

XI.

National Institute of Dental and Craniofacial Research

INTRODUCTION

The National Institute of Dental and Craniofacial Research (NIDCR) is the lead agency for dental, oral, and craniofacial research in the United States. Much of this research is guided by international collaborations, as scientists team up throughout the world to solve health problems that affect populations in all countries. International health is a priority area for the National Institutes of Health (NIH) and for NIDCR. On entering the 21st century, NIDCR is seeking research solutions globally. The Director of NIDCR has emphasized that the Institute aims to be as inclusive as possible and to engage all who are interested in working toward improved quality of life for all persons, as it relates to dental, oral, and craniofacial health. NIDCR's broad constituent base and research portfolio are reflected in its new name, which became effective on October 21, 1998, during fiscal year 1999 (FY 99). Formerly known as the National Institute of Dental Research, the Institute now has a name that better represents its mission to the public and research community, both domestically and globally.

The Office of International Health (OIH) coordinates and promotes international activities at NIDCR. The Office was established in March 1998 within the Office of the Director, NIDCR. During FY 99, OIH engaged in a broad strategic planning effort to develop a mission statement, objectives, and strategies to implement its major functions: liaison with global agencies and national organizations involved globally; coordination and facilitation of NIDCR bilateral and multilateral agreements; facilitation of international workshops and associated research agendas; leadership as a World Health Organization (WHO) Collaborating Center for Epidemiology, Prevention, and Treatment of Oral Diseases and Conditions; and dissemination of information about international collaborative research and research training opportunities.

Three themes for NIDCR's international activities during FY 99 were globalization, research networks, and funding partnerships. Scientific research is a global endeavor. Researchers who previously worked in relatively isolated laboratories on single topics are now linked globally with colleagues to study complex problems. Efforts to map the human genome are driving much of this interaction and will continue to focus biomedical and behavioral research in the 21st century, as scientists tease apart the complexities of gene-behavioral-environmental relationships.

During FY 99, OIH conveyed the importance that NIDCR places on the globalization of science at many international venues, seeking to stimulate global interest in, and support of, research on oral health. Major presentations were given at international meetings in Africa, Europe, the Middle East, South America, and Southeast Asia. OIH, in cooperation with the NIDCR Division of Extramural Research, also launched a major initiative to globally link scientists from both industrial and developing countries in teams that would conduct collaborative research on questions requiring international attention. In March 1999, NIDCR issued a Request for Applications for 2-year International Collaborative Oral Health Research (ICR) planning grants and received almost 30 applications, which will be reviewed and funded during FY 00. The grants will support the planning of biomedical, epidemiologic, and behavioral studies in areas that require international research. This new funding mechanism is a significant innovation at NIDCR and the NIH to stimulate investigator-initiated grant applications from networks of researchers worldwide. It is hoped that recipients will subsequently submit applications to conduct full-scale studies, which NIDCR would fund together with other national and international partners.

Also to stimulate the globalization of research and research collaborations, NIDCR

explored the possibility of including foreign dental students in the Institute's intramural Summer Dental Student Award program. No mechanism exists for granting U.S. visas to foreign students, which would enable them to participate in the NIH research environment. In a related activity, NIDCR stimulated a partnership with the International Federation of Dental Education Associations, the International Association for Dental Research (IADR), and the American Association of Dental Schools to explore the development of a short-term research training program for foreign students that would be offered at NIDCR and other U.S. dental research institutions. This program, once developed, would be supported by NIDCR and other funding sources. The training of young scientists is a critical part of building research networks.

Fostering international networks of researchers and institutions is a major goal of NIDCR's ICR agenda. During FY 99, NIDCR continued to refine and focus this agenda. One of the priority areas is craniofacial anomalies, particularly cleft lip and cleft palate. The Institute continued to stimulate international collaborative research in this area. In October 1998, OIH staff emphasized the need for research networks and partnerships at the International Task Force Meeting on Craniofacial Anomalies, in Bauru, Brazil. In November 1998, NIDCR hosted a highly successful international symposium, entitled *Toward a Molecular Understanding of Craniofacial Morphogenesis*, at the NIH, in Bethesda, Maryland. The meeting was attended by almost 400 participants. In June 1999, the Director, NIDCR, presented the keynote lecture at the 6th European Craniofacial Congress, in Manchester, England. This meeting made possible additional gatherings of researchers to plan for global collaborative initiatives in the study of craniofacial anomalies.

A major development was the successful review of a contract proposal from WHO to

support planning and care services for international collaborative research in craniofacial anomalies. Approved in August 1999, this proposal has specific objectives for planning and development of research protocols, databases, and network infrastructure to sustain and coordinate collaborative biomedical, epidemiologic, and behavioral studies. Supported activities will include epidemiology and surveillance studies and clinical trials for prevention and treatment. NIDCR anticipates funding the contract during FY 00.

In September 1999, NIDCR hosted another international meeting, the Workshop on Prevention of Craniofacial Anomalies, to identify research strategies for preventing cleft lip and palate. Also during FY 99, OIH staff observed craniofacial research and surgical care activities of Operation Smile in the Philippines. NIDCR intramural and extramural scientists participate in this organization's mission trips, during which they are able to conduct research on the nutritional and genetic aspects of oral clefting. OIH is exploring opportunities for partnerships with Operation Smile and with Smile-Train, a nonprofit group devoted to training health care workers to repair cleft lip and palate, beginning in China.

Assessing optimum levels of fluoride for humans is another priority area in the ICR agenda. A key activity in this area during FY 99 was the meeting entitled International Collaborative Research on Fluoride: Research Needs Workshop, which was held at the NIH, in Bethesda, in May 1999. The purpose of the workshop was to develop an agenda for international research on fluoride. The workshop was organized and hosted by NIDCR in collaboration with other NIH components, the Centers for Disease Control and Prevention (CDC), industry, and IADR. The participants, who came from 17 countries, benefited from the opportunity to network with each other and recommended an agenda of 10 priority areas.

Other topics in the ICR agenda are infectious diseases and emerging infectious diseases, such as human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS); orofacial gangrene (noma); oral cancer; biomaterials and biomimetics; and reduction of disparities in health, through health promotion efforts.

NIDCR is promoting collaborative research in all these areas.

Partnerships in funding are essential for supporting broad-scale international research efforts. NIDCR has promoted these partnerships as a way of leveraging its own research dollars to accomplish research goals for the benefit of all. During FY 99, OIH began a focused effort to develop an expanded network of potential funding partners. OIH prepared a strategic implementation plan for network development and initiated preliminary and exploratory discussions with public, private, and nonprofit organizations in the United States and worldwide. The range of organizations includes, but is not limited to, the European Union, the German Ministry of Science and Technology, the Japanese Development Agency, the Korea Institute of Science and Technology, the United Nations' Foundations, the U.S. National Science Foundation, and the World Bank.

NIDCR's efforts to foster globalization of research, research networks, and partnerships in funding are detailed in this chapter. 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

Animal Model for Bone Studies

In FY 99, a team of investigators in Canada, Denmark, and Italy and researchers in NIDCR's Matrix Metalloproteinase Unit and the Craniofacial and Skeletal Diseases Branch collaborated to generate a knockout mouse deficient in membrane type 1 matrix metalloproteinase (MT1-MMP). This family of enzymes is believed to play a major role in cellular development and remodeling processes. The essential nature of the gene became manifest when the scientists characterized the phenotype of the MT1-MMP-deficient mouse. The animal exhibited dwarfism, osteopenia, arthritis, and connective tissue disease resulting from reduced turnover of collagen. This animal model will be used to determine whether the membrane-associated matrix metalloproteinase (MMP) produces these effects independently or exerts effects on other MMPs. The scientists

reported this significant finding in *Cell*, in October 1999.

Genes for Taste Receptors

Also during FY 99, two scientists from the United Kingdom, working in NIDCR's Oral Infection and Immunity Branch, discovered and sequenced the first genes for taste receptors. These receptor proteins (TR1 and TR2) are responsible for recognition of sweet and bitter substances. The transmembrane proteins, which signal via a G protein pathway, share only 40% homology, a finding that highlights the selectivity of each receptor. TR1 is associated with fungiform papillae, which are scattered, knob-like projections on the tongue, whereas TR2 is associated with circumvallate papillae, which are the largest projections, arranged in a "V" pattern at the front of the tongue. The scientists have provided a vital tool for tracing the development and migration of taste receptors and an approach to discovering related genes. This important discovery was published in *Cell*, in February 1999; the report was selected as the lead article, and a figure from the article was displayed on the cover.

SUMMARY OF INTERNATIONAL PROGRAMS AND ACTIVITIES

Country-to-Country Activities and Bilateral Agreements

In FY 99, OIH staff continued their involvement in 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 are participating in efforts to implement community water fluoridation in Russia. In collaboration with CDC and WHO, OIH staff coordinated and reported on training activities for water plant personnel. Although circumstances in Russia forced postponement of fluoridation activities in Moscow, Russian oral health authorities identified two other locations that could serve as demonstration project sites. OIH helped to arrange a site assessment and a training visit during FY 99. Other organizations cooperating in this effort are the U.S. Agency for International Development; the Office of International and Refugee Health, U.S. Department of

Health and Human Services; the Fogarty International Center (FIC); the Russian Ministry of Health; the Russian Dental Association; and multinational corporations.

At the request of the German government, NIDCR scientists met with German scientists for preliminary discussions on potential areas of cooperation in research on chronic pain.

Activities With International and Multinational Organizations

World Health Organization

The Associate Director for International Health serves as Director of the WHO Collaborating Center for Epidemiology, Prevention, and Treatment of Oral Diseases and Conditions. OIH staff participated in a meeting of the WHO Collaborating Centers of the Americas, in Washington, D.C., in October 1998. Staff also collaborated with WHO on organizing the second meeting of all WHO Collaborating Centers for Oral Health, which will be held at the NIH, in Bethesda, Maryland, in April 2000.

NIDCR continued to work closely with WHO to stimulate collaborative research on noma, craniofacial anomalies, fluoride, and other global oral health issues. In 1994, WHO launched an International Action Network Against Noma, and NIDCR's WHO collaborating center is part of this network. In February 1999, OIH and WHO staff traveled to Niger to review the problem of noma with national health authorities. The team visited noma centers and hospitals and conducted an assessment of laboratories and agencies that could help with data collection and analysis of biological samples. The extent of the problem was greater than expected, both in number and severity of cases. The Niger Minister of Health is giving high priority to research and health promotion related to this devastating disease, which is rampant among impoverished and malnourished children in Africa. To enhance awareness of the disease and discuss research directions, NIDCR sponsored a seminar on international collaborative research on noma, at NIDCR, in Bethesda, in June 1999, and is facilitating research on noma in collaboration with FIC.

In addition, OIH staff helped to organize and participated in a meeting hosted by the WHO Regional Office in Africa to discuss implementation of a new Regional Oral

Health Strategic Plan. At this meeting, in Harare, Zimbabwe, in March–April 1999, the 10-year plan was adopted by the region's ministers of health. It will facilitate research, service, and educational training projects on the African continent. The plan includes noma, oral cancer, and HIV, which are also areas of emphasis in NIDCR's ICR agenda.

For a visit of the WHO Director General to the NIH in October 1998, OIH staff contributed briefing materials conveying NIDCR's long and successful collaboration with WHO. During this visit, the Director, NIDCR, discussed specific NIDCR initiatives being pursued with WHO. OIH staff also participated in preliminary discussions with the NIH and with CDC and WHO on strategies for control of tobacco use. At the request of WHO, OIH staff contributed comments and suggestions for implementation of WHO's Global Strategy in Oral Health, and OIH stimulated linkages between WHO and other organizations (e.g., Global Forum for Health Research) on research areas of interest to NIDCR.

To celebrate WHO's International Collaborative Studies of Oral Health Outcomes (ICS-I and ICS-II), OIH staff participated in a symposium, in Taipei, Taiwan, in June 1999. For the annual meeting of the American Public Health Association, in Washington, D.C., in November 1998, OIH staff conducted a symposium on the policy implications of ICS-II for oral health care in the United States.

The Associate Director for International Health also continued to serve on the Technical Advisory Group for the Regional Advisor for Oral Health, Pan American Health Organization (PAHO). At a March 1999 meeting in Washington, D.C., data and program initiatives related to dental caries in the Americas were reviewed favorably by the advisory group. Particularly significant were data showing reduction in the prevalence of dental caries where fluoride programs had been instituted. NIDCR's role and activities as a WHO collaborating center were highlighted at the meeting. The Associate Director also continued to represent NIDCR, as well as the American Dental Association (ADA) and Fédération Dentaire Internationale (FDI), at meetings of the Directing Council of PAHO.

European Union

Through the European Union-U.S. Science Technology Agreement, NIDCR worked with FIC to foster relations with the European Union as a potential funding partner for international research on oral health. In October 1998, OIH staff met with European Union staff in Brussels, Belgium, to advise them on international collaborative activities in research on oral health.

Fédération Dentaire Internationale

The NIDCR Director and staff gave scientific presentations at the 1998 World Dental Congress of the Fédération Dentaire Internationale, in Barcelona, Spain, in October 1998. The Associate Director for International Health helped to plan this meeting and participated in an FDI special consultation meeting on strategic planning for global promotion of oral health. She continued to serve as a consultant to FDI's Congress and Education Committee and contributed substantially to the planning of future World Dental Congresses, which will be held in Mexico City, Mexico, in 1999; in Paris, France, in 2000; and in Kuala Lumpur, Malaysia, in 2001. The Associate Director also served as the representative of IADR's Behavioral Scientists and Health Services Research Group to the FDI General Assembly.

In March 1999, during the annual meeting of IADR in Vancouver, British Columbia, OIH staff met with the Executive Director of FDI to discuss cooperation between NIDCR and FDI to enhance international collaborations and to leverage funds to support these activities. FDI is establishing a Fund for Developing Countries, which may become an activity of mutual interest and serve to enhance NIDCR's partnership with FDI. In August 1999, NIDCR staff participated in FDI's 2nd World Conference on Oral Health Promotion, at the Royal College of Physicians, London, England.

International Association for Dental Research

The 77th annual meeting of IADR was held in Vancouver in March 1999. More than 50 NIDCR staff members 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. The Associate Director for International Health chaired a meeting of a new IADR Committee on Tobacco. Participants discussed a potential role and activities for IADR in global promotion of the prevention of tobacco use and the cessation of smoking among youth.

In conjunction with the meeting, OIH staff attended the annual meeting of the Council of the International Federation of Dental Education Associations, a potential partner in the globalization of research. Staff described NIDCR's effort to globalize research on oral health and to develop a research training program in the United States for international students. After the IADR meeting, OIH staff participated in a conference entitled Successful Aging Through Oral Health, which was sponsored by IADR's Geriatric Oral Research Group, another potential partner for NIDCR's global interests.

During FY 99, the Associate Director for International Health worked with the IADR Regional Development Committee to enhance research in developing nations. She also presented reports on the opportunity for international scientific collaboration at meetings of the East and South African Division of IADR, held in Nairobi, Kenya, in October 1998; the Egyptian Division of IADR, in Cairo, in December 1998; and the Continental European Division of IADR, in Montpellier, France, in September 1999.

American Dental Association

The Associate Director, NIDCR, continued to serve as consultant to the ADA Council on Annual Sessions and International Relations.

Fogarty International Center

The Associate Director represented NIDCR at the NIH international representative meetings coordinated by FIC, and OIH staff worked closely with FIC to advance training in research on AIDS and emerging infectious diseases.

Other Activities

Health promotion is an important part of NIDCR activities, and Institute staff provide leadership in this area, both domestically and internationally. During FY 99, a health promotion specialist in NIDCR's Office of Science Policy and Analysis was the invited

keynote speaker at a meeting of the Asian Academy of Preventive Dentistry, in Chiang Rai, Thailand, in November 1998. She spoke on the use of partnerships in advocacy for oral health promotion and disease prevention. In related talks, she addressed the role of health care providers in prevention and early detection of oral cancer and the role of health promotion in preventing dental caries.

The specialist also was an invited speaker at the 8th International Symposium on Oral Health Development, in Seoul, Korea, in August 1999. While in Korea, she participated in two symposia on community water fluoridation. During FY 99, she also facilitated visits to U.S. sites by Japanese researchers and dental practitioners and by a Korean reporter communicating on water fluoridation, and she worked with several NIDCR Guest Researchers and visitors from Brazil, Japan, Korea, and Thailand.

Another staff member in the Office of Science Policy and Analysis continued her doctoral-level studies at University College Medical School, London, England, where she is focusing on health promotion and health disparities. The investigator collaborated on a research grant from the Department of Health, United Kingdom, to develop and test outcome measures for primary care settings, and she consulted with researchers in numerous countries, including Brazil and New Zealand.

In addition, the Associate Director for International Health participated in the first International Symposium on Developing International Collaboration on Oral Health Promotion, in Fukuoka City, Japan, in July 1999. The symposium was cosponsored by NIDCR, the Fukuoka Dental Association, and Kyushu University. The Associate Director presented opportunities for international collaborative research that could benefit developing nations, nations in transition, and industrial countries.

In September 1999, OIH staff participated in the 3rd Congress of the European Association of Dental Public Health, in Strasbourg, France. This association offers linkages to potential funding partners, such as European Ministries of Health. OIH cohosted a symposium in conjunction with the congress, to identify key areas of research in dental public health and community clinical practice over the next 5 years. Staff

described NIDCR's global mission and participated in work groups on promotion and epidemiology of oral health.

In addition to these health promotion activities, NIDCR pursues other global opportunities. In October 1998, the Institute cosponsored an international conference on the role of saliva in oral health, in Beijing, China. About 100 Chinese and U.S. researchers exchanged information on salivary research and discussed possibilities for collaboration. Staff described NIDCR programs on salivary research and visited the Beijing Dental School's research and clinical facilities.

In June 1999, OIH staff attended the annual meeting of the Global Health Council. Formerly known as the National Council for International Health, this U.S.-based, nonprofit organization is identifying and communicating priority international health problems to national and international bodies in both the public and private sector. OIH is exploring potential funding partnerships with the council. Staff from NIDCR and ADA will serve on the council's newly created advisory committee on oral health.

In September 1999, the Associate Director for International Health participated in the third meeting of the International Advisory Committee for the D. W. Cohen Middle East Center for Dental Education, in Jerusalem, Israel. The regional center, affiliated with Hebrew University and Hadassah School of Dental Medicine, Jerusalem, supports short-term training for students and continuing education courses for health professionals throughout the region.

In addition, OIH staff explored potential collaborations in meetings at NIDCR with representatives from the University of Chile; the Ministry of Science and Technology of the government of India; and the Korea Institute of Science and Technology. Staff also hosted and provided NIH and NIDCR orientations to international groups from the Chinese Ministry of Health and the Medical University of Lodz, Poland. In January 1999, staff cosponsored an NIDCR seminar on quality-of-life measurements from an international perspective.

An important opportunity for 2000 is the World Congress on Medicine and Health, which will be held during the World Exposition EXPO 2000, in Hannover, Germany, on July 21–August 31, 2000. With the Asso-

ciate Director's advice, given during FY 99, the congress will include speakers on dental and craniofacial issues, and 1 day will be devoted to topics on oral health. The Director of NIDCR's Division of Intramural Research will be a major speaker on this day.

Extramural Programs

In FY 99, extramural activities included 9 foreign grants, 22 domestic grants with foreign components, and 1 domestic contract with a foreign component. Of these, four foreign grants and nine domestic grants with a foreign component were new awards in FY 99. In addition, NIDCR cofunded 16 awards with FIC and provided a supplemental grant for one cofunded award. This extramural international activity directly involved research institutions and scientists in 28 countries and Taiwan. The countries were Argentina, Australia, Belgium, Brazil, Canada, China, the Czech Republic, Denmark, England, France, India, Israel, Japan, Jordan, Mexico, New Zealand, Niger, Nigeria, the Philippines, Portugal, Senegal, Sudan, Sweden, Thailand, Trinidad, Turkey, Vietnam, and Zimbabwe. 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 support for two foreign grants and nine domestic grants with a foreign component. Among the six NIDCR scientific programs, this international activity was the second largest in number of awards and dollars, 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.

Comprehensive Research Centers

In FY 99, NIDCR initiated funding for two Comprehensive Oral Health Research Centers of Discovery to study craniofacial development and disease. One of the new Centers

of Discovery is located at the University of Iowa, Iowa City. The work at this Center will involve a number of projects, including a study conducted in collaboration with investigators at Zhabei Eye Hospital, Shanghai, China. The scientists will search for genetic causes of cleft lip and cleft palate in 150 families from the Shanghai region and 145 families at sites in Columbus, Ohio, and Pittsburgh, Pennsylvania. The families have multiple members affected with cleft lip and cleft palate. An additional 100 patients and their parents will be examined in Shanghai for genetic linkages. 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, potentially, lead to therapies to prevent cleft palate.

Another Center of Discovery is located at Johns Hopkins University, Baltimore, Maryland. Researchers will study 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 investigators at clinical sites in Buenos Aires, Argentina; Prague, Czech Republic; and Mexico City, Mexico. At the four sites, which include Baltimore, researchers will be conducting a genome-wide search among families who have multiple members affected with oral clefts to identify genes contributing to the risk of this malformation. The information gained may indicate strategies for preventing these birth defects or for identifying persons at highest risk.

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. With NIDCR support, an investigator at the University of Aarhus, Denmark, is using Danish cases of these conditions and control groups for these analyses. During FY 99, he analyzed biological samples collected in Denmark from 300 cases of cleft lip and palate and from 600 control subjects. The investigator found that maternal smoking was associated with a moderately increased 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, is working with investigators at the University of Calgary, Alberta, 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 European white populations. This research effort was expanded in Shanghai, China, and was enlarged to allow comparison with a white population in West Bengal, India.

A fourth study funded by NIDCR in FY 99 is being conducted by an investigator at Ohio State University, Columbus. In collaboration with a researcher at Jordan University of Science and Technology, Irbid, this investigator is focusing 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, will be advantageous for identifying genetic loci for these conditions. To date, eight families with nonsyndromic clefts have been enrolled in the study, as well as one family with unidentified syndromic clefts. The scientists are focusing on genetic markers on chromosome 4.

In some single-gene craniofacial disorders, clefting malformations are associated with mutations of the gene for transforming growth factor α (TGF- α). A researcher at the University of Iowa, Iowa City, has documented associations between these malformations and TGF- β_3 , as well as interactive effects between TGF- α and smoking. These findings resulted from NIDCR-supported research conducted initially in the Philippines and then extended to Denmark, Japan, and Vietnam. Other genes linked to clefting malformations also could be identified.

These and other researchers aim to clarify the genetic and environmental aspects of cleft lip with or without cleft palate, but still others are focusing on improving 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. Because of evidence suggesting that this aberration is related to surgical repair of the lip, NIDCR continues to support a prospective, randomized, controlled study to assess outcomes of two surgical techniques for repairing cleft lip. This research involves collaboration between investigators at the University of Florida, Gainesville, who are using one technique, and investigators at the University of São Paulo, Brazil, who are using another method. The study will include 200 infants who have cleft lip and 200 who do not have the disorder.

As part of this study, other investigators at the same institutions are comparing the effects on the level of speech competence of two surgical approaches for repairing cleft lip and palate. Recruitment of about 600 children for this prospective, randomized, clinical trial continued in FY 99. The grant was recently renewed for completion of the trial. The findings could provide a "gold standard" for evaluating and comparing speech benefits from new or modified surgical procedures and approaches for cleft lip and palate.

Biom mineralization

Researchers at four institutions are cooperating in a new multidisciplinary program project to obtain fundamental information on the development of mineralized tissues, such as tooth enamel. The institutions are Forsyth Dental Center, Boston, Massachusetts; McGill University, Montreal, and the University of Montreal, Quebec; and the University of Texas Health Science Center, San Antonio. The Canadian researchers are contributing 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 disease in mineralized tissues; and developing procedures for regenerating damaged and diseased mineralized tissues.

A scientist at the Weizmann Institute of Science, Rehovot, Israel, continues 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 99 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 three foreign grants and five domestic grants with foreign components. In collaboration with two FIC programs, NIDCR also cofunded 3 awards under the AIDS International Training and Research Program (AITRP) and 13 awards under the International Training and Research Program in Emerging Infectious Diseases (ITREID) and provided a supplemental grant for 1 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 importance of research on HIV infection, AIDS, and other emerging infectious diseases worldwide. 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 99, NIDCR awarded two new grants to foreign investigators 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 investigators will enhance understanding of the formation of plaque and the possibilities for designing therapeutic strategies based on establishing nonpathogenic dental plaque.

For one of the studies, investigators at the University of Toronto, Ontario, and the University of Florida, Gainesville, are collaborating to elucidate the genetic transformation of the bacteria *Streptococcus mutans* in plaque; this bacteria is a primary cause of

dental caries. The conditions favoring a physiological state that allows bacteria to incorporate foreign DNA ("competence") has not been studied in biofilms. The investigators will determine the optimum conditions for transformation of the bacteria, define the mechanisms of competence, and apply novel approaches to search for genes expressed during the growth of biofilm.

The second study is being conducted by a team of researchers in Sydney, Australia, from the Institute of Dental Research, the University of New South Wales, and Macquarie University. Two powerful and complementary new technologies will be used to monitor changes in gene expression during colonization and maturation of a model biofilm. The researchers will focus on the interactions between two adherent bacteria in the oral cavity, *Streptococcus gordonii* and *S. mutans*, both of which can be genetically manipulated. They have studied pertinent parameters, including regulatory mechanisms of surface adhesins; influence of extracellular sugar polymers; response to unfavorable (e.g., acidic) environments; and effects of key enzymes.

Another new award in FY 99 was received by an investigator at the University of Tennessee, Knoxville, who is working with investigators at the University of Otago, Wellington, New Zealand, on a complementary study. The goals of this research are to develop simple quantitative measures of the composition and physiology of oral plaque; to examine the variations and relationships in plaque "communities"; and to characterize the shifts in composition and physiology dependent on nutrients and the oral environment. Ecologically induced shifts are presumed to be key factors in the progression of periodontal disease. A better understanding of these processes in normal and pathogenic plaque communities will clarify cause-and-effect relationships in both periodontal disease and dental caries and will underpin the development of new intervention strategies. The two laboratories bring different but complementary areas of expertise to this project.

NIDCR also supported a new collaboration between scientists at Forsyth Dental Center, Boston, and the University of Göteborg, Sweden. The aim is to control periodontal infections by suppressing or eliminating the organisms that cause them. The

scientists in Sweden have access to clinical specimens useful for the study. In related studies, the scientists are assessing the efficacy of antimicrobial methods for preventing or eliminating the complex of bacteria (*Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Treponema denticola*) that appears to be a major contributor to the periodontitis common among adults. They also are evaluating adverse effects (i.e., emergence of undesired or antibiotic-resistant bacterial species) from these procedures and ecological relationships of bacteria in the oral cavity.

Comprehensive Research Center

One of the Comprehensive Oral Health Research Centers of Discovery established by NIDCR in FY 99 is at the University of Washington, Seattle, where investigators are addressing the basis of oral and craniofacial health and susceptibility to disease in children, as a key to lifelong oral health. The research projects are grouped into four clusters: innate host defense and mucosal health, periodontal disease, craniofacial disorders, and caries. Two projects involve foreign investigators and sites. In one project, a 5-year clinical trial, the effectiveness of a motivational interviewing approach in preventing early childhood caries will be evaluated among more than 200 healthy infants (12–18 months of age) and their mothers from the Punjabi-speaking community in Surrey, British Columbia. The experimental group of mothers will receive counseling sessions and follow-up telephone calls and postcards, in addition to an educational pamphlet and video presentation. The investigator, who is from the University of British Columbia, Vancouver, hypothesizes that the infants will have significantly fewer early childhood caries and less caries disease and that the mothers will have greater change in behavior and better parenting practices, compared with a control group. 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, will conduct 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. Specifically, this researcher will investigate the clinical features of the disease, microbial patterns of transmission, and risks to oral and systemic health. This project complements additional ongoing studies of women's oral health and disease that are being conducted by the researcher at the University of Dakar, 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 is collaborating with investigators at the University of Dakar on another new project, to evaluate the association between oral sex and HIV infection among female commercial sex workers in Senegal. This project complements other studies being conducted by the researcher in Senegal. It will expand understanding of the spread of HIV in Africa and of the biology of oral transmission of HIV. Specific aims are to describe the frequency and factors involved in the practice of fellatio among the workers and the association between these practices and their HIV status; to provide insights into the risk of HIV transmission from infected workers to uninfected male partners during fellatio; and to determine whether use of low-cost topical treatments for oral inflammations associated with HIV decreases the frequency and quantity of HIV in oral secretions.

In another joint study between the University of Washington and the University of Dakar, investigators continued a clinical epidemiology study of oral lesions in heterosexual women and men in Senegal who may or may not be infected with human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), or both viruses. Oral lesions are found in a substantial number of HIV-infected persons, and the incidence of HIV infection is greater in developing countries such as Senegal. However, few systematic studies of the epidemiology of HIV-associated oral disease in the populations of these countries have been undertaken, and no published data on the risk of oral pathology associated with HIV-2 are available. The investigators are using and building on a clinical infrastructure already

developed for cross-sectional and cohort studies of HIV and neoplasia.

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. During FY 99, researchers at the University of California School of Public Health, Berkeley, collaborated with the chair of the Department of Oral and Maxillofacial Surgery at the University of Zimbabwe Medical School, Harare, to develop a teaching curriculum for short-term training on HIV/AIDS. Faculty from the University of Maryland, Baltimore, joined local health care workers in Rio de Janeiro, Brazil, and Port of Spain, Trinidad and Tobago, to conduct training on the biology, pathogenesis, and dental management of HIV disease. Also, two dentists from Brazil came to the University of Maryland on a 30-day research fellowship, which resulted in development of several collaborative research protocols. At the University of Washington, Seattle, two dentists, from Senegal and Thailand, attended the university's Summer Institute in Clinical Dental Research Methods. While there, they developed pilot studies for research grant applications and explored possibilities for training that could lead to a degree for colleagues in their countries.

Herpesvirus

Scientists at Eastman Dental Institute, University of London, England, continued to explore the oral implications of human herpesvirus 8 (HHV-8). They are applying a highly sensitive and technically difficult technique to identify cell types in the oral mucosa that support HHV-8 infection; to clarify the association of HHV-8 with Kaposi's sarcoma and HIV; and to evaluate the role of saliva in transmission of HHV-8. The scientists have substantial archival samples of oral tissue.

Emerging Infectious Diseases

During FY 99, 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 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 noma, a devastating disease, among children in four African countries. In Nigeria, the grantee from the University of Maryland, Baltimore, teamed with investigators at the Nigerian Institute for Medical Research, Lagos, to conduct fieldwork in three deprived rural communities, where they examined 75 children. Twelve of the children had acute necrotizing gingivitis, and almost one-third of them had had measles in the preceding month. An association between measles infection and subsequent occurrence of noma has been well documented. The investigators also are exploring other factors that may contribute to immunosuppression (e.g., oral bacteria and fungi, malnutrition, and impoverished living conditions). In Niger, the grantee completed preparations for a study that will begin in FY 00. He conducted the feasibility study; identified research teams and partners in three study sites; adapted the core protocol for local conditions; drafted a sociological questionnaire; pilot tested instruments and procedures; and began collecting information from local health care workers. Education and information materials were distributed, and a training campaign was launched to reach all primary health care centers in Niger. This research is being conducted in collaboration with WHO and other international organizations. During FY 99, findings from the study were presented in six articles coauthored by the grantee and published in international journals.

Neoplastic Diseases

International activities in NIDCR's Neoplastic Diseases Branch included support for a new 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.

Genetic Markers of Oral Cancer

In a new study, investigators in Canada are evaluating the temporal patterns of clonal changes in oral lesions of patients at high risk of oral cancer. Two hypotheses are being tested: (1) that loss of alleles in cells scraped from these lesions independently predicts

risk of lesions progressing abnormally in patients who do not have cancer and (2) that information obtained from cells scraped from former cancer sites can be used to predict the clinical outcome during follow-up for patients with oral cancer. The research team, consisting of investigators from Simon Fraser University, Burnaby, British Columbia, and Vancouver General Hospital, the British Columbia Cancer Agency, and the University of British Columbia, Vancouver, is building on previous work with archival tissue samples. The samples in the present study will be collected over a 2-year period from 50 patients with oral dysplasia and 100 patients with squamous cell cancer and 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 new study, investigators from the University of Michigan, Ann Arbor, are leading 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 the Institut Curie, Paris, France, in efforts (1) to determine whether overexpression and mutation of the p53 gene predicts the response to chemotherapy, the preservation of organs, or survival and (2) to discover how an effect is influenced by expression of the Bcl-2 and Bcl-x proteins, which block cell death. These hypotheses, suggested by previous studies, will be tested in *in vitro* experiments with known cell lines and in tumor samples obtained from patients before and after organ-sparing therapy and from patients receiving 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 therapies are critically needed.

In addition, NIDCR continued support to a researcher at Moffitt Cancer Center, Tampa, Florida, for a proposed collaboration with a researcher from Khartoum, Sudan, to identify persons who may be genetically susceptible to oral cancer, because of abnormalities in genes that encode enzymes in-

involved in the metabolism of tobacco carcinogens and alcohol. The researchers plan (1) to compare the polymorphisms in these genes with mutations in the p53 gene, a known tumor suppressor, and (2) to examine race as a possible factor in susceptibility to oral cancer that is associated with specific genotypes for carcinogen-metabolizing enzymes. Populations in Sudan have an unusually high frequency of oral cancer.

Genes for Gingival Fibromatosis

An investigator at the University of Pittsburgh, Pennsylvania, has 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 are from the Center for Human Genetics, Louvain, Belgium; the State University of Campinas, Piracicaba, Brazil; 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, provided consultation 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. During FY 99, they identified two distinct genes on chromosome 2 that cause hereditary gingival fibromatosis.

Chronic Diseases

NIDCR's international activities in the Chronic Diseases Branch included two foreign grants and one 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 joint (TMJ) disorders. 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).

Pain Mechanisms

Two scientists at the University of Toronto continued their long-term studies to clarify brain stem mechanisms involved in dental

and orofacial pain. Current findings from one scientist indicate that injury to the tooth pulp induces changes in neuroplasticity in brain stem neurons participating in sensory reception. The changes involve a central sensitization that has been described previously in models of spinal cord injury. This sensitization results in development of hyperalgesia (enhanced sensitivity to pain) and the spread of the pain to uninjured areas. In new, related studies, the scientist will examine specific anatomic sites in the central nervous system that may be responsible for the changes in neuroplasticity in the neurons and will explore how these sites interact with each other.

The second scientist is completing studies of the reflex pathways in the trigeminal subnucleus caudalis. His research, which has enhanced understanding of the relationship between nerve fibers in the TMJ and pain reception in the central nervous system, supports the importance of the central nervous system in pain reception. He is analyzing results and preparing reports for publication.

Temporomandibular Joint Function

Investigators at the University of Washington, Seattle, and the University of British Columbia, Vancouver, continued to work together on the development of experimental models for studying the function and mechanics of the TMJ. The investigators are enhancing understanding of the biochemical environment of the TMJ and elucidating the effects of alterations in this environment on remodeling or degeneration of the joint. For their studies, they have been using the TMJ of pigs, which they have determined to be a relevant model for humans.

Biomaterials, Biomimetics, and Tissue Engineering

NIDCR's international activities in the Biomimetics, Tissue Engineering, and Biomaterials Branch included one domestic grant with a foreign component. 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. To stimulate international collaborations, the Chief of the Branch participated in the first international workshop on Self-Assembling Peptide

Systems in Biology, Medicine, and Engineering, in Heraklion, Greece, in July 1999. She chaired a session on biomedical engineering and informed participants about NIDCR's research program.

Utility of Dental Treatments

In FY 99, NIDCR provided support for a new 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 technology under development; tooth extraction; and root canal therapy. The two measurement 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 McMaster University, Hamilton, Ontario, who is contributing to the development and pilot testing of the measurement tools, training of interviewers and the research team, and analysis of data. The researchers will conduct 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 one foreign grant, one domestic contract with a foreign component, and three domestic grants with a foreign component. This program had the third largest amount of international activity in the Division of Extramural Research, reflecting 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 Older Adults

In FY 99, NIDCR supported a new, small research grant awarded to a newly independent scientist at the University of Toronto, who is conducting a secondary analysis of existing data to evaluate a hypothetical association between tooth loss and age-related hearing loss in healthy men. The hypothesis is that adults who retain a significant number of their natural teeth with tooth and periodontal integrity have better hearing acuity than adults who have no teeth. This hypothesis is based on evidence suggesting that orofacial and auditory sensory information is processed interactively. The scientist, working with scientists at the Veterans Health Administration Boston Outpatient Clinic and Tufts University, Boston, Massachusetts, and the University of North Carolina, Chapel Hill, is analyzing data from 20 years of follow-up in the Veterans Affairs' Normative Aging and Dental Longitudinal Studies. The study participants were approximately 1,230 men aged 21–80 years residing in the Boston area. Delineation of an association between tooth loss and age-related hearing loss would provide a foundation for intervention studies to improve oral health and hearing acuity in adults.

In a continuing study, researchers at the University of Washington, Seattle, and the University of British Columbia, Vancouver, are testing whether a simple home care regimen of rinsing with chlorhexidine and fluoride reduces tooth loss in an ethnically diverse population of adults aged 60–75 years who previously had oral disease and irregular dental care. Beginning the 3rd year of this 5-year clinical trial, the researchers continued recruiting, enrolling, and examining participants at both sites. All baseline data have been collected for 700 participants in Seattle and 400 participants in Vancouver, and the first round of 1-year follow-ups, which will continue for 5 years, has started. About 49% of the Seattle participants are in ethnic minority groups (African Americans, Asians, or Hispanics), and many of the Vancouver participants are Indo-Chinese recruited from senior centers.

Cross-Cultural Studies of Pain

Investigators at the University of Washington are also completing 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. Their specific conclusions include the following: (1) Pain beliefs associated with ethnicity and differences in health care systems affect the perceived need for anesthetics. (2) The characteristics or causes of dental anxiety can differ by ethnicity and the dental health care system, dentists' beliefs, or patients' expectations. (3) Expressions of suffering reflect different social environments and cultural assumptions about pain, suffering, and the availability of social supports for the patient with pain (e.g., workmen's compensation, welfare support, and insurance coverage). Enhanced understanding of the effect of cultural influences on perceptions of pain and 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

Investigators 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 com-

posites 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 99, the investigators completed annual follow-up visits of all the children enrolled in the study. The data will be analyzed, and results will be identified during the next several years of this study, which uses a blinded protocol. Interim analyses addressing demographic and oral health issues reveal that the children have additional carious lesions at a rate higher than expected, which may be due to lack of fluoridation combined with generally inadequate oral health behaviors.

Amalgam and Resistant Bacteria

In the 2nd year of a contract, a researcher at the University of Washington 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. The researcher is obtaining and assaying oral and urine samples from 150 of the children participating in the Casa Pia Study. Reporting preliminary results, the researcher has 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 to verify these observations.

International Meetings

In FY 99, NIDCR supported 17 international conferences, many of which were held in the United States. The four Gordon Research Conferences addressed the following topics: collagen, molecular cell biology, salivary glands and saliva, and the biology of spirochetes. Other conferences held in the Unit-

ed States focused on access to health information for older adults, receptors for growth factors, bone research, technologically advanced materials with biomedical application, cell and molecular biology of bones and teeth, and microbial pathogenesis. NIDCR also supported the 5th Hinman Student Research Symposium, which will be held in Memphis, Tennessee, in October 1999, and which attracts dental students from outside the United States.

Conferences supported by NIDCR and held in other countries included the International Congress of Virology, in Sydney, Australia; the U.S.-Latin American Conference on Saliva and Oral Health, in Santiago, Chile; the first international workshop on Self-Assembling Peptide Systems in Biology, Medicine, and Engineering, in Heraklion, Greece; and the Conference on *Haemophilus*, *Actinobacillus*, and *Pasteurella*, in Johannesburg, South Africa.

In addition, about 70 NIDCR scientists and science administrators traveled to 34 countries and Taiwan to participate in international meetings or meet with research colleagues. 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 made presentations at local universities, visited scientific laboratories to discuss or conduct collaborative research, and provided consultation on matters of oral health.

Both the Director and the Scientific Director of NIDCR extended leadership to this scientific outreach. In recognition of his contributions to research on oral health globally, the Director received an honorary degree from the University of Montreal; was invited to present the keynote address at the 6th European Craniofacial Congress and to receive an honorary fellowship in the Craniofacial Society of Great Britain, in Manchester, England; and served on the Swedish Medical Research Council to perform an international evaluation of Swedish dental research. The Director also participated in the International Symposium on Craniofacial Morphology, in Witten, Germany; the FDI World Dental Congress, in Barcelona, Spain; and annual meetings of the American Academy of Pediatric Dentistry, in Toronto, the

American Academy of Esthetic Dentistry, in Vancouver, and the American Academy of Maxillofacial Prosthetics, in Victoria, British Columbia.

The Scientific Director presented a seminar at the 40th annual meeting of the Japan Association of Oral Biology, in Nagoya, and a lecture on trends in biological science at the Danish Dental Society Symposium, in Aarhus. While in Denmark, he discussed ongoing collaborative research projects and reports that are in preparation with Danish scientists. The Scientific Director also traveled to Montreal to collaborate on a study and prepare a research report for publication.

Intramural Programs and Activities

The NIDCR Division of Intramural Research continued its international leadership and outreach during FY 99. Scientists throughout the Division performed the following functions: 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 to other scientists and laboratories around the world; reviewed research grants for foreign institutions; served as external advisors for young investigators at foreign institutions; coauthored numerous publications with foreign scientists; and published reports of research in many international journals.

In addition, approximately 90 foreign scientists worked with U.S. investigators in NIDCR's intramural laboratories. These scientists represented 29 countries, and more than one-third (35%) came from two countries: China and Japan. Another 35% came from six other countries: Australia, India, Israel, Italy, Korea, and Spain. About 30% came from 21 other countries: Argentina, Belgium, Bulgaria, Canada, Denmark, Egypt, France, Germany, Greece, Hungary, Iran, Mexico, Mongolia, the Netherlands, New Zealand, the Philippines, Poland, Russia, Switzerland, Thailand, and the United Kingdom. NIDCR intramural investigators also collaborated on research projects at institutions in 25 countries and Taiwan. In addition to many of the countries mentioned here, the investigators worked with scientists

in Austria, Brazil, Burkina Faso, Chile, Egypt, Ireland, Norway, and Sweden.

Craniofacial Developmental Biology and Regeneration Branch

Researchers in NIDCR's Craniofacial Developmental Biology and Regeneration Branch are studying the cell biology and molecular biology of craniofacial development and regeneration, as well as developmental mechanisms. During FY 99, scientists in the Cell Biology Section focused on development and regeneration of salivary glands, angiogenesis, and metastasis of tumor cells. The ultimate aim for the 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. Addressing this problem, scientists from Israel and New Zealand used a cultured human submandibular gland cell line and developing submandibular glands from a mouse as complementary model systems. They identified and located integrin receptors involved in cell adhesion and formation, identified several genes that induce cell differentiation, and elucidated signaling mechanisms and the activity of growth factors.

Researchers from Korea sought to identify molecular events, signals, and genes involved in the formation of blood vessels and to discover novel angiogenic and antiangiogenic factors that may be important clinically. They demonstrated that thymosin β_4 promotes dermal and corneal wound repair in rats, an effect that will be tested on wounds that do not heal in patients. They also defined active sites important for endothelial cell interactions in the basement membrane matrix. Angiogenesis involves dissolution of this underlying support structure, migration and proliferation of endothelial cells, and synthesis of new basement membrane. In addition, the scientists discovered that human soluble intercellular adhesion molecule 1 (ICAM-1) is angiogenic. Increased levels of this protein are found in certain diseases associated with increased blood vessel formation (e.g., diabetes and cancer), and tumor cells containing the gene for this protein form larger tumors.

Investigators from Germany, Israel, and

Spain are making progress in understanding factors involved in the growth and spread of cancer cells. Knowing that a component of the basement membrane, laminin 1, promotes malignant behavior, they screened more than 700 peptides derived from laminin 1 and found two that are highly active with tumor cells. Both peptides increase colonization of the lung with tumor cells, and one causes metastases to the liver. In collaboration with a researcher at the University of Pittsburgh, Pennsylvania, the investigators modified the structure of the laminin peptide YIGSR to derive stable peptides with enhanced antiangiogenic, antitumor, and antimetastatic activity. In other experimental studies, the investigators are clarifying organ-specific metastases. For example, they determined that osteonectin is the active factor in bone extracts that promotes invasion of breast and prostate cancer cells, and they made progress in identifying a highly potent factor in brain tissue that promotes invasion of breast cancer cells. For these and other studies, they have developed 12 prostate tumor and epithelial cell lines.

During FY 99, Branch staff presented these major research findings at international meetings in Greece, New Zealand, the United Kingdom, and the United States.

In the Developmental Mechanisms Section, scientists from Bulgaria, China, Israel, Japan, Mexico, and the Netherlands identified novel 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 organization of the cytoskeleton. The scientists 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. Key advances include describing the regulatory functions of a new human tumor-suppressor protein (PTEN), which has been implicated in 10%–50% of many types of cancer; identifying proteins involved in the adhesion of cells and organization of the cytoskeleton as novel signaling molecules; and making progress in developing

an artificial salivary gland and identifying a peptide that may be useful in an AIDS vaccine. The Chief of the Section is an elected council member of the International Society for Matrix Biology; serves as a member of the international scientific review board of the Department of Molecular Pathology and Medicine, San Raffaele Scientific Institute, Milan, Italy; and was an invited speaker at research symposia in Canada and the United Kingdom.

In the Molecular Biology Section, investigators from China, Denmark, Japan, and Spain participated in research that resulted in the creation of a mouse model for skeletal dysplasia. Using gene-targeting approaches, they created mice that lacked 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 perlecan-deficient mice have both skeletal and craniofacial abnormalities and die soon after birth. The skeletal abnormalities, which are due to defects in cartilage development, are similar to those found with thanatophoric dysplasia type I, which is fatal and is known to be caused by mutations in a growth factor gene. The results suggest (a) that there is a link between perlecan and the activity of growth factors in formation of the skeleton and (b) that perlecan has multiple roles in development. The research is partially supported by a grant from Seikagaku Corporation, Japan. The Chief of the Section presented findings at two symposia in Japan.

Craniofacial Epidemiology and Genetics Branch

Major research projects in NIDCR's Craniofacial Epidemiology and Genetics Branch include gene mapping and classic epidemiologic studies of oral and nasopharyngeal cancer. Researchers from China had major roles in these projects and collaborated with researchers in Taiwan to study populations in Taiwan. Branch investigators also extended significant effort to analyze tissue samples from populations in Greece and Puerto Rico for a case-control study of oral cancer. Part of this work involved development and testing of improved methods for detecting human papillomavirus. In addition, scientists from Argentina, Iran, and Poland contributed to large-scale, genome-scanning studies of nonsyndromic cleft lip

and palate, early-onset periodontitis, and Kartagener's syndrome.

A researcher from Norway continued to collaborate with NIDCR researchers on studies of early-onset periodontitis, to identify and quantify risk factors for the disease, which affects adolescents and young adults. Findings during FY 99 strengthen suggestions of oral and systemic associations in health and disease. The researchers determined that levels of antibody to *Porphyromonas gingivalis* in blood serum are similar to those in the oral cavity's gingival crevicular fluid and that high levels of immunoglobulin G (IgG) and immunoglobulin A (IgA) antibody reactivity to *P. gingivalis*, *P. intermedia*, and *Actinomyces actinomycetemcomitans* may promote tissue destruction. In addition, they found that, compared with whites, African Americans have higher serum concentrations of IgG1, IgG2, and IgG3. Furthermore, IgG3 levels were positively correlated with a person's generalized periodontal status and a higher rate of disease progression, whereas elevated serum levels of IgG2 may be correlated with localized periodontal destruction and a slow rate of disease progression.

The Norwegian researcher also collaborated on the NIDCR component of the Baltimore (Maryland) Longitudinal Study of Aging, conducted by the National Institute on Aging. In this study, NIDCR scientists are identifying and quantifying risk factors for periodontal disease and other indicators of oral health. During FY 99, they demonstrated that use of cigars or pipes is associated with increased prevalence, extent, and severity of periodontal disease in older persons. This finding is particularly timely because use of cigars increased dramatically in the United States during the 1990s.

Craniofacial and Skeletal Diseases Branch

Investigators in NIDCR's Craniofacial and Skeletal Diseases Branch are focusing their research on determining the developmental processes involved in the formation of skeletal elements, the remodeling and maintenance of these elements after birth, and alterations in these processes in disease states. Translation of research findings into clinical studies and applications continues to be a major area of emphasis. All these efforts are accomplished with participation of foreign

scientists in the Branch and collaboration with scientists throughout the world.

Bone marrow stromal cells, which have the ability to form bone, cartilage, stroma that supports hematopoiesis, associated fat cells, and perhaps other connective tissues are important mediators of skeletal metabolism after birth. During FY 99, two scientists, from Australia and Italy, worked on the Skeletal Genome Anatomy Project to facilitate gene discovery and to identify changes in the pattern of gene expression of skeletally derived cells as a result of developmental age and disease. From more than 8,000 clones that have been sequenced to date, several novel genes were identified and are being analyzed for their potential role in skeletal metabolism. The scientists anticipate that this research will lead to the use of molecular engineering technology to introduce genes that direct normal skeletal metabolism or correction of a genetic defect in osteogenic cells.

In studies of the function and regulation of human genes, finding ways to overcome the extreme inefficiency of transferring DNA into human cultured cells is a major challenge. To devise new methods to meet this challenge, a researcher from Switzerland investigated two procedures that use adenoviruses, for their applicability to marrow stromal cells and trabecular bone cells. The researcher demonstrated that adenoviruses containing a reporter gene accomplish substantial gene transfer, thus indicating that they are ideal for these unique cell types. The researcher also found that adenoviruses modified with polylysine efficiently transferred genes into multilayer, highly differentiated nondividing cells.

An investigator from the Netherlands used the same technology to "rescue" estrogen responsiveness in cells unable to make the estrogen receptor (ER) alpha. Use of this technology restored activation of the ER alpha-dependent gene by estrogen. Further research showed that estrogen responsiveness could be regulated by treatment with TGF- β 1, a major regulator of skeletal metabolism. Treatment with TGF- β also affected production of interleukin 6 (IL-6) in ER-rescued cells; IL-6 influences the formation of cells that destroy bone. This approach is the first of its kind to clarify the relationship of growth factors to the function of ER in human bone-forming cells.

Branch investigators also continued studying two classes of matrix proteins that are highly enriched in bone, the small leucine-rich proteoglycans and proteins that mediate cell-matrix interactions. During FY 99, the investigators completed experiments started by a visiting professor from Germany. They demonstrated that the pattern of expression of a receptor that internalizes two small proteoglycans, biglycan and decorin, overlaps areas where these two closely related proteoglycans are synthesized and that decorin contributes to the development of capillaries. Related studies, conducted previously with investigators in Liège, Belgium, showed that the cell-binding protein, bone sialoprotein (BSP), is expressed by metastatic cancers and that BSP can recruit new blood vessels. This property may explain why tumors usually express high levels of BSP. During FY 99, a scientist from Germany was instrumental (1) in the finding that BSP levels in blood serum are elevated in several cancers that metastasize to bone and (2) in studying the potential role of BSP in cancer cells' evasion of immune surveillance.

In addition, scientists from Israel and Italy continued to collaborate with colleagues in Israel and Italy on the design and implementation of three NIH protocols for studying and treating fibrous dysplasia of bone. Although the prevalence of this disease is not known, it is a significant health problem. The NIH protocols, initiated during FY 97-FY 99, constitute the largest and most definitive clinical study of this disease. Pertinent to this study is the finding by an investigator from Italy that a major defect of fibrous dysplastic bone is a lack of bone matrix mineralization (osteomalacia), which leads to softening of the bone, deformation, and fracture. Studies to determine the nature of this defect in mineralization are continuing.

Branch staff participated in international meetings in Australia, Canada, China, France, Germany, Italy, and the Netherlands. One scientist received a Young Investigator Award at the 3rd International Congress on Osteoporosis, in Xi'an, China, in March 1999.

Gene Therapy and Therapeutics Branch

Investigators in NIDCR's Gene Therapy and Therapeutics Branch apply genetic tools to elucidate the function and dysfunction of

salivary glands. In the Gene Transfer Section, they use technologies that make it possible to transfer genes to salivary glands for treating local or systemic diseases and to address significant biological questions. During FY 99, scientists from different countries focused on adapting and using optimal vectors to carry target genes into salivary cells for investigative purposes. In a major project, scientists from China, Israel, and Japan demonstrated that native salivary epithelial cells in the body recognize transgene-encoded determinants for sorting and directing secretory proteins into saliva or into the bloodstream. This finding has major implications for the use of salivary cells as target sites for treatments based on the delivery of gene-based proteins. A report of this research will be published in *Human Gene Therapy* in FY 2000. In addition, a scientist from Israel studied the interactions among salivary cells, matrix proteins, and biodegradable polymer substrates. This research, which contributed significantly to the Branch's efforts to develop an artificial salivary gland, will be reported in *Tissue Engineering* in FY 2000.

In addition to research activities, the Chief of the Section participated in international meetings in Canada, China, Japan, and Korea; lectured at institutions in China and Japan; served as associate editor for the *European Journal of Dental Education* and as editor for the Americas for the journal *Oral Diseases*; was an external referee for promotion and tenure at Hebrew University, Jerusalem; and provided invited commentary on the present and future state of dentistry for *Lancet*. Another scientist in the Section was the recipient of a fellowship from the Japan Society for the Promotion of Science.

Investigators in the Membrane Biology Section study the structure, function, and regulation of membrane transport proteins involved in the secretion of exocrine fluid. Of special interest is the secretory Na⁺ K⁺ 2 Cl⁻ cotransporter, the ion transport system responsible for most of the fluid secretion by many salivary glands. During FY 99, scientists from India, Japan, and Mongolia participated in studies to define the phosphorylation sites involved in regulation of the cotransporter and to understand the topology of the cotransporter. The scientist from Japan made considerable progress in expressing this mammalian protein in yeast,

for the purpose of obtaining sufficient amounts of the protein for studies of its structure. Membranes prepared from genetically transformed yeast contain levels of the cotransporter somewhat greater than those found in the parotid gland of rats, which is one of the richest mammalian sources of this protein. The scientist is now attempting to purify this recombinant protein in functional form. Researchers in this Section also continued to pursue their long-time interest in elucidating the function of the intracellular IP3 receptor. In addition, they presented lectures at institutions and meetings in Japan and Korea.

Research in the Secretary Physiology Section is directed toward understanding the mechanisms that regulate calcium signaling in salivary gland cells. During FY 99, scientists from India and Japan contributed to studies of the calcium influx mechanism in these cells, a major research focus of the Section. They also participated in investigations of the possible role of calcium signaling in salivary gland dysfunction resulting, for example, from radiation treatments of the head and neck. Conducted in collaboration with scientists in Austria and Israel and funded by the U.S.-Israel Binational Foundation, this research is focused specifically on understanding the effect of transition metal ions in radiation-induced damage.

Oral Infection and Immunity Branch

Researchers in NIDCR's Oral Infection and Immunity Branch are studying infectious and parasitic diseases. They are seeking to understand infectious pathogens, host defense mechanisms, and pathogen-host interactions and to develop therapeutic interventions. During FY 99, an investigator from China studied the molecular biology of oral bacteria, and scientists from France and the Philippines focused on the genetics of these bacteria. In related studies, Branch staff collaborated with researchers in Canada, Denmark, France, Germany, Japan, New Zealand, Sweden, and the United Kingdom, and one investigator was an ad hoc reviewer for four international journals.

Development of vaccines continued to be an area of emphasis. A scientist from China contributed to development of a new generation of vaccines for pertussis, and a scientist from Egypt focused on hepatitis C vaccines from transgenic plants. This re-

search was complemented by extensive collaborations with other scientists in Canada, Egypt, France, Japan, and the United Kingdom. In studies of anthrax toxin, scientists from China, Iran, Korea, and Russia clarified the three-dimensional structure of the lethal factor in the toxin; used fusion proteins from the toxin to induce cellular immunity to HIV; identified critical domains in the lethal factor; identified the receptor for the toxin; modified the protective antigen for the toxin to redirect the toxin to tumor cells; and characterized the biochemical changes that lead to toxin-induced cell death. Collaborative research with laboratories in Canada, India, Japan, and the United Kingdom complemented this work.

In studies of autoimmunity, an investigator from Japan participated in studies to clone, sequence, and characterize novel genes from pancreatic islet cells. Collaborations with scientists in Canada, Germany, and the United Kingdom focused on elucidating autoantibodies in persons with diabetes, including identical twins, and on viruses as triggers of diabetes. The Branch's senior investigator in this area is a member of the board of directors of the Paul Ehrlich Foundation, Frankfurt, Germany.

Another focus of the Branch is the molecular biology of taste, smell, and pheromone reception. A researcher from the United Kingdom was instrumental in the discovery and sequencing of the first genes found for taste receptors. (See also the section on "Highlights of Recent Scientific Advances Resulting From International Activities.") In another research area, scientists from China, France, Japan, and Korea investigated the receptors and molecular mechanisms for signal transduction in cells. This research was conducted with scientists in Brazil and Israel. The Branch's senior investigator in this area served as a reviewer for a grant awarded by the Israel Research Council.

Scientists from Australia, China, and Greece contributed to research on the mechanisms of acute and chronic inflammatory disease. They focused on several areas: genetic regulation of secretory leukocyte protease inhibitor, which is an antiviral, antibacterial, and antiproteolytic molecule; generation of a mutant mouse model for studying the actions of this inhibitor; mechanisms of HIV entry into cells and potential antiviral intervention strategies; the contri-

bution of programmed cell death (apoptosis) to the evolution of acute and chronic inflammatory disease; and mechanisms for administering and controlling gene therapy in experimental models of tumors and autoimmune disease. The Branch's senior investigator in this area served as president of the Society for Leukocyte Biology.

In related work, collaborations continued with a researcher at Centre Muraz, Bobo Dioulasso, Burkina Faso, under a contract awarded to an investigator at the University of Minnesota, Minneapolis. The researchers are examining the role of specific and innate host factors that contribute to resistance or susceptibility to vertical retroviral transmission of HIV from pregnant women to their infants. They are developing and using methods and assays to explore the immunologic and viral characteristics of oral and intestinal fluids and tissues in U.S. women and of breast milk and serum in African women. Breast-feeding is encouraged in Africa but has been discouraged in the United States and other industrial countries. The results of the study may provide valuable insight for the potential development of vaccines against HIV infection.

Another collaboration is under way with a researcher in the United Kingdom. This research is focused on the mechanisms of wound healing.

Oral and Pharyngeal Cancer Branch

In NIDCR's Oral and Pharyngeal Cancer Branch, 15 scientists from seven countries contributed to studies of the molecular basis for squamous cell carcinogenesis. The countries represented were Argentina, Italy, Japan, Korea, Spain, Thailand, and the United Kingdom. The molecular basis of cancer is a major research focus in the Branch, and scientists are studying the normal and aberrant functioning of molecules that participate in the transduction of signals for cell proliferation. Certain alterations of proteins involved in mitogenic signaling are known to exert profound effects on cellular behavior, including malignant transformation. The scientists have uncovered novel pathways leading to the promotion of cell growth. They previously showed that cell-surface receptors linked to G proteins can behave as potent ligand-dependent oncogenes. Building on this work, they recently made a number of discoveries in signal

transduction, including the observation that signaling from G protein-coupled receptors to the nucleus involves the integration of numerous signals transduced by a highly complex network of mitogen-activated protein (MAP) kinases, rather than resulting from the stimulation of a single linear cascade of protein kinase.

Using this information, investigators addressed the nature of the angiogenic pathways regulated by a herpesvirus gene associated with Kaposi's sarcoma (KSHV), specifically the G protein-coupled receptor encoded by open reading frame 74 of the KSHV gene. They found that this receptor enhances the expression of vascular epithelial growth factor (VEGF) by stimulating the activity of hypoxia initiation factor (HIF) 1 alpha, which binds to and activates transcription from a hypoxia response element within the VEGF promoter, and that this stimulation involves phosphorylation of the inhibitory domain of HIF-1 alpha by the mitogen-activating phosphorylation kinase signaling pathways. The investigators also dissected a novel pathway whereby integrins and G protein-coupled receptors signal to distinct MAP kinases through focal adhesion kinase. In addition, they identified a key role for phosphoinositol 3 (PI3) kinase beta in the activation of cell survival pathways by G protein-coupled receptors 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 PI3 kinase and Akt. (E-cadherins are surface adhesion molecules that play a major role in aggregation-dependent cell survival in head and neck squamous cell carcinoma [HNSCC].) This novel molecular mechanism indicates that the interaction among neighboring cells may ultimately control the fate of normal and transformed epithelial cells. In related work, the investigators also sought to identify the key amino acid residues critical for binding of spermidine and nicotinamide adenine nucleotide, in efforts to gain insight into the complex molecular interactions involved in deoxyhypusine synthase reaction, which is essential for protein synthesis and cell growth.

Branch scientists 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 identify molecular markers of the progression of disease and to develop new approaches for treating oral malignant tumors. During FY 99, NIDCR investigators worked with the National Cancer Institute on the establishment of the Head and Neck Cancer Genome Anatomy Project. This team of investigators has submitted six complementary DNA (cDNA) libraries from HNSCC lines and normal and immortalized gingival keratinocytes. The information in these libraries, which is available to the public, suggests that there is a distinct pattern of gene expression in these cells, and it has yielded 39 previously unidentified cDNA clones. Six new cDNA libraries from microdissected normal and cancer tissues contain a wealth of information. Preliminary analysis of 513 clones in these libraries has led to identification of 187 potentially novel genes. More information on DNA sequence will be generated shortly, and investigators are proceeding to engineer stage-specific cDNA libraries and are using DNA chip screening methods to evaluate the pattern of expression of novel and known genes.

In another study, Branch scientists are developing an in vitro model of oral carcinogenesis by using immortalized human gingival keratinocytes (IHGK). During FY 99, scientists introduced various human protooncogenes, including *c-myc*, *N-ras*, *K-ras*, and *craf*, as well as the viral oncogenes *MSV-H-ras* and *MSV-H-ras V12*, into IHGK cells. They selected oncogene-transfected cells and compared the properties of these cells with those of normal human gingival keratinocytes and IHGK cells. They also investigated several aspects of the molecular mechanisms involved in the growth and terminal differentiation of normal human gingival keratinocytes.

A major limitation of research on HNSCC is the lack of suitable animal models for testing the validity of current genetic paradigms of tumorigenesis and for exploring the effectiveness of treatments and chemopreventive approaches. In collaboration with NIDCR's Gene-Targeting Core Facility, Branch scientists recently generated transgenic mice that carry the tetracycline-inducible system (*tet*-on receptor) and the receptor for the avian leukosis virus (*tv-a* receptor), targeted to the basal layer of stratified epithelium by using the cytokeratin 5

promoter. For the tetracycline-inducible system, transgenes of interest, including candidate oncogenes, will be expressed under the control of a tetracycline-responsive promoter. In relation to the receptor for the avian leukosis virus, various genes can now be expressed in vitro and in vivo in the skin and oral mucosa by using highly effective avian retroviruses. With these biologically relevant animal models, the scientists can perform in vivo tests of the transforming potential of activated alleles of newly discovered signaling molecules, alone or with coexpression of dominant interfering mutants of various tumor-suppressor genes. This research is expected to unravel the mechanisms responsible for squamous cell carcinogenesis and to facilitate the search for alternative therapies for oral cancer.

In addition to these research efforts, Branch investigators worked with investigators in laboratories in Argentina, Germany, Italy, Korea, the Netherlands, and Spain. The Chief of the Branch served as grant reviewer for various research-funding institutions in Argentina, Austria, Canada, Germany, Israel, Italy, and the United Kingdom. The participation of foreign investigators in the Branch was supported, in part, by the Ministerio de Educación y Cultura's NATO (North Atlantic Treaty Organization) Science Fellowship Program of Spain; the Ramon Areces Foundation, Madrid, Spain; and the Ministry of University Affairs, Prince of Songkla University, Hat Yai, Thailand.

Pain and Neurosensory Mechanisms Branch

NIDCR's Pain and Neurosensory Mechanisms Branch conducts multidisciplinary research to improve understanding and treatment of pain. The program integrates basic and clinical research. During FY 99, a pathologist originally from Hungary and recently from the United Kingdom participated in studies to understand the molecular basis of pain transduction, including the heterologous expression of wild-type and mutated receptors of the vanilloid receptor family. These are the very first molecules in the pain transmission pathway from the pain nerve endings in the skin and deep tissues to the spinal cord and then to the brain. Through the pathologist's efforts, the Branch was able to establish the presence of the vanilloid receptor in plasma membrane

and endoplasmic reticulum. The scientists imaged the activity of the wild-type and mutant receptors by using in vivo, online confocal microscopy coupled with fluorescent tagging of vanilloid 1 receptors and contrasting tags for intracellular organelles, as well as calcium-sensitive dyes. They demonstrated that this very sophisticated system can be used to examine cellular responses triggered by the vanilloid ligands. They are continuing this work to establish a thermal-controlled cell for testing parallel effects of mutations on heat transduction. The scientist from Hungary and the United Kingdom has greatly assisted the Branch in fine tuning of its neuroanatomy research program.

In addition to this and other research conducted in the Branch, NIDCR investigators participated in the 9th World Congress on Pain, in Vienna, Austria, in August 1999, presenting an invited address and four research abstracts.

Immunopathology Section

Scientists in NIDCR's Immunopathology Section are defining the pathological effects of inflammatory processes on connective tissue. During FY 99, a scientist from India contributed to understanding of the signaling pathways involved. The scientist continued to delineate specific MAP kinases in the signal transduction pathway for induction of phospholipase, prostaglandin H synthase 2, and MMPs in human monocytes. This study also continued to focus on identifying and characterizing the regulation of monocyte MT1-MMP and its relationship to activation of the MMP-2 produced by stromal cells in bone marrow and tumor cells.

Matrix Metalloproteinase Unit

During FY 99, scientists in NIDCR's Matrix Metalloproteinase Unit focused on the role of membrane-associated MMPs, specifically MT1-MMP, in the activation of other MMPs. In collaboration with scientists in Canada, Denmark, Italy, and Spain, the NIDCR scientists made a major advance by genetically engineering a mutant mouse deficient in active MT1-MMP. (See the section on "Highlights of Recent Scientific Advances Resulting From International Activities.") These scientists are continuing to probe the complexity of this system by reconstitution experiments to define the parameters of MMP activation in general.

Molecular Structural Biology Unit

During FY 99, two investigators, from India and Japan, participated in a targeted, antiviral research program conducted by NIDCR's Molecular Structural Biology Unit. The investigator from Japan developed new methods for investigating slow, cooperative molecular motions of the backbone and side chains of proteins and then applied these techniques to characterize the molecular motions of various domains of the HIV-1

protease, free in solution and bound to potent inhibitors. In recognition of this work, the investigator has been invited to present a lecture at the XIXth International Conference on Magnetic Resonance in Biological Systems, to be held in Florence, Italy, in August 2000. The research was supported in part by the Human Science Foundation of Japan and was complemented by additional studies with other investigators in Japan.

In related work, the investigator from

India initiated investigations of the effect of inhibitor binding on the hydrogen bonds within HIV-1 protease. This study is focusing on the question of whether inhibitor binding may affect hydrogen bonds that stabilize formation of the free protease dimer. The investigator also characterized the dynamics and structure of the C-terminal domain of the anti-HIV protein, MAP 30, and the hydrogen bonds in this protein.