included two domestic grants with a foreign component, one of which was a new award in FY 00. The aim of the Branch is to encourage research on the development and utility of natural and synthetic materials used to repair, regenerate, restore, and reconstruct oral and craniofacial tissues and organs.

Tissue Engineering and Regeneration

In FY 00, NIDCR supported a new award for the design of scaffold architecture to facilitate healthy regeneration of bone. The grantee, at the University of Michigan, Ann Arbor, is collaborating with an investigator at the Swiss Federal Institute of Technology, Zürich, to verify that scaffold architecture influences early vascularization and deposition of tissue matrix, as well as long-term bone and mechanical stiffness, and that early vascularization and mineralization lead to formation of long-term bone and sufficient mechanical stiffness. Three different scaffolds constructed of hydroxyapatite will be tested in a minipig mandible model. Numerous studies indicate that a biomaterial scaffold can significantly influence the outcome of tissue-engineering treatments. The results from this study will help researchers to optimize the performance of biomaterial scaffolds through computational design to enhance the structure and function of regenerated tissue.

Choosing Among Dental Treatments

NIDCR also supported a study to test the feasibility and validity of two methods for measuring a population's preferences and choices for three types of dental treatment: dentin regeneration, a new technology; tooth extraction; and root canal therapy. The two methods, healthy time equivalent and willingness to pay, have been useful in health economics and clinical decision making but have not been applied in dentistry. This study, based at the University of Michigan, Ann Arbor, includes a health economist from MacMaster University, Hamilton, Ontario, who contributes to the development and pilot testing of the measurement tools, training of interviewers and the research team, and analysis of data. The investigators are conducting telephone and home interviews with two population samples of more than 400 adults each, with or without dental insurance, in southeast Michigan. This study is the first to test a model for adopting or choosing dental treatments. The results will be useful to health care providers and policy makers when they select among alternative treatments and health outcomes.

Clinical, Behavioral, and Health Promotion Research

International activities in NIDCR's program of Clinical, Behavioral, and Health Promotion Research included two foreign grants, one domestic contract with a foreign component, and six domestic grants with a foreign component. This program had the second largest amount of international activity, in terms of dollars, within the Division of Extramural Research, which reflects the Institute's emphasis on clinical research. International studies in this area can be particularly informative because they address the interactive roles of socioenvironmental, behavioral, genetic, and biomedical factors in dental, oral, and craniofacial diseases and conditions in a variety of cultural settings.

Oral Health in Children

In FY 00, NIDCR awarded two new International Collaborative Oral Health Research Planning Grants to plan large, international studies of oral health in children. One of the 2-year grants was awarded to a researcher at the University of Dundee, Scotland, to develop detailed plans and preliminary data for collaborative research on childhood dental caries, a significant health problem worldwide for ethnically diverse, socioeconomically deprived populations. The researcher will coordinate the activities of 32 established scientists in 10 countries. The consortium includes five WHO Collaborating Centers and receives support through WHO's European Regional Office. For the planning grant, the aims are to identify and set priorities for key research questions; determine the nature and virulence of caries-associated microflora and the effects of fluoride on this flora; develop measures of familial and cultural perceptions and beliefs that contribute to development of caries; and identify associations between caries in early childhood and access to and use of health care services. The exploratory studies involve children at high risk of caries, from five ethnic groups—Africans and African Americans, Asians of the Indian subcontinent, Chinese, Hispanics, and whites.

Another planning grant was awarded to an investigator at the University of Medicine and Dentistry at New Jersey, Newark, to

plan a research protocol to develop, standardize, and validate a culturally sensitive measure of oral health–related quality of life in children. The investigator has assembled a research team from Canada, Denmark, France, New Zealand, Sweden, the United Kingdom, and the United States. This team is organizing an extended coalition of clinicians and researchers to generate and pretest items; developing methods for pretesting items in different countries; and preparing the research proposal for development and validation of the instrument. Limited information is available on the oral health–related quality of life in children, partly due to the lack of psychometrically sound measures for use in children. Development of the measure could accelerate opportunities to study variations in children's oral health in different cultures and health care systems.

In addition, NIDCR provided new support to an investigator at the University of Pittsburgh, Pennsylvania, to study the transmission of *Streptococcus mutans* from mothers to infants. The study will be a longitudinal, randomized, clinical trial conducted in Bauru, São Paulo, in collaboration with investigators at the University of São Paulo, Brazil. The specific aims are to determine whether salivary levels of *S. mutans* can be reduced in mothers of young infants by a variety of interventions (e.g., restorative procedures, topical fluorides, and oral hygiene); whether the interventions reduce or delay acquisition of the bacteria in infants; and whether the children's incidence of caries is subsequently reduced. Dental decay is the most prevalent affliction of children worldwide, and the incidence of caries is still high in newly industrial countries such as Brazil, especially among lower-income groups. Studies suggest that decay and the establishment of *S. mutans* in the oral cavity can be reduced or prevented in young children by treating mothers who are highly infected, before eruption of the children's primary teeth. The Bauru community offers a good test site: it lacks water fluoridation, has a high level of unmet needs for dental care, is in a sugar cane region offering frequent access to sugar, and boasts the leading dental school in Central and South America.

Oral Health in Older Adults

A scientist at the University of Toronto completed a secondary analysis of existing data, to evaluate a hypothetical association between tooth loss and age-related hearing loss in healthy men. The hypothesis was based on evidence suggesting that the processing of orofacial information and the processing for auditory information are interactive. The investigator, in collaboration with researchers at the Veterans Health Administration Boston Outpatient Clinic and Tufts University, Boston, Massachusetts, and the University of North Carolina, Chapel Hill, analyzed data from a 20-year follow-up in the Veterans Affairs Normative Aging and Dental Longitudinal Studies. The study participants were approximately 1,230 men residing in the Boston area. Findings indicate that changes in dental predictors (i.e., losing two or more teeth since baseline, with several different cutoff points for baseline measures) were significantly associated with decreased hearing sensitivity at follow-up. Loss of molar tooth support also was significantly associated with greater hearing declines from baseline, which the investigator suggested may be attributed to changes in the vertical dimension of occlusion and associated changes in acoustic immitance. The data suggest that preservation of natural teeth may contribute to aural health in older adults, which may in turn help to prevent or reduce social and functional disability.

In a continuing study, researchers at the University of Washington, Seattle, and the University of British Columbia, Vancouver, tested whether a simple home-care regimen of rinsing with chlorhexidine and fluoride reduced tooth loss in an ethnically diverse population of adults aged 60–75 years who previously had oral disease and irregular dental care. In FY 00, the 4th year of funding, the researchers continued to perform follow-up on the participants at both sites. All baseline data for this 5-year clinical trial have been collected for 700 participants in Seattle and 400 participants in Vancouver, and retention rates at the 2-year follow-up are higher than expected. About 49% of the Seattle participants are ethnic minorities (African Americans, Asians, and Hispanics), and many of the Vancouver participants are Indo-Chinese recruited from senior centers.

Cross-Cultural Studies of Pain

In FY 00, investigators at the University of Washington, Seattle, completed a collaboration with investigators in China, Denmark, Sweden, and Taiwan, in a 5-year study to evaluate how patients and health care providers view acute and chronic pain. The investigators used medical anthropology and psychometric methods to obtain and compare qualitative and quantitative data on perceptions and remedies for pain associated with tooth drilling, persistent facial or jaw pain, or labor and childbirth. The study demonstrated the usefulness of these methods for understanding the cultural contexts of pain reactions and remedy preferences. The results show that ethnicity has a major influence on both perceptions of pain descriptors and perceived needs for remedies, whereas professional socialization has more influence on perceptions of remedies. Dental and medical professionals did not differ from patients in their perceptions of pain description, but they did differ in their perceptions of remedies. The investigators also identified cultural cognitions and nuances that have clinical and theoretical relevance. Enhanced understanding of the effect of cultural influences on perceptions of pain and on strategies for coping with pain will be useful for improving communications between patients and providers about diagnosis and treatment of pain.

Effects of Dental Amalgam on Health

Researchers at the University of Washington, Seattle, and the University of Lisbon, Portugal, continued their collaboration in the Casa Pia Study of Dental Amalgams in Children. This clinical trial involves 500 children, aged 8–10 years, who are students at the Casa Pia Schools in Lisbon and who need extensive dental restorative treatment. All the children receive dental care and are randomly assigned to one of two treatment groups. Tooth restoration is performed with dental amalgam in one group and with composites in the other group. The objective is to determine whether dental amalgam affects health. Children in the mixed dentition stage offer a special opportunity for study, because dental amalgam is the material most frequently used for restoration of their teeth and they may be most susceptible to its effects on health. During FY 00, the researchers completed the 2nd year of follow-up and started follow-up visits for the 3rd year. Data will be analyzed and results will be identified during the next several

years of this blinded study. Interim analyses of data on demographic and oral health issues revealed that the children have additional carious lesions at a rate higher than expected, which may be due to lack of fluoridation and generally poor oral health behaviors.

Amalgam and Resistant Bacteria

Another investigator at the University of Washington, Seattle, continued to assess the effect of dental amalgam on the development of resistance by oral bacteria to antibiotics or mercury. Specific aims include determining whether treatment with dental amalgam or composites alters the prevalence of oral bacteria that are resistant to antibiotics or mercury and whether acquisition of resistant bacteria in the oral cavity is linked with enteric acquisition of such bacteria. In FY 00, the investigator completed the 2nd year of follow-up of oral and urine samples from 140 of the 150 children participating in the Casa Pia Study. Reporting preliminary results, the researcher identified bacteria associated with periodontal disease that appear to be resistant to antibiotics and has proposed a genetic mechanism for acquiring resistance to mercury and antibiotics. These results also indicate a mechanism by which mercury from amalgam may contribute to development of antibiotic resistance in many bacteria, including those outside the oral cavity. Additional studies are under way.

Cleft Lip and Cleft Palate

In addition, researchers at the University of Florida, Gainesville, and the University of São Paulo, Brazil, compared the effects on the level of speech competence of two surgical approaches for repairing cleft lip and cleft palate. Recruitment into this prospective, randomized, clinical trial continued in FY 00. The findings could provide a gold standard for evaluating and comparing speech benefits from new or modified surgical procedures and approaches for repairing cleft lip and cleft palate.

International Meetings

In FY 00, NIDCR supported 22 international conferences, all of which were held in the United States. Three Gordon Research Conferences addressed biomineralization, cellular and molecular mycology, and periodontal disease. NIDCR supported the World Congress on Osteoporosis 2000, in Washington, D.C., and the 6th World Biomaterials Congress, in Minneapolis, Minnesota.

In collaboration with the NIH Office of Rare Diseases, NIDCR sponsored the international workshop entitled Enhancing Clinical Research in Sjögren's Syndrome: Critical Issues and the international conference entitled Noma: Building a Research Infrastructure for Developing Countries. The NIH Office of Dietary Supplements also supported the meeting on noma.

Other NIDCR-supported conferences focused on small genomes, post-transcriptional control of gene expression, alternative approaches to managing carious lesions, mucosal injury in cancer patients, saliva in health and disease, microbial polysaccharides, oral manifestations of HIV infection, biofilms, enterococci, nutrition and oral infectious disease, genomics and bioinformatics, and mouse molecular genetics. NIDCR also provided support for the annual Hinman Student Research Symposium, in Memphis, Tennessee, which attracts dental students from throughout the world.

In addition, more than 60 NIDCR scientists and science administrators traveled to 28 countries to participate in international meetings or meet with research collaborators. They gave invited lectures and keynote speeches; chaired scientific sessions; attended planning meetings; presented courses and seminars; discussed collaborative studies; and exchanged information on research advances and opportunities in dental, oral, and craniofacial health. They also presented talks at local universities, visited scientific laboratories to discuss or conduct collaborative research, and provided consultation on matters of oral health.

Both the NIDCR Director and the NIDCR Scientific Director extended their leadership to this scientific outreach. The NIDCR Director delivered a keynote address on Future Trends in Dentistry, at the International Dental Exhibition and Meeting, in Singapore, in April 2000, and in May, lectured on the Prospects for Dental Science, Education, and Practice in the 21st Century, at the 22nd Asia Pacific Dental Congress and the 19th General Meeting of the Japanese Association for Dental Science, in Tokyo. In March, he provided a videotaped lecture for the 1st Smile Train Cleft Lip and Palate Symposium, in Beijing, China. He also spoke at the 7th DMA (Dental Manufacturers of America, Inc.) World Dental Trade Conference, in Chicago, Illinois.

The Scientific Director participated in the conference on Dental Plaque Revisited, at the University of London, England; the International Conference on Microbial and Model Genomes, in Paris, France; the Endo 2000 Seminar, in Toronto; the International Workshop on Oral Manifestations of HIV Infections, in Skukuza, South Africa; and the Medicine Meets the Millennium conference, in Hannover, Germany. In organizing a 2001 Gordon Research Conference, he traveled to Pisa, Italy, to review a potential site for the conference.

Also, the Director, Division of Extramural Research, was a keynote speaker at the Satellite Symposium on the Physiology and Pathophysiology of Salivary Glands and attended the annual meeting of the Chilean Society of Physiological Sciences, in Santiago, in late September–early October 2000.

Intramural Programs and Activities

The NIDCR Division of Intramural Research is a major participant and leader in international research. During FY 00, NIDCR intramural scientists offered their expertise in many ways. They collaborated with investigators at foreign laboratories; organized and chaired sessions at major international meetings; presented invited lectures and research findings; provided consultation on the direction and progress of research; supplied important biological reagents and other materials to scientists and laboratories around the world; reviewed research grants for foreign institutions; provided leadership for professional societies; served as editors of international journals; coauthored numerous publications with foreign scientists; and published reports of research in numerous international journals.

In addition, more than 90 foreign scientists, representing 27 countries, collaborated with U.S. investigators in NIDCR's intramural laboratories. Approximately 25% of the scientists came from Japan, and another 20% came from China and Israel. Countries represented by 1–5 scientists each included the following: Argentina, Australia, Belgium, Bulgaria, Canada, Denmark, France, Germany, Greece, Hungary, India, Iran, Italy, Korea, Mexico, Mongolia, the Netherlands, New Zealand, the Philippines, Russia, Spain,

Thailand, Turkey, and the United Kingdom. NIDCR intramural investigators also collaborated on research projects at institutions in 24 countries. In addition to many of the countries mentioned here, the investigators collaborated with scientists in Austria, Brazil, Burkina Faso, Chile, Ireland, Poland, South Africa, Sweden, and Switzerland, as well as Taiwan.

Craniofacial Developmental Biology and Regeneration Branch

Researchers in NIDCR's Craniofacial Developmental Biology and Regeneration Branch are exploring fundamental questions about mechanisms of development, organization of tissues, differentiation of cells, and cancer. During FY 00, scientists from France, Israel, Japan, Korea, New Zealand, and Spain contributed to research in the Cell Biology Section. They focused on the effect of the extracellular matrix on development and in cancer, specifically the differentiation of salivary glands and the growth and metastasis of tumor cells. The ultimate aim for research on salivary glands is to establish a biologically based, targeted approach for treating salivary hypofunction and regenerating secretory tissue. Reduction or loss of salivary function occurs with Sjögren's syndrome, head and neck irradiation, and use of certain medications. Using high-density cDNA (complementary DNA) arrays, the scientists identified a gene that is induced during differentiation of human submandibular gland cells in the presence of laminin (a component of the basement membrane) and that regulates the size and rate of formation of acini (small, sac-like dilatations) in the oral cavity. The scientists also identified several genes that are important in gland development.

In addition, scientists in the Branch used more than 600 overlapping peptides to define the active sites on laminin 1 that are important for interactions among endothelial cells and for growth and metastasis of tumor cells. They identified several active sites and their cellular receptors, including four sites that are specific to endothelial cells. In collaboration with scientists in the Branch's Molecular Biology Section, they screened more than 700 overlapping peptides derived from laminin 1 and found two that are highly active with tumor cells, in the lung and liver. In collaboration with scientists at the National Institute of Bioscience and Technology, Japan, and the University of Pittsburgh, Pennsylvania, scientists in the Section continued efforts to simulate and enhance the anti-angiogenic, antitumor, and antimetastatic activity of the laminin peptide YIGSR. They also continued studies to explain why breast and prostate cancer cells preferentially metastasize to bone. They identified the active factor in bone extracts as osteonectin and found that it activates proteases in breast and prostate cancer cells, but not in cells that do not metastasize to bone or bone extracts. In animal experiments, osteonectin caused an increase in the total number of metastases. The ultimate aim is to develop new treatments for cancer.

Also in the Molecular Biology Section, scientists from China, Denmark, Japan, and Spain continued research on skeletal dysplasia (abnormal development), using a mouse model deficient in perlecan, a component of extracellular matrix that is thought to affect the growth and differentiation of cells by modulating the activities of growth factors. The scientists previously created this mouse model. The perlecan-deficient mice have both skeletal and craniofacial abnormalities and die soon after birth. The findings suggest (a) a link between perlecan and the activity of growth factors in formation of the skeleton and (b) multiple roles of perlecan in development. The research is partially supported by a grant from Seikagaku Corporation, Japan.

During FY 00, the Head of the Section presented research findings at international meetings in France, Israel, Japan, the United Kingdom, and the United States. She served on the editorial board of the International *Journal of Biochemistry and Cell Biology* and was internationally elected to the board of the Metastasis Research Society.

In the Developmental Mechanisms Section, scientists from Bulgaria, China, Israel, Japan, Mexico, and the Netherlands continued to focus on molecular mechanisms involved in specific interactions of cells with extracellular matrix or other cells. They explored how these interactions are translated into signal transduction within cells and during organization of the cytoskeleton. The researchers are elucidating the regulation of these processes in the adhesion and migration of cells, the invasion of tumor cells, and other processes critical to embryonic development, malignant conditions, and AIDS. Understanding of these processes could lead to new approaches for prevention and treatment of disease, including tissue engineering and vaccines. Work continued on describing the regulatory functions of the human tumor-suppressor protein PTEN, which is implicated in 10%–50% of many different cancers; identifying proteins involved as novel signaling molecules in the adhesion of cells and the organization of the cytoskeleton; developing an artificial salivary gland; and identifying a peptide that may be useful in an AIDS vaccine. The Head of the Section is an elected council member of the International Society for Matrix Biology and a member of the international scientific review board of the Department of Molecular Pathology and Medicine, San Raffaele Scientific Institute, Milan, Italy.

Craniofacial Epidemiology and Genetics Branch

NIDCR's Craniofacial Epidemiology and Genetics Branch seeks to improve and promote dental, oral, and craniofacial health through research in epidemiology and health promotion. Major research projects include gene mapping and classic epidemiologic studies of oral and nasopharyngeal cancer. Researchers from China had major roles in these projects and collaborated with investigators in Taiwan to study populations in Taiwan. This research is supported under an NIDCR contact with investigators at National Taiwan University, Taipei. Branch investigators also collaborated with colleagues at the International Agency for Research on Cancer, Lyon, France, on a genetic epidemiologic study of oral cancer. In other significant efforts, Branch investigators analyzed population samples of oral cancer, from both patients and control subjects, in Puerto Rico and Greece. They also focused on large-scale genome scanning in patients with nonsyndromic cleft lip and cleft palate, early-onset periodontitis, and the craniofacial disorder Kartagener's syndrome. An investigator from Iran contributed to the Branch's efforts. In related research, the Branch collaborated with investigators at Hebrew University, Jerusalem, Israel, and the Institute of Human Genetics, Poznan, Poland.

During FY 00, the Chief of the Branch gave presentations at four international

gatherings in France, Switzerland, and the United Kingdom. He addressed the genetic and environmental aspects of periodontal diseases, cleft lip and cleft palate, and oral cancer.

Craniofacial and Skeletal Diseases Branch

NIDCR's Craniofacial and Skeletal Diseases Branch conducts research on bone and disorders of the skeleton. Researchers are characterizing the cells, genes, and macromolecules in bone, tooth, and cartilage tissue; identifying the mechanisms of growth and development in health and disease; elucidating the processes that govern regulation of skeletal metabolism; and describing the mineral structure and mineralization processes in bones and teeth.

Scientists from Belgium and China are contributing to research in the Molecular Biology of Bones and Teeth Unit. During FY 00, foreign scientists made substantial progress in understanding the molecular events that control the expression and function of matrix genes made by mineralized tissues. They developed new techniques to examine mRNA (messenger RNA) expression and novel means to transfer genes into human skeletal cells. Part of this research led to the discovery of a key matrix gene, Big-3, which may control the function of bone cells in normal states and in pathological conditions such as melorheostosis, a form of osteosclerosis of the long bones.

The work of the foreign researchers was also critical in determining the role of small proteoglycans in the structure and function of the musculoskeletal system. Using a transgenic mouse model lacking the small proteoglycan biglycan, they discovered the basis for decreased bone mass and showed that it was caused by multiple defects in bone marrow stromal fibroblasts, the precursors of osteogenic cells. They also created knockout mouse models to test the hypothesis that multiple small proteoglycans may act cooperatively in the skeletal system. The mice developed numerous defects in the connective tissue and, interestingly, may offer a new animal model for studying diseases such as Ehlers-Danlos syndrome, osteoporosis, and osteoarthritis.

In addition, the Head of the Unit is a coinvestigator on a binational Israel-U.S. grant to examine the function and regulation of genes for the enamel matrix. She was a member of the organizing committee for two major international meetings on bone, and she chaired a session on the mechanisms of bone formation, at the 78th annual IADR meeting in Washington, D.C., in April 2000.

During FY 00, researchers in the Protein Chemistry Unit, including a scientist from Turkey, expanded a collaboration with investigators at the University of Liège, Belgium. This team of researchers showed that one of the matrix proteins for bone and teeth, bone sialoprotein (BSP), which they previously discovered, can mediate the migration and attachment of endothelial cells in humans. Even more important, they showed that BSP also can promote angiogenesis, giving an advantage to tumor cells that express BSP. This finding may suggest possibilities for developing reagents to block this activity. The Head of the Unit made a presentation describing this research at the 2nd North American Symposium on Skeletal Complications of Malignancy, in Montreal, Quebec.

Also in FY 00, foreign scientists in the Skeletal Biology Section and the Clinical Studies Unit made significant progress in understanding the phenotypic properties of bone marrow stromal cells in health and disease. Studies revealed that these cells arise from fully committed osteogenic cells and that they can shift from one phenotype to another, depending on microenvironmental factors. In anticipation of potential clinical applications, the scientists also showed that the cells can regenerate bone in a variety of model systems. Because of the cells' central role in skeletal metabolism, the scientists are continuing to explore the effect of certain mutations on the phenotypic abilities of these cells. For example, studies of fibrous dysplasia of bone, caused by a mutation in a regulatory protein, indicate that in this disease, normal bone and bone marrow are replaced by abnormal bone and that the fibrotic marrow does not support blood formation. The findings indicate that mutated bone marrow stromal cells accumulate in the marrow and are unable to differentiate fully into osteogenic cells, causing the formation of abnormally shaped and undermineralized bone trabeculae. These factors probably contribute to the fractures and deformities that occur in fibrous dysplasia of bone.

The Head of the Unit, who also is Chief of the Branch, is a member of the international scientific review board of the Department of Molecular Pathology and Medicine, San Raffaele Scientific Institute, Milan, Italy. During FY 00, she was an invited speaker at conferences in Australia and Israel and attended the annual meeting of the Endocrine Society, in Toronto, Ontario.

Gene Therapy and Therapeutics Branch

The NIDCR Gene Therapy and Therapeutics Branch is focused on advancing fundamental biological science from the laboratory into the clinic. Investigators combine expertise in oral medicine and epithelial biology, specifically transport physiology and signal transduction between cells, with development and use of in vivo gene transfer technology to treat pathological conditions affecting the salivary glands (e.g., Sjögren's syndrome and irradiation damage). In FY 00, scientists from Bulgaria and Italy, in the Biology Unit, studied two new viral isolates of adeno-associated virus (AAV4 and AAV5) as natural mutations of other viral serotypes, to understand the biology of this genus of virus and as potential vectors for gene transfer. The Head of the Unit reported on this research at an international workshop on parvovirus in Quebec.

In the Gene Transfer Section, scientists from Canada, Israel, Japan, and the Netherlands used various technologies (a) to transfer genes into the salivary glands, as treatment for local or systemic diseases, and (b) to address significant biological questions. They focused on the adaptation and use of optimal vectors to carry target genes into salivary cells. The scientists continued to improve understanding of the mechanism by which the hybrid adenoretroviral vector, AdLTR-luc, is able to mediate integration into genomic DNA and achieve stable transgene expression. Using AAV vectors, they showed that long-term transgene expression (more than 2 months) can be achieved after gene delivery with these vectors and that biologically significant levels of the transgene product are found in the serum. They also continued to make progress toward developing an artificial salivary gland.

The Head of the Section, who also is Chief of the Branch, held the Jack Lewin Epstein Memorial Lectureship at Hebrew University, Jerusalem, where he also served as an exter-

nal referee for promotion and tenure. In addition, he was a visiting professor at Tokyo Dental College, Chiba, Japan, and an invited speaker at meetings in Japan, Korea, and Spain.

In the Membrane Biology Section, a researcher from Mongolia succeeded in deriving a topology model for the membrane-spanning domain of the mammalian salivary Na^+ K^+ 2 Cl^- cotransporter. The model indicates the presence of 12 membrane-spanning segments and represents the first such study of this gene family of transport proteins. In related work, a researcher from Japan has made considerable progress in expressing the cotransporter in yeast, as a means of obtaining sufficient protein for study of its structure. Membranes prepared from transformed yeast contain levels of the cotransporter that are 10 or more times higher than those observed in the rat parotid gland, which is one of the richest mammalian sources of this protein. The researcher is now attempting to purify the recombinant protein. The Head of the Section was an invited speaker at two international conferences, in Japan and Korea.

In the Secretory Physiology Section, a team of investigators, including a scientist from India and collaborators in Austria, Chile, Ireland, and Israel, studied specific components and mechanisms of fluid secretion from salivary glands. During FY 00, they focused on the physiological significance of the transient receptor potential protein (Trp 1) in the store-operated calcium entry (SOCE) mechanism. Trp 1 has been proposed as a component of the SOCE channel, but the exact mechanism regulating Trp 1 is not known. The investigators' findings suggest that a region of Trp 1 may modulate SOCE to restrict the amount of calcium entering human submandibular gland cells. Using Trp 1 as a tool to examine the role of calcium influx in stimulated salivary gland fluid secretion in rats, they also showed that in vivo expression of Trp 1 in submandibular glands induces an increase in stimulated fluid secretion by increasing SOCE in acinar cells. This finding provides direct evidence that SOCE has a role in stimulated fluid secretion from the salivary glands.

Researchers in NIDCR's Sjögren's Syndrome Clinic, including an investigator from the Netherlands, are contributing to international efforts to develop classification criteria, gene therapies, outcome measures, and epidemiologic data to increase understanding of Sjögren's syndrome. In addition, researchers from the Clinic collaborated with investigators at the University of Lund, Malmö, on a population-based study of Sjögren's syndrome, conducted in Olmsted County, Sweden. The results show that the primary form of the disease is not associated with increased mortality, although the secondary form may be associated.

The Head of the Clinic is a member of the American-European Consensus Group for Classification Criteria for Sjögren's Syndrome. During FY 00, he participated in modification of these criteria and collaborated with an investigator at the University of Birmingham, England, to develop a framework for outcome measures for clinical investigations. The Head of the Clinic was an invited speaker at the University of Toronto; the University of Bergen, Norway; and the Swedish Rheumatology Society, Halmstad. He also moderated a session at the Sjögren's Syndrome Outcome Measures Consensus Conference, held in conjunction with the European Rheumatology Research Conference, at Oxford University, England.

Oral Infection and Immunity Branch

Researchers in NIDCR's Oral Infection and Immunity Branch study infectious and parasitic diseases, the leading cause of death worldwide. Their research addresses the causes, diagnosis, treatment, and prevention of infectious and inflammatory diseases; the functional and molecular organization of infectious organisms; the cellular, biochemical, and molecular components of inflammatory and immune responses underlying pathogen–host interactions; the physiological mechanisms of host defense; and interventions to benefit the host.

During FY 00, scientists from France and the Philippines, in the Bacterial Communications Unit, enhanced understanding of oral streptococci and developed new techniques (a) to investigate these bacteria, the predominant early colonizers of freshly cleaned enamel surfaces, and (b) to explore the structure of microbes in vivo. The scientists developed the first oral streptococcal strain to express green fluorescent protein, for use as a species-specific marker in model oral biofilms; showed that fluorescent streptococci can be used to elucidate the spatial architecture of microbial communities in dental plaque in vivo; and enhanced understanding of streptococci's ability to adhere tightly to tissue surfaces in the oral cavity. Scientists in the Unit were invited speakers at symposia in Israel and the United Kingdom and reviewed grants for research institutions in Canada and New Zealand.

In the Bacterial Toxins and Therapeutic Unit, investigators from China, Korea, and Russia contributed to determining the structure and function of proteins in anthrax toxin. They developed highly effective systems for expression and purification of the lethal factor protein, in amounts sufficient to determine the crystal structure, and produced a variety of structural variants to study the relationship of structure to function. In separate studies, the investigators used insertional mutagenesis in eukaryotic cells to identify cellular genes required for toxin action and located genes involved in binding and endocytosis of the toxin. They also created unique mutants of the protective antigen protein, for the specific killing of cells that express certain cell-surface proteases, and this achievement suggests a route to development of antitumor agents. The investigators have begun to use genomic sequence data to identify key virulence factors produced by *Bacillus anthracis*. Also, they are using analyses of gene expression to identify changes in macrophages treated with anthrax toxin.

In the Cellular Immunology Section, scientists from Australia, China, Greece, and the United Kingdom developed a transgenic mouse in which the gene for secretory leukocyte protease inhibitor (SLPI) has been inactivated. This knockout mouse exhibits aberrant wound healing, which can be reversed by exogenous SLPI. Additional studies focused on the role of TGF-β in arthritis and pathways of apoptosis (cell death).

In collaboration with a scientist at Centre Muraz, Bobo Dioulasso, Burkina Faso, under a contract awarded to a principal investigator at the University of Minnesota, Minneapolis, researchers continued to study the role of specific and innate host factors contributing to resistance or susceptibility to vertical retroviral transmission of HIV from pregnant women to their infants. The researchers are developing and using methods and assays to explore the immunological and viral characteristics of oral and intesti-

nal fluids and tissues in U.S. women and of breast milk and serum in African women. Breast-feeding is encouraged in Africa but is discouraged in the United States and other industrial countries. Important and unanticipated new data generated under this contract show that increased levels of SLPI in vaginal fluids are associated with significantly lower rates of perinatal transmission of human immunodeficiency virus type 1 (HIV-1). The data suggest that increased levels of selected innate mucosal immune factors such as SLPI may contribute to a natural antiretroviral defense. The continuing study will explore the degree of infectivity or inhibitory activity in the fluids of women who transmit HIV-1 compared with that in the fluids of women who do not transmit HIV-1, as well as other functional aspects.

Another collaboration, with a scientist in the United Kingdom, is focused on mechanisms of wound healing. The Head of the Cellular Immunology Section served as president of the Society of Leukocyte Biology; cochair of an international meeting on host–parasite interactions, held in Turin, Italy; and member of an international review committee of the Canadian Arthritis Network Center of Excellence. She also chaired a symposium on HIV, in Florence, Italy, and gave presentations at meetings of two professional societies, in Toronto, Ontario, and Harrogate, England.

In the Experimental Medicine Section, scientists from China were involved in efforts to clone, sequence, and characterize novel genes from pancreatic islet cells. They focused efforts on IA-2 and IA-2β, which are members of the protein tyrosine phosphatase family and major autoantigens in insulin-independent (type 1) diabetes mellitus. Autoantibodies to IA-2 and IA-2β appear years before the onset of clinical diabetes and are highly predictive markers for the development of the disease. A scientist from Japan conducted related work, and complementary studies were performed in collaboration with investigators in Canada and the United Kingdom. In addition, the Head of the Section served as a member of the board of the directors of the Paul Ehrlich Foundation, Frankfurt, Germany.

During FY 00, foreign investigators also contributed to research conducted in the Receptors and Signal Transduction Section. Representing China, France, Japan, and Korea, they characterized intracellular molecular mechanisms involved in the release of inflammatory mediators from cells. Their experiments demonstrated that the protein tyrosine kinase Syk is essential for the immunoglobulin receptor–induced release of inflammatory mediators. The investigators identified the molecules regulated by Syk and defined the mechanism of regulation of Syk after aggregation on the immune receptor. In other studies, they found that the focal adhesion protein tyrosine kinase (FAK) plays an important role in immunoglobulin receptor–induced secretion. Additional studies were conducted in collaboration with investigators in Brazil.

In the Taste and Smell Unit, scientists from the United Kingdom identified a large family of G protein–coupled receptors (GPCRs) involved in perception of bitter taste. By being able to sense bitter taste, animals are protected against ingesting poisonous compounds. This family of receptors encodes proteins (T2Rs) found only on the surface of specific cells within taste buds, and when some of the genes for these proteins were inserted into model systems, the proteins mediated responses to specific bitter compounds. Together, these results validate T2Rs as receptors for bitter taste. It appears that each cell within the taste buds that contains one T2R actually has the entire family of receptors on its surface, accounting for the uniform bitter taste that is evoked by unrelated toxic compounds. By identifying functionally defined taste receptors, scientists gain molecular tools for marking specific taste receptor cells, defining signaling pathways, dissecting receptor specificity, generating topographic maps, and tracing neuronal connections and circuits. An in-depth understanding of taste mechanisms could lead to improved treatments and outcomes (i.e., better-tasting drugs and patients' increased adherence to treatment regimens). (See also the section on "Highlights of Recent Scientific Advances Resulting From International Activities.")

Oral and Pharyngeal Cancer Branch

Scientists in NIDCR's Oral and Pharyngeal Cancer Branch are conducting basic research on the normal and aberrant functions of tissues, cells, and molecules, as they relate to oral disease and other disease states. Special emphasis is given to studies of the cause, diagnosis, treatment, and prevention of oral tumors. During FY 00, 13 foreign scientists in the Cell Growth Regulation Section explored the molecular basis of cancer by studying the function of molecules participating in the transduction of signals for cell proliferation. The scientists represented Argentina, Italy, Japan, Mexico, Spain, Thailand, and the United Kingdom. They continued to uncover novel pathways leading to the promotion of cell growth, building on previous findings and discoveries about the role of GPCRs in signal transduction. Using the information gained, the investigators continued to address the nature of the angiogenic pathways regulated by a gene for Kaposi's sarcoma–associated herpesvirus (KSHV), specifically the GPCR encoded by open reading frame 74 of KSHV. In addition, they explored a key role for phosphoinositol 3 kinase beta in the activation of cell-survival pathways by GPCRs through Akt, a key component of these pathways. Furthermore, they showed that E-cadherins can initiate outside–in signal-transducing pathways that regulate the activity of mitogen-activated protein kinase, a key component of pathways for cell proliferation and survival. E-cadherins are surface adhesion molecules that have a major role in aggregation-dependent cell survival in head and neck squamous cell carcinoma (HNSCC) cells.

Investigators in the Branch also are addressing the ravaging problem of oral cancer. The ultimate aims of this research are to elucidate the genetic changes that contribute to the evolution of oral neoplasia and to use this knowledge to develop molecular markers of the progression of disease and new approaches for treating oral malignancies. During FY 00, investigators continued their collaboration with the National Cancer Institute, NIH, on the Head and Neck Cancer Genome Anatomy Project. Using bioinformatic tools, they analyzed available nucleotide sequences and identified at least 132 genes that may have a role in the pathogenesis of HNSCC or that may represent suitable markers for early detection of the disease or for targeted pharmacological intervention. Using a laser microscopy technique, the investigators procured epithelial cells from a representative set of tumors and matching normal tissues and, with complex cDNA probes and DNA arrays, explored possible patterns of expression of cancer-related

genes in HNSCC. They found that HNSCC exhibits a distinctive pattern of expression of differentiation markers; signal-transducing and cell-cycle regulatory molecules; growth and angiogenic factors; and matrix-degrading proteases.

Researchers in the Branch are collaborating on similar studies with scientists in Chile, Germany, Italy, and Spain and are providing reagents to laboratories throughout the world. In addition, they served as grant reviewers for research institutions in Argentina, Canada, Germany, Israel, and Italy and participated in international meetings in Germany and the United Kingdom.

In the Molecular and Cellular Biochemistry Unit, scientists from Belgium and Korea conducted structure–function studies of deoxyhypusine synthase, the first-step enzyme in the biosynthesis of hypusine in the eukaryotic initiation factor 5A. Their studies provide further insight into the mechanism and complex molecular interactions of deoxyhypusine synthase reaction. Studies to better understand the physiological function of the eukaryotic initiation factor 5A are under way. In addition, the scientists derived cell lines of immortalized human gingival keratinocytes from normal human gingival keratinocytes, for comparative studies with HNSCC cells. They showed different responses to high calcium levels, TGF-β, and tissue plasminogen activator. They also determined that the small, proline-rich protein 1 may play an important role in the barrier function of normal oral epithelium, and they examined cellular changes occurring in immortalized human oral keratinocytes transfected with several oncogenes. The investigators are collaborating with scientists in Korea on related studies of deoxyhypusine synthase and differentiation and transformation of keratinocytes.

In the Proteases and Tissue Remodeling Unit, researchers from Denmark studied proteolytic modification of the extracellular matrix in remodeling of physiological tissue and in cancer. They focused on understanding the basic functions of the plasminogen activation cascade and of other matrix-degrading serine proteases in these processes. Specifically, the researchers studied the mechanism of initiation of the plasminogen activation cascade; investigated the functional association between the plasminogen activation system and matrix metalloproteases; determined the role of plasmin in involution of the mammary gland; generated mice deficient in a novel protein associated with the receptor for urokinase plasminogen activator; and analyzed serine protease expression in the progression of squamous cell carcinoma and the healing of incisional skin wounds. These studies resulted in potentially important insights for designing innovative strategies for therapeutic intervention in the progression of cancer. The researchers conducted additional studies of plasminogen, in collaboration with researchers in Denmark and France, and discussed research findings at a symposium in Australia.

Pain and Neurosensory Mechanisms Branch

NIDCR's Pain and Neurosensory Mechanisms Branch conducts multidisciplinary pain research in the laboratory and the clinic. Laboratory research is aimed at understanding the generation, transmittal, and amplification or attenuation of pain signals; developing animal models of inflammation that mimic chronic neuropathic pain in humans; and identifying factors involved in the expression of endogenous pain. Clinical investigations are focused on developing better methods for assessing pain; understanding mechanisms of acute and chronic pain; developing new methods of pain control; and imaging pain in the central nervous system.

In the Cellular Neuroscience Section, in FY 00, a scientist from Japan contributed to studies of the development of nociceptive pathways. Using an animal model of peripheral inflammation in the neonate, investigators identified a reorganization of pain pathways in the spinal cord, which occurred only in the early neonatal period and in response to persistent pain and tissue injury. The changes appear to be permanent and likely reflect activity in the nociceptive pathways during critical developmental periods when the nervous system is programmed to develop permanent connections. In response to noxious stimuli, the animal also exhibited behavioral changes, suggesting a functional change in the animal's response to pain as an adult. Research is under way to determine the critical factors responsible for these changes. (See also the section on "Highlights of Recent Scientific Advances Resulting From International Activities.")

In the Clinical Measurement and Mechanisms Unit, foreign scientists investigated pain mechanisms in animal models and humans. During FY 00, a scientist from China discovered unexpected, fast, bidirectional nerve conduction between the spinal cord and the dorsal root ganglion. He observed velocities higher than 300 m/second and an average velocity of 220 m/second, much greater than the previously measured velocities of 120 m/second in the mammalian nervous system. In a related study, the scientist and others in the Unit showed that stimulation of brain sites involved in descending analgesia actually increased antidromic activity—nerve conduction that travels the "wrong way," from the spinal cord to peripheral receptors. This finding suggests the action of a previously unknown mechanism for central-to-peripheral nerve conduction, in which pain-evoked activity in peripheral primary afferent nerves is blocked when central, descending systems are activated.

Investigators from Australia and Germany contributed to studies of the cerebral response to blunt pressure applied to the thumb. Tenderness to blunt pressure is a main clinical symptom in diseases such as fibromyalgia. The investigators showed that painful pressure activates a network related to the side of the body stimulated, as well as specific responses in the left thalamus and right prefrontal cortex of the brain. Their results challenge the current concept of a unitary pain circuit, demonstrate activation of unique networks in qualitatively different conditions and during brief periods of time, and show that orbital activation is associated with increased psychophysical measures of unpleasantness. To resolve conflicting results in the literature on pain, the investigators also started a study to examine the physiological and cognitive mechanisms involved in placebo analgesia in response to topical anesthetics. The Head of this Section is the editor of the international journal *Pain* and presented invited lectures in Austria and Israel.

In the Clinical Trials Unit, a Canadian anesthesiologist completed a 3-year clinical pain research fellowship during FY 00. He finished three studies in which he was the first to demonstrate relief of pain by an

α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainate antagonist and showed different degrees of effectiveness of this and other antagonists in facial neuralgia. The investigator has assumed a faculty position at Queens University, Kingston, Ontario, and has received funding in Canada to conduct clinical trials on neuropathic pain. Unit investigators also collaborated with a researcher at Hebrew University and Hadassah Medical Center, Jerusalem, Israel, and with another NIDCR intramural scientist to localize a gene predisposing to neuropathic pain, on a small area of one chromosome.

In the Neuronal Gene Expression Unit, a scientist from Hungary participated in research on the molecular basis of pain signaling arising from tissue injury, inflammation, nerve injury, and painful heat. This research is focused on the vanilloid receptor 1, which is the first molecule in the pain transmission pathway from the periphery to the spinal cord and is located in the pain nerve endings in the skin and deep tissues. The scientist's contributions helped to establish the presence of the receptor in both plasma membrane and endoplasmic reticulum and to image its functional activity. Through his efforts, the Unit has been able to exploit a sophisticated imaging system to examine molecular functions and cellular responses.

In addition to this research, Branch investigators participated in the International Association for the Study of Pain; presented an invited lecture at the 9th International Dental Congress on Modern Pain Control, in Jerusalem, Israel, in May 2000; participated in the planning of an NIH-German Collaborative Workshop on Pain; and were invited speakers at pain research meetings in Grand Cayman Island, Spain, and the United Kingdom.

Functional Genomics Unit

During FY 00, scientists from India and Japan, working in the Functional Genomics Unit, identified molecular mechanisms involved in neuronal phosphorylation and their potential role in neurodegenerative diseases. They also explored the effect of these mechanisms on brain development, neuronal migration, and neuromuscular junctions in transgenic mouse models, which they developed. They are elucidating the regulation of the mechanisms during embryonic development and aging. In addition, the scientists sought a more precise understanding of the role of TGB-β in autoimmune disorders. These studies may eventually lead to new research approaches to prevention and treatment of some neurodegenerative diseases and autoimmune disorders. Scientists in the Unit collaborated on related studies with scientists in Canada, France, India, Japan, Sweden, and the United Kingdom and were invited speakers at two research institutions in Japan.