National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1990

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National Institute of Dental Research

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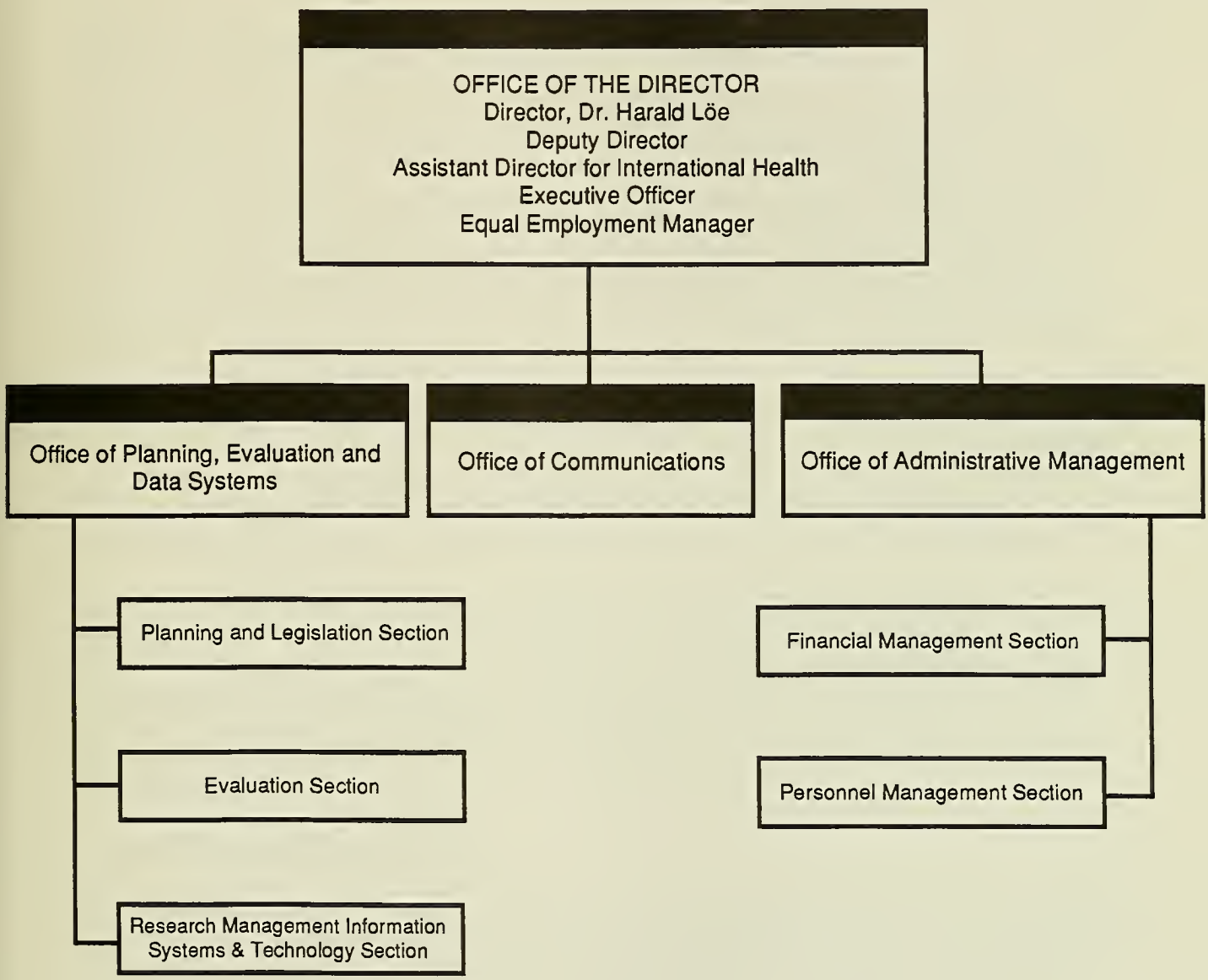
INTRODUCTION

FY 1990 marked the beginning of a new decade and the starting point for new directions in NIDR research. The eighties painted a picture of dramatic reductions in caries among school children and substantial improvements in the oral health of middle-aged America. Emerging from this picture, however, is a more subtle image of those who did not share in these successes.

While the younger segments of society have benefited greatly from advances in dental science, older Americans have not been so fortunate. They continue to suffer high levels of caries, periodontal disease and toothlessness. Also warranting attention in the new decade are individuals at high-risk for oral disease: the handicapped, institutionalized, immunocompromised and others who come under the umbrella of "special care" patients. These populations are the primary targets of NIDR's research thrust into the nineties, an effort which has as its ultimate goal "Teeth for Life" for all.

Office of the Director

NATIONAL INSTITUTE OF DENTAL RESEARCH



OFFICE OF THE DIRECTOR
NATIONAL INSTITUTE OF DENTAL RESEARCH

Research Directions and Policy

The NIDR entered the decade with a new *Long-Range Research Plan for the Nineties,* "Broadening the Scope." The plan advances two major initiatives that have become high priorities for the Institute. The first reflects concern for "special care" patients—those whose oral health is affected by well-known systemic diseases such as diabetes, cystic fibrosis, AIDS and others, as well as patients with rare or "orphan" diseases including scleroderma, osteogenesis and dentinogenesis imperfecta and the ectodermal dysplasias. Also included among special care patients are individuals who have suffered oral complications because of cancer chemotherapy or radiation to the head or neck. All told, these patients add up to millions of Americans.

NIDR's second major initiative is embodied in the *Research and Action Program for Improving the Oral Health of Older Americans and Other Adults at High-Risk.* The goals of this program are the elimination of toothlessness among future generations of Americans and the prevention of further deterioration of the oral health of individuals with already compromised dentition—with a target date for accomplishment set for the next 20 years.

The Research and Action Program emphasizes the importance of identifying individuals of any age who are at risk for oral health problems for whatever reason—dental, medical or socioeconomic. Thus, the program complements the Special Care Patient initiative but adds demographic factors such as age, education and income in determining risk. The combined research advances from these activities will clearly extend beyond the immediate target populations to the benefit of all Americans and will reinforce the theme for the 1990s: "Teeth for Life."

Program Management and Direction

In FY 1990, the Director provided oversight for broad Institute-wide planning of research and research training grants and contracts for the both the extramural and intramural programs. He held annual strategic planning meetings with each program component, reviewing past accomplishments and immediate and longer-range initiatives. The Director conducted periodic updates and on-course corrections in areas of trans-NIDR special interest ranging from specific use of the contracts mechanisms to scientific issues related to AIDS, minority health initiatives, and the Research and Action Program.

Extramurally, the Director oversaw the preparation and issuance of all program announcements. Of special note were solicitations for clinical core dental research centers and for research on taste and smell, temporomandibular disorders, and anti-HIV factors in saliva. The Director participated with other NIH components and outside agencies and organizations in efforts to develop innovative approaches to funding, such as fostering collaborations between academia and industry and establishing partnerships with foundations. Plans are under way to organize the first NIH government-industry-university research conference focused entirely on extramural support.

The Director served as Chairman of a World Health Organization/Fédération Dentaire Internationale Joint Working Group on International Collaboration for Oral Health Research aimed at establishing a multinationally funded trust to support research which requires a collaborative effort among countries.

The Director represented the Institute at the first meeting of the newly formed PHS Oral Health Coordinating Committee. The meeting addressed the NIDR Research and Action Program, focusing on coordination and the role other agencies can play in implementing this program.

The Director launched a new initiative with the National Cancer Institute to explore future collaboration in areas of mutual research interest. Dr. Löe met with the Director, NCI, to identify opportunities in both the extramural and intramural programs for collaborative efforts in such areas as oral cancer, oral effects of cancer treatments, prevention activities, joint training programs, and other relevant fields.

The Director oversaw the development of the annual Congressional justification and testified at Senate and House Appropriations Committee hearings, defending the President's budget and highlighting the year's research advances with emphasis on their implications for improved oral health.

Serving as Acting Director of the Epidemiology and Oral Disease Prevention Program (EODPP), the Director provided leadership in initiatives focused on aging, at-risk and minority populations; and advanced interagency collaborations in AIDS research between NIDR and the Walter Reed Army Institute of Research, and in epidemiological studies with the Department of Veterans Affairs. During this fiscal year, the Director aggressively recruited a distinguished dentist-epidemiologist to head EODPP. In his position as Acting Chief, Periodontal Diseases Section, EODPP, the Director continued as senior investigator in ongoing longitudinal studies of the natural history of periodontal disease in man.

In the Intramural Research Program (IRP), the Director selected an internationally known expert in the field of extracellular matrices as the new chief of the Laboratory of Developmental Biology and Anomalies. He also reviewed all components of the IRP with special attention to progress and plans presented by younger staff. The Board of Scientific

Counselors positively reviewed the Institute's Bone Research Branch and the Laboratory of Microbial Ecology during this fiscal year.

In FY 1990, the Director served as a member of the NIH Science Education Advisory Group, the NIH AIDS Executive Committee, and the NIH General Expense Budget Review. As a member of the NIH Performance Review Board, the Director also took part in the Senior Executive Service performance review for NIH.

Employee Opportunity

The Director reaffirmed the Institute's commitment to equal employment opportunity through a variety of activities and programs during FY 1990. The Director approved the NIDR's Federal Equal Opportunity Recruitment Program (FEORP) plan for 1989-1992 and led discussions with Executive Staff to heighten awareness of both the FEORP plan and the Merit Promotion Program throughout the Institute.

The Director's encouragement of supervisors and managers to recruit and employ minorities and women enhanced the NIDR profile by 5 percent in the Commissioned Corps category, by 2 percent in the General Management (GM) series, and by 5 percent in the General Schedule (GS) category. In addition, a total of three women and minority interns in NIH management and personnel intern programs were selected for assignment to the NIDR Executive and Personnel Offices.

The Director established an NIDR internal group to review the status of research relating to minorities and to plan new efforts. He initiated development of a tracking system to provide thrice yearly reports on minority-related research and efforts to recruit minorities into oral health research or to NIDR staff. A National Advisory Dental Research Council subcommittee will provide oversight.

While actively recruiting minority candidates for the position of EEO manager, the Director ensured continuity of equal employment opportunity activities by appointing the Executive Officer as acting EEO manager and maintaining current chairmanship of the EEO program advisory committee.

A Junior Fellowship for Administrative Positions was established during this fiscal year and a minority selected to participate. Partial support was also provided for the National Hispanic Youth Institute and for the NIH Disability Employment Awareness Program. Focusing on the handicapped, the Director arranged for a presentation to NIDR administrative staff on the Marriott Foundation for People with Disabilities project and selected an intern in this pilot project for assignment in the NIDR's intramural research program.

Under the Director's leadership, total FY 1990 minority program support was $1,040,572, a 79 percent increase over the previous fiscal year. This was accomplished through NIDR support of a variety of minority-related research and training mechanisms, including the MARC and MBRS programs, Minority High School Student Research Program, Intramural

NRSA Institutional Training Program, minority research supplement awards, and direct grant support to a minority institution.

Organizational Activities

In FY 1990, the Director oversaw a reorganization within OD's Office of Planning, Evaluation and Communications (OPEC), formerly comprised of the Planning and Evaluation Section, Research Data and Management Information Section, Public Inquiries and Reports Section (PIRS), and the Special Assistant for Special Projects. Under the reorganization, PIRS became the Office of Communications, and the remaining sections formed the new Office of Planning, Evaluation and Data Systems (OPEDS). OPEDS now includes the Planning and Legislation Section, Evaluation Section, and the Research Management Information Systems and Technology Section.

Under the leadership of the Director, internal controls and monitoring related to budget, positions and foreign travel were greatly enhanced. Procurement activities improved significantly, and centralization of the process was completed. Emphasis was placed on property accountability and required paperwork, and the Institute volunteered to participate in a pilot wall-to-wall property inventory.

The Director held a "Town Meeting" in spring 1990 in the Lister Hill Auditorium to provide an opportunity for employees to ask questions and raise issues of concern. He and Executive Staff provided detailed answers to these questions and oversaw the preparation and NIDR-wide distribution of the Town Meeting Summary in a special issuance of the Institute's EEO Bulletin.

The Director chaired regular meetings of the Executive Staff and Small Staff (immediate OD directors) and periodically attended program-level staff meetings to assure cognizance of management issues at all levels of the Institute and to be available to staff for open discussions. In accordance with the Ethics Reform Act, the Director conducted an ethics training session for all senior Institute staff.

Professional and Public Communications

A major concern in FY 1990 involved the premature release of data concerning fluoride carcinogenicity based on toxicology studies in rats and mice. The Director promptly alerted NIH and department officials and assembled a team of NIDR experts on this subject. He oversaw the development and distribution of data and position papers on water fluoridation; established a system of communications among dental professional groups and Public Health Service agencies to ensure smooth coordination of responses to fluoride information inquiries from a broad range of sources; provided technical information and advice to staff of the National Toxicology Institute, the sponsor of the study, about the release of the data; and pledged NIDR's continuing commitment to fluoride research and monitoring activities.

The Director addressed numerous professional dental research, education and practitioner audiences during FY 1990, using the occasions to describe NIDR research progress and plans and their implications for dentistry of the future. The Director held a forum at the joint meeting of the International and American Associations for Dental Research to exchange ideas and update the research community with respect to NIH and NIDR policies and activities.

The Director planned and conducted a highly successful 1-day research review program for visiting dental specialists. As a result, he is directing plans for a 3-day continuing dental education program to be held at the NIH in spring 1991.

During FY 1990, the Director conducted press and television interviews on a variety of dental subjects and appeared in a 1-hour documentary film on dental research produced by the American Fund for Dental Health for distribution to public television outlets. He also released information and data on major public health issues such as the effects on the oral tissues of smokeless tobacco use and the oral complications of cancer therapies. In other activities, the Director met with representatives of health voluntary organizations concerned with the oral effects of systemic diseases.

Honors and Awards

During this fiscal year, the Director was appointed Commander of the Royal Norwegian Order of Merit and was presented with a medal from King Olav V at the Norwegian Embassy in Washington, D.C. He also received Honorary Doctorates from the Medical University of South Carolina and the University of Detroit and an Honorary Membership in the International Academy of Periodontology.

Publications

Brown, L.J., Oliver, R.C., and Löe, H. 1990. "Evaluating Periodontal Status of U.S. Employed Adults." J. Am. Dental Assoc. 121:226-232.

Löe, H. 1990. "Teeth for Life: Oral Disease Prevention in Research and Practice." International Dental Journal 40:74-78.

Löe, H. and Drury, T. 1990. "Future NIDR Initiatives in Risk Assessment." In *Risk Assessment in Dentistry,* ADA Publications, Washington, D.C. pp. 301-302.

Löe, H., Listgarten, M.A., and Terranova, V.P. 1990. "The Gingiva: Structure and Function." In *Contemporary Periodontics*. Genco, R., Goldman, H.M., and Cohen, D.W. (Eds.) The C.V. Mosby Company, St. Louis, pp. 3-32.

Löe, H. and Morrison, E. 1990. "Epidemiology of Periodontal Disease." In *Contemporary Periodontics*. Genco, R., Goldman, H.M., and Cohen, D.W. (Eds.) The C.V. Mosby Company, St. Louis, pp. 106-116.

Löe, H. and Dubner, R. 1990: "Pain Research: From Laboratory to Clinic." Philip Journal, 7(4):193-198.

Presentations

Remarks. NIDR Awards Ceremony, Bethesda, MD, Oct. 5, 1989.

"Complications of Diabetes in Native Americans: Periodontal Disease." Conference on Diabetes in American Indians and Alaska Natives, Mesa, AZ, Nov. 15, 1989.

Speech at Norwegian Embassy upon acceptance of Norwegian Royal Order of Merit, Washington, D.C., Nov. 17, 1989.

"Sri Lanka Study and Update on Incidence of Periodontal Disease in the U.S." Rounds to periodontal residents, USPHS, Bethesda Naval Hospital, Bethesda, MD, Nov. 29, 1989.

"Changing Disease Patterns—Their Impact on Dentistry." Yankee Dental Congress, Boston, MA, Jan. 19, 1990.

"Dental Research and the Practicing Dentist." Yankee Dental Congress, Boston, MA, Jan. 19, 1990.

"Return to the Future: Several Views of Biomedical Research." Celebration of Research Symposium, San Antonio, TX, Jan. 23, 1990.

"Can Basic Research Affect the Clinical Management of Periodontal Disease?" Lecture Magistralis to 5th International Congress of the Italian Society of Periodontology, Rome, Italy, Feb. 17, 1990.

Opening Statement. Senate Appropriations Hearings, Washington, D.C., Feb. 20, 1990.

"NIDR's Perspectives on Implantology Research." Symposium on Implant Dentistry Technology Assessment, AADR, Cincinnati, OH, March 7, 1990.

Opening Statement. House Appropriations Hearings, Washington, D.C., March 13, 1990.

"Changing Trends in Oral Health" and "The Changing Face of Dentistry." Tufts University School of Dental Medicine, Dean's Seminar Series, Boston, MA, April 10, 1990.

"Current Avenues and Advances in Dental Research." Pan American Health Organization, Seminar for Dental Deans of Spain, Washington, D.C., April 11, 1990.

Participation in panel discussion at PAHO Headquarters, Washington, D.C., April 13, 1990.

Introduction to Kreshover Lecture, NIH, Bethesda, MD, May 30, 1990.

"The Broadening Scope of Dentistry." Commencement Speech, University of Detroit School of Dentistry, Detroit, MI, June 3, 1990.

"The Prevalence and Severity of Periodontal Disease in the U.S." D. Walter Cohen Periodontal Symposium, Philadelphia, PA, June 7, 1990.

"Research Developments Affecting Oral Health by the Year 2000." Second International Conference on Preventive Dentistry and Epidemiology, Karlstad, Sweden, June 11-14, 1990.

"Dental Science in a New Age." Clinical Center Grand Rounds, NIH, Bethesda, MD, Aug. 1, 1990.

Chair Symposium I, "Periodontics Today." 78th Annual World Dental Congress, Singapore, Sept. 10, 1990.

"The Changing Face of Dentistry." Keynote Address to the International Association of Periodontology Meeting, Istanbul, Turkey, Sept. 17, 1990.

OFFICE OF THE DEPUTY DIRECTOR

The Deputy Director shares responsibility with the Director, NIDR, in the direction and management of the Institute's programs and activities, and has full authority to act on his behalf. With the Director, the Deputy represents the DHHS and the NIH on matters pertaining to the Institute's programs and budget, and ensures effective liaison with other Federal agencies, professional organizations, and the dental research community.

Institute Management

The Deputy Director participated in the development of the FY 1992 budget proposal, and the preparation for and defense of the President's 1991 budget, including participating with the NIDR Director at the House of Representatives Appropriations Subcommittee hearings and the development of responses to the questions of the House and Senate Appropriations Subcommittee members.

The Deputy Director serves as the Performance Recognition Group Manager for all Performance Management Recognition System employees and as the Employee Performance Management System Budget Manager for the Office of the Director employees. He also serves as a member of the review group for all NIDR quality increase and bonus recommendations for EPMS employees and approves performance awards for PMRS employees.

The Deputy Director has been actively involved in the recruitment process for a permanent Director of the Epidemiology and Oral Disease Prevention Program.

The Deputy Director continues to serve as the Executive Secretary of the Institute's bimonthly Executive Staff meetings and the Office of the Director small staff meetings. In his Chief-of-Staff role, he has ensured the timely completion of resulting action item assignments.

NIDR Advisory Council and Committee Activities

The Deputy Director oversees the committee management function of the NIDR. The NIDR has four formally chartered advisory committees, one of which is the National Advisory Dental Research Council. The Deputy Director serves as the Executive Secretary for this Council. He was responsible for the conduct of the January, May and October meetings in FY 1990.

NIDR and NIH Representation Activities

In the absence of the Director, the Deputy Director represented NIDR at the weekly NIH Institute/Center/Division (ICD) meetings. He also represented NIDR at the NIH ICD Deputy Directors meetings. The Deputy Director is a founding member of this group which has substantially increased communications among the respective ICDs and has facilitated both the resolution of some problems and cooperative involvement on special initiatives.

Liaison Activities

The Deputy Director served as the NIDR liaison consultant to the National Affairs Committee of the American Association for Dental Research, the Council on Dental Research of the American Dental Association, the Association of State and Territorial Dental Officers, the National Dental Association, the American Association of Public Health Dentistry, and the Dental Section of the American Public Health Association. Additionally, the Deputy maintained very close coordination with the Washington offices of the American Dental Association, the American Association of Dental Schools, American Dental Hygienists' Association, and the American and International Associations for Dental Research. The Deputy Director represented the NIDR by participating in the annual meetings of these associations, as well as the Annual Conference of Dental School Deans.

U.S. Public Health Service (PHS) Dental Responsibilities and Activities

In addition to his NIDR responsibilities, Dr. Littleton serves as Deputy Chief Dental Officer, PHS. In this role, he assists the Chief Dental Officer in providing leadership and coordination of PHS national and international oral health activities, and dental professional affairs of the Office of the Surgeon General; represents the Surgeon General to local, state, national and international groups and professional societies; provides advice and guidance on matters such as recruitment, retention and career development of PHS dental personnel; and performs other duties as assigned by the Surgeon General. He has spent considerable energy in assisting in the oversight of dental activities of the PHS and carrying out a broad range of assignments.

Liaison with national dental organizations is an important activity. The Deputy Chief Dental Officer served as the alternate delegate to the House of Delegates of the American Dental Association and as delegate to the American Association of Dental Schools.

In his role as Deputy Chief Dental Officer and as an ex-officio member of the Surgeon General's Dental Professional Advisory Committee, he was actively involved in a number of important PHS initiatives.

As both NIDR Deputy Director and Deputy Chief Dental Officer, he was instrumental in establishing an interagency mechanism (Oral Health Coordinating Committee) for pursuing national adult oral health initiatives. Under this initiative, he has led the NIDR in establishing an association with other agencies that has resulted in the specification of national goals and

objectives, comprehensive agency assessment of relevant oral health activities, budgetary redirection in support of the initiative, and proposals for achieving the goals and objectives.

The Deputy Chief Dental Officer helped organize a major national symposium at the meetings of the American Association of Dental Research and the American Association of Dental Schools to effectively inform the dental research and educational communities concerning the status of fluoride toxicity studies and the PHS role. Much misleading and potentially harmful information had been disseminated by special interest groups so that it was especially important to report the research data to the scientific community and detail the initiatives undertaken by the Department of Health and Human Services in response to the preliminary study findings. This session, attended by more than a thousand scientists, was assembled under severe time constraints and served to clarify events to date, outline the steps the department had initiated to address the controversy, and allay many concerns.

Professional Activities

The Deputy Director maintains his academic appointment as a Professorial Lecturer in the Department of Community Dentistry, Georgetown University School of Dentistry. In this role, he conducts seminars and lectures and participates in other faculty activities. He serves on the Editorial Review Board of the *Journal of Dental Education,* as a special reviewer for the American Fund for Dental Health, and as a member of the Fund's Hillenbrand Fellowship Committee. In addition, he maintains membership in a broad range of professional associations and actively participates in their meetings.

Presentations

Welcome to the Future. Convocation Address, School of Dental Medicine, State University of New York at Stony Brook. May 20, 1990.

Adult Oral Health Promotion: Coordination of Efforts to Improve Oral Health. Annual Meeting of the Association of State and Territorial Dental Directors and the National Oral Health Conference, San Diego, CA. April 3, 1990.

Research Training Opportunities in Dentistry. 26th Annual Dental Students Conference on Research, School of Dentistry, University of California School of Dentistry. April 1, 1990.

The Excitement of Entering a Profession in Transition. District of Columbia Dental Society Spring Meeting, Washington, D.C. March 25, 1990.

Update on the National Toxicology Program Fluoride Toxicity Study. Oral Medicine Fellowship Rounds, National Institutes of Health, Bethesda, Maryland. March 15, 1990.

Panelist, Special Symposium on Fluoride. Annual Meetings of the American Association of Dental Schools and American and International Associations for Dental Research, Cincinnati, OH. March 7, 1990.

Symposia Co-Moderator, Can People with AIDS Get the Dental Care They Need? 52nd Annual Meeting of the American Association of Public Health Dentistry, Honolulu, HI. November 2, 1989.

Dental Public Health - The Trends and Status of Federal Programs. 117th Annual Meeting of the American Public Health Association, Chicago, IL. October 24, 1989.

Career Options in the Uniformed Services and Public Health. 2nd Annual AADS/ICD Careers Conference in Dentistry, Northwestern University, Chicago, IL. October 14, 1989.

OFFICE OF THE ASSISTANT DIRECTOR FOR INTERNATIONAL HEALTH

The Assistant Director for International Health is responsible for coordinating global oral health initiatives, ongoing programs and activities, and facilitating their progress as well as promoting communication among U.S. dental investigators and scientists abroad. During Fiscal Year 1990, major effort was expended on initiating relevant international activity related to the Long-Range Research Plan for the Nineties.

International Collaboration for Oral Health Research (ICOHR)

ICOHR is an integral part of the NIDR Long-Range Plan for the Nineties and represents a research agenda of questions which require or would be greatly enhanced by international collaborative approaches to study design and conduct. The document also includes recommendations to facilitate the initiation and implementation of such research. Activities during the current fiscal year to further this initiative included: meetings with NIDR intramural and extramural staff to develop research proposals; report to the Fogarty International Center Advisory Board on activities in progress or planned; correspondence and other communication with potential extramural investigators in the U.S. or abroad to effect strengthened capacity to conduct collaborative research; formal papers describing ICOHR to the American Association of Public Health Dentistry (Hawaii) and to the Southeast Asian Division, International Association for Dental Research (Singapore) and in the course of Extramural Program seminars given at the Medical College of Georgia, the University of Pittsburgh and the University of Rochester; incorporation of incentives for international collaboration in the Request for Applications for the clinical core dental research centers and the updated announcement on program project grants.

Perhaps one of the more ambitious projects has been the initiation by the Director and the Assistant Director for International Health of the Fédération Dentaire Internationale/World Health Organization Joint Working Group 15 on ICOHR. This group, composed of dental research representation from each of the seven economic summit countries, the European Community and the International Association of Dental Research, has as its objective to garner financial support to sustain some ICOHR projects. Having convened in June 1990 in Geneva, a plan was outlined to test the feasibility of utilizing initially four areas to promote funding: fluorides; oral cancer; orofacial anomalies and dental biomaterials. Expert panels will be convened early during fiscal year 1991 to develop promotional materials which will be reviewed in February 1991 by the Joint Working Group. Modelled after the Human Frontiers Science Program, the goal will be to set up an international trust fund to be located at the WHO which can supplement sources of support to be found nationally.

International Collaborative Study of Oral Health Outcomes (ICS-II)

Continuing the implementation of the ICS-II involving the NIDR, the Centers for Disease Control, the Indian Health Service, the World Health Organization and its subcontractor the Center for Health Administration Studies at the University of Chicago, data collection has proceeded in New Zealand, the USSR, Poland, and in US sites among Native-American populations and in the Baltimore metropolitan and non-metropolitan areas. A meeting of organizers and site coordinators was held in March in Cincinnati, Ohio, and in September in Singapore. Funds have been made available through the US-India Rupee Fund for sites in India. A research grant application has been submitted to the NIH for a replication in Israel and for supplementary support for analyses of the older age group samples cross-nationally (to the NIH and or the Agency for Health Care Policy and Research, PHS). The NIDR portion of support is dedicated to the Baltimore replication: one contract to Research Triangle Institute and another contract to the Oral Health Unit of the World Health Organization.

Organizational Liaison: World Health Organization (WHO)

NIDR continued in its role as the WHO Collaborative Center for Epidemiology, Prevention and Treatment of Oral Diseases and Conditions. Aside from the ICOHR and ICS-II programs which vitally involve WHO, the NIDR participated actively in the sixth and seventh coordinating meetings for WHO Centers dealing with oral manifestations of HIV infection: a meeting held in March in Cincinnati, Ohio; and one meeting held in September in Singapore. The NIDR assumed the lead responsibility for developing minimal essential data required for epidemiology and surveillance purposes. A core protocol has been developed for the purpose of conducting natural history studies. The Epidemiology and Oral Diseases Prevention Program (EODPP) staff with a contractor drafted the document and is integrating comments from the coordinating body members.

In conjunction with the American Dental Association, EODPP staff have reviewed and pilot-tested the WHO Surveillance System for Documenting Oral Manifestations of HIV Infection. The same staff also have substantially contributed to the Fédération Dentaire Internationale/WHO Joint Working Group 14 on AIDS in the areas of epidemiology and surveillance and have utilized both communication networks to obtain critical reviews and to share other related information on basic research, research related to infection control, disease prevention and health promotion. EODPP assisted Extramural Program staff in administering the NIDR supplement to the Fogarty International Center's training grant at the University of California—for a short course to train oral epidemiologists for AIDS studies. The WHO networks, once again, were effectively utilized to inform audiences of this two-day course which attracted foreign scientists from South America, Africa and Thailand.

Both the dental staff of the Pan American Health Organization and of WHO headquarters in Geneva have been helpful in identifying potential research resources in Eastern European and in Latin American countries—leads which are being explored and hopefully will eventuate in collaborative research activity between US dental scientists and these foreign investigators.

The Director and the Assistant Director for International Health participated in a meeting convened by WHO in Gex, France during July to begin discussions regarding a blueprint for oral health in the first half of the 21st century. This blueprint, once developed, should guide the Oral Health Unit's short and long-range planning activities in areas covering oral health status and trends, projections of treatment needs and manpower requirements as well as health promotion efforts and research capacity building.

Organizational Liaison: Federation Dentaire Internationale (FDI)

NIDR staff continued to play an active role in the FDI for the primary purpose of science transfer to clinicians and for maintaining essential communication networks to international communities of dental professionals, clinicians as well as scientists, educators and administrators. The Director of NIDR serves as Chairperson of Joint Working Group 15 (ICOHR) of the Commission on Oral Health Research and Epidemiology (CORE) of which the Assistant Director for International Health is a member. The latter staff person completed her assignment to develop guidelines for oral health promotion as the remit for Working Group 3 of CORE. The report was published in the *International Dental Journal* during FY 1990. Joint Working Group 14 on AIDS, also a CORE activity, is covered by the Chief, Soft Tissue, Craniofacial Defects and Pain Section, EODPP.

The Assistant Director for International Health also serves on the Scientific Programme Committee for the FDI and has contributed to program development for Singapore (September 1990), for Milan (October 1991) and for Berlin (September 1992). Suggestions are submitted for the FDI speakers databank in all areas of dental research of interest to clinical and public health audiences. She also chaired a special session of the Singapore meeting on "New Frontiers in Patient Care" which was organized by an EODPP staff member and the FDI affiliated organization, Behavioral Scientists in Dental Research. She also represents the latter organization to the FDI General Assembly.

The Health Science Administrator for the Restorative Materials Area, Extramural Program, NIDR completed her term as chairman of the Commission on Dental Products but continues as a consultant to that FDI Commission as well as a delegate to the International Standards Organization which met in Beijing during FY 1990.

Organizational Liaison: International Association for Dental Research (IADR)

Aside from ICOHR initiatives which continued to receive visibility at the NIDR exhibit at the IADR meeting, the Assistant Director for International Health was the Distinguished Lecturer on this subject at the Southeast Asian Division meeting in Singapore in September 1990. She also serves on the IADR-FDI Scientific Advisory Committee.

Organizational Liaison: American Dental Association (ADA)

The Council on ADA Sessions and International Relations continued to utilize the advice of the Assistant Director for International Health on matters related to the FDI, WHO, and its

newest venture with Health Volunteers Overseas (HVO). There is now a distinct operating unit, Dental Volunteers Overseas (DVO), and the Assistant Director for International Health has been participating in meetings of the Steering Committee which is considering start-up programs in the Caribbean. During FY 1991, it is expected that specific sites will be identified, volunteers recruited, trained and utilized. It is also anticipated that the ADA and FDI secretary-treasurer will participate in the dissemination and promotion of the FDI Guidelines for Oral Health Promotion.

Organizational Liaison: Fogarty International Center (FIC)

The Assistant Director represented the NIDR at the NIH-ICD International Representatives meetings held every six weeks and coordinated by the FIC; submitted the NIDR annual international report as a contribution toward the NIH annual report document; transmitted NIDR staff comments on a variety of circulated documents including WHO, PAHO and the WHO Western Pacific Regional Office plans and reports; attended and presented an NIDR report at the winter FIC Advisory Board meeting; and participated in a WHO priority setting meeting convened by FIC. Comments were facilitated on one project for the US-Yugoslav Joint Fund on Scientific and Technical Cooperation.

FIC staff have assisted NIDR staff in the two-day training course on AIDS for oral epidemiologists held in San Francisco in June 1990. NIDR international travel is coordinated with FIC staff and such travel is reviewed for appropriateness by the Assistant Director. FIC staff specifically have facilitated the Taiwanese interest in dental research, new initiatives in Latin America and Eastern Europe, a request to the Director to receive a foreign decoration, one application for waiver of the exchange visitor foreign residence requirement and a variety of questions raised by staff grantees concerning regulations and policies governing administrative matters in the conduct of international research.

Bilateral Health Initiatives

US-Israel

NIDR staff participated in reviewing applications for research support to the US-Israel Binational Science Foundation. These reviews for program relevance and priority are coordinated by the Assistant Director for International Health and forwarded through the Fogarty International Center. Technical assistance was provided during the year to Israeli investigators submitting to NIH and/or PHS support for their research projects.

US-Italy

A new proposal was submitted during FY 1990 to facilitate NIDR intramural collaboration between the Clinical Investigations and Patient Care Branch and the University of Bari to support work on the purification and reconstruction of the $Na^+/K^+/2Cl$-cotransporter from rabbit parotid acinar cell basolateral membranes. Two other projects remain active in the Bone Research Branch of the Intramural Program.

US-USSR

For the first time dental health was included in the agenda of the US-USSR Joint Committee for Health Cooperation. The agreement, which also contained a dental initiative, was signed in June 1990 by the Assistant Secretary for Health, DHHS and the Soviet Minister of Health. The USSR intends to inaugurate this aspect of the agreement by hosting in Moscow at the end of April 1991 a joint symposium on oral manifestations of HIV infection and secondary immunodeficiency. In addition, potential collaboration in the areas of dental materials, pain research and oral health of aged populations will be explored.

Other International Activities

During this fiscal year, the Assistant Director provided orientation to the NIDR for visitors from a number of countries including Britain, China, Nigeria, South Africa, the USSR, and Yugoslavia. She also provided written responses to inquiries from Australia, Britain, Egypt, India, Israel, Mexico, Netherlands, Norway, Saudi Arabia, Scotland, Turkey and from WHO on behalf of Hungary. Requests for information ranged from research training opportunities, research grant eligibility and procedures, potential job applicants, assistance in publication preparation, conference support, background documents about extent of international oral health activities, to ideas for research initiatives.

The Assistant Director participated in the planning of a symposium, "Towards Coordination of US Organizational Initiatives for Global Oral Health," to be held in conjunction with the American Association for Public Health Dentistry during FY 1991.

She was also conferred Honorary Fellowship in the International College of Dentists in November 1989.

Publications

Cohen, L. K., "Leadership Role of Dental Associations," *International Dental Journal,* February 1990, Vol. 40, No. 1, pp. 48-52.

Cohen, L. K., "Promoting Oral Health: Guidelines for Dental Associations," *International Dental Journal,* April 1990, Vol. 40, No. 2, pp. 79-102.

Presentations

Cohen, L. K., "International Collaboration for Oral Health Research," Paper presented at 52nd meeting of the American Association of Public Health Dentistry, Honolulu, Hawaii, November 3, 1989.

Cohen, L. K., "International Collaboration for Oral Health Research," Paper presented as Distinguished Lecturer for the 5th Scientific Meeting of the International Association for Dental Research - Southeast Asian Division, Singapore, September 8, 1990.

OFFICE OF PLANNING, EVALUATION AND DATA SYSTEMS (OPEDS)

This office, formerly the Office of Planning, Evaluation and Communications (OPEC), was reorganized in 1990. The new *Office of Planning, Evaluation and Data Systems* (OPEDS) develops and coordinates science program and policy activities related to planning, evaluation, analysis, and legislation; and maintains the NIDR research project information systems and serves as the NIDR focus for ADP technology. The Office also provides essential support services to the Director of the Institute in developing documentation for speeches, presentations and publications. OPEDS includes: Office Chief, Planning and Legislation Section, Evaluation Section, and Research Management Information Systems and Technology Section (RMISTS).

PLANNING AND LEGISLATION SECTION

The Planning and Legislation Section, OPEDS, develops and coordinates science policy and program planning for the Institute, including annual plans; Long-Range Research Plan; Biennial Reports; and special policy and planning efforts relating to specific research issues or initiatives. The section also functions as the Institute's legislative liaison, and assists in the liaison with health voluntary organizations and dental professional groups.

Planning

A major activity of the section was completion and preparation for publication of the *NIDR Long-Range Research Plan for the Nineties*, "Broadening the Scope." The manuscript was edited and revised following reviews by the National Advisory Dental Research Council (NADRC) and other advisory groups and consultants. Editorial changes also were made to reflect new studies and reviews of the safety of fluoride conducted by the National Toxicology Program of the National Institute of Environmental Health Sciences, the National Cancer Institute, and the Office of the Assistant Secretary for Health.

Other major planning activities served to advance new initiatives stated in the Long-Range Research Plan concerning patients at high risk for oral health because of systemic disease (e.g., diabetes, ectodermal dysplasias, osteogenesis imperfecta) or disease treatment (e.g., radiation or chemotherapy for cancer). Staff addressed meetings of health voluntary organizations and professional groups who have formed a National Alliance for Oral Health. Staff cooperated in efforts of the American Dental Association to collect informational materials for the general public and health care providers concerning oral health problems associated with systemic disease.

Staff coordinated development of the contributions of NIDR and NADRC to the FY 1989-90 Biennial Reports of the Director, NIH as requested by Congress. Staff developed and coordinated materials for the annual briefing session with the Acting Director, NIH, highlighting new initiatives, reviewing the status of ongoing initiatives, and discussing issues that may affect scientific programs. Staff, working with the RMISTS and Budget Office, prepared special reports on NIDR funding of research in such areas as rehabilitation research and minority health. Staff prepared NIDR responses to the special initiative to develop an NIH-wide Strategic Plan and to new directives and program priorities established by the Secretary, HHS.

Legislation

As the NIDR legislative contact with the Division of Legislative Analysis (DLA) in the Office of the Director, NIH, staff responded to proposed legislation in Congress in such areas as the use of animals in research, reviewed testimony prepared for Congressional hearings, for example, on the human genome mapping initiative, and commented on legislation proposed by NIH and other Public Health Service agencies. Staff informed DLA of oral health issues raised by the public, the media, or members of Congress and their staffs.

Other Activities

Planning and legislation staff worked with the Director, NIDR and Budget Office in preparing the FY 1991 Congressional Justification, opening statements, and Congressional questions for the record. Staff worked with the Director, NIDR in preparing oral presentations and articles for a variety of audiences. Staff attended special meetings of intramural staff and extramural research scientists convened at the request of the Director, NIDR, to address the future scope of NIDR research activities. Staff is preparing a report based on these discussions, the Long-Range Research Plan, and other information, to discuss the impact of the expanded mission of the Institute on its current structure and functions, as well as implications for research manpower training and career development, dental education and practice.

Staff served as a member of the following groups or committees:

- NIH Planning and Evaluation Officers
- NIH Animal Welfare Steering Committee
- NIA/DVA/NIDR Collaborative Project on Oral health in Aging

Presentations

Joan Wilentz

"NIDR and Special Patient Issues"—Priorities for the Nineties presented at the National Alliance for Oral Health meeting in Bethesda, June 1990.

"Planning Activities at the NIDR"—meeting of Robert Wood Johnson Fellows, Bethesda, July 1990.

"Priority Program Initiatives at the NIDR"—American Association of Dental Schools Legislative Workshop, September, 1990.

Assistance to the Director, NIDR in preparing the following speeches:

- Conference on Diabetes in American Indians and Alaska Natives/Phoenix
  Complications of Diabetes in Native Americans: Periodontal Diseases

- Yankee Dental Congress/Boston
  Dental Research and the Practicing Dentist and *Changing Disease Patterns—Their Impact on Dentistry*

- Celebration of Research Symposium, University of Texas/San Antonio
  Return to the Future: Several Views of Biomedical Research

- Fifth International Congress of the Italian Society of Periodontology/Rome
  Lectura Magistralis: *Can Basic Research Affect the Clinical Management of Periodontal Diseases?*

- International/American Association for Dental Research/Cincinnati
  Reactor Comments: *NIDR Perspectives on Implantology Research*

- House and Senate Appropriations Subcommittees/Washington
  Opening Statements and Highlights

- Tufts University School of Dental Medicine Dean's Seminar/Boston
  Changing Trends in Oral Health and the Changing Face of Dentistry

- Pan American Health Organization Seminar for Spanish Dental Deans/Washington
  Current Avenues and Advances in Dental Research

- University of Detroit School of Dentistry Commencement Address/Detroit
  The Broadening Scope of Dentistry

- Second Annual Conference on Preventive Dentistry and Epidemiology/Karlstad, Sweden
  Research Developments Affecting Oral Health by the Year 2000

- NIH Clinical Center Grand Rounds/Bethesda
  Dental Science in a New Age

- Fourth Meeting of the International Academy of Periodontology/Istanbul
  Keynote Address: *The Changing Face of Dentistry*

Article:

- Editorial for Philip Journal/Munich
  Goals of Dental Medicine in the Year 2000

EVALUATION SECTION

The Evaluation Section is responsible for developing and coordinating program evaluation activities for the NIDR, including the Annual Evaluation Plan and assessment of the content, significance, and effectiveness of all NIDR programs.

During FY 1990, the NIDR began development of an evaluative bibliometric information system, in collaboration with staff from the NIH Library, a contractor, ROW Sciences, Inc., and a dental COSTEP student. The purposes of this system are: (1) to enable the NIDR to track the research output of its grantees and the citations received by this output, and (2) to monitor the orofacial research activity of the international dental research community. The system is based on information about research articles found in MEDLINE and supplemented, when necessary, by journal lookups.

To accomplish the first purpose, the following data are being compiled for all articles resulting from NIDR support through the R01, P01, and P50 extramural research grant mechanisms: title of publication; all authors, their institutions and countries; all sources of support acknowledged (NIDR and other) in the article; journal title, volume, pages, year of publication; and assigned MeSH keywords. Using the Science Citation Index, total citations to every article are computed. Data from MEDLINE are obtained through GRATEFUL MED, and then downloaded and stored using askSam, an information management software package. All publications are classified according to the NIDR Long-Range Research Plan. Coding is based on a thesaurus, developed by NIDR and NIH Library staff, for major research areas covered in the Plan; the thesaurus is based on MeSH keywords. The first project in which this database will be used compares the quantity and quality of research output from the Specialized Centers (P50) program to that of a cohort of program project grants (P01) and traditional research grants (R01), controlling for research area and duration of funding.

To address the second purpose, similar procedures are employed to compile data from all articles in the most outstanding international dental research journals. So far, this has been accomplished for publications in the years 1985-1988. Information will be used to compare research among various countries, institutions, and investigators.

Other evaluation research projects begun in FY 1990 include the following: (1) determining the research accomplishments and current status of individuals who were supported by the NIDR during research training between 1980-1988 through the NRSA program (institutional or individual fellowship award); (2) developing a methodology to evaluate, on an ongoing basis, individuals who have completed training under the Dentist Scientist Award; (3)

assessing the citation patterns to articles authored by NIDR intramural research scientists; and (4) developing a model for projecting the needs for oral health research manpower, by specific area, in the year 2000.

Staff also developed the FY 1991/1992 NIDR Evaluation Plan.

Staff served as a member of the following groups or committees:

- Evaluation Technical Review Committee, NIH
- Planning Committee for report on "Educating Dentists For The Future," Institute of Medicine, National Academy of Science
- Technical Review Panel, "Evaluation of General Clinical Research Centers Program," NCRR, NIH
- Workshop on "The Future of Research in Dental Public Health"
- Workshop on "Dental Informatics: Strategic Issues for the Dental Profession"

Publications

J.A. Reese and J.A. Lipton. "Contribution of Women Dentists to Dental Research," *International Dental Journal,* 40: 139-141, 1990.

Presentations

"Future Directions for NIDR-Funded Pain Research," NIDR Clinical Pain Meeting, January 31, 1990

"Dental Informatics," at Clinical Staff Fellowship Conference, Clinical Investigations and Patient Care Branch, Intramural Research Program, NIDR, February 8, 1990

"Planning and Evaluation of Research Activities at the NIDR," Grants Associate/HSA Seminar Series, NIH, February 16, 1990

"International Comparison of Research Performance at Dental Institutions," Invited presentation at the Annual Meeting of the American Association for Dental Research, March 7, 1990

"Policy Applications of NIH Evaluation Research for the Sociology of Science and Technology," American Sociological Association Annual Meeting, August 13, 1990

"Measuring Scientific Research Activity: A Comparison of Bibliographic and Survey Data," American Sociological Association Annual Meeting, August 14, 1990

Staff also coordinated:

- Development of "New Approaches to Computer-Assisted Diagnosis and Decision-Making in Dentistry," a symposium sponsored jointly by the American Association for Dental Research and American Association of Dental Schools, March 7, 1990

- A tour of the NIH for members of the American Sociological Association at their annual meeting, August 13, 1990

RESEARCH MANAGEMENT INFORMATION SYSTEMS AND TECHNOLOGY SECTION (RMIST)

The Research Management Information Systems and Technology Section (RMIST) is responsible for the collection, retrieval, reporting and management of data related to NIDR research. In close collaboration with other NIDR sections (i.e., Financial Management, Grants Management, Contracts Management, Extramural Research Program, Intramural Research Program, and Epidemiology and Oral Diseases Prevention Program), and NIH components (e.g., Division of Research Grants and Division of Financial Management), data are collected on all NIDR projects, and reports are generated to reflect the substantive and fiscal posture of the Institute and to assure consistency with NIDR data reported by NIH.

To meet the research and management information needs of the Institute, RMIST continues to create and maintain computer databases on NIDR research and to prepare reports about NIDR scientific, programmatic, and budgetary activities. To meet the NIDR's needs of the 1990s, RMIST will put increasing emphasis on developing online databases that can be queried directly by end users and serve as a resource to all of NIDR regarding automated data processing and information technology including hardware, software, and online services. In FY 1990, RMIST established two units to reflect this new mission: the Research Projects Databases and Analysis Unit, and the Systems and Technology Unit. Activities of both units are described in the following paragraphs.

Technical Reports

The recurring need for certain kinds of information and the need for an easily accessible archival record prompted the development of several printed technical reports on a fiscal year basis. In FY 1990, initiatives were undertaken to improve consistency. New systems were implemented by the NIDR to update the DRG databases to make them consistent with the NIDR databases (e.g., the AIDS field in the IMPAC was reviewed for the first time and updated). A new series was started by RMISTS, entitled "Reporting Concepts," which documents the decision rules used in many NIDR reports, particularly definitions that are mandated by the NIH (e.g., what types of grants to include in "Other Research").

The *National Institute of Dental Research Programs* is a comprehensive collection of charts and tables which list and display, in a variety of formats, all of the projects supported by the

Institute. The "Programs" booked was revised substantially in FY 1990 to display the information in a format more consistent with NIH requirements and with other NIDR publications, such as budget reports.

The *Annual Report,* required by the National Institutes of Health, and its companion, the *NIDR Code Book,* provides us with not only a yearly record of activities but is the source of data elements on projects that are essential to the information needs of the Institute. The *NIDR Indexes* provide information about these projects by subject using the Computerized Retrieval of Information on Scientific Projects (CRISP) thesaurus of terms. *Trainees and Fellows Supported by the National Institute of Dental Research* provides the only hard-copy record of that very important area of support.

DENTALPROJ

DENTALPROJ is a data file of ongoing dental research projects supported by the National Institutes of Health, the Department of Veterans Affairs, Department of Defense, and the Centers for Disease Control. These projects have been indexed by specially trained scientific indexers using the Medical Subject Headings, or MeSH keywords, employed in MEDLINE. NIDR is the only component of the NIH that provides the scientific community this unique method to access information about research in progress.

In FY 1990, new dissemination activities were undertaken including publishing a pamphlet describing DENTALPROJ, a workshop at the Annual meeting of the American Association of Dental Schools, and online demonstrations on a wide screen at the annual meetings of the International Association of Dental Research and the American Association of Dental Schools.

NIDR ONLINE

The NIDR ONLINE system was introduced in 1986 to improve the transfer of information from NIDR to its constituency. NIDR ONLINE is an electronic bulletin board that contains news from NIDR, telephone directories, and current grant information.

In FY 1990, we added the full text of all current NIDR Requests for Applications and Requests for Proposals. In FY 1990, new dissemination activities were undertaken, including publishing a pamphlet describing NIDR ONLINE, a workshop at the Annual meeting of the American Association of Dental Schools, and online demonstrations at the annual meetings of the International Association of Dental Research and the American Association of Dental Schools.

Conflict of Interest System

In response to a request initiated by the Grants Management Section, a computerized Conflict of Interest System was developed for the Extramural Programs. The new system automatically identifies any Council Members whose affiliations and/or Institutions are in

conflict with a particular Grant being reviewed at Council. This automated approach represents significant improvement in efficiency over the existing manual method.

Computer Generated Council Books

RMIST has also been involved in the automation of the National Advisory Dental Research Council Books. The first NIDR computer generated Council book was transmitted in May for testing purposes, and we plan on permanently instituting this procedure for the October Council. This pilot project was made possible through extensive consultation and collaboration with the Division of Research Grants (DRG), the NIDR Grants Management Section, and the NIDR Scientific Review Branch.

Intramural Procedures

RMIST has designed and instituted a new system to track the NIDR Intramural and Epidemiological Research Projects and to calculate the cost for each project using a more accurate method. The cost for each project is determined by an algorithm that uses the Manyears per Project to allocate the total costs per Laboratory.

NIDR ADP Committee

RMIST staff serve as the Chairman and executive staff of the NIDR ADP Committee. In FY 1990, this committee initiated and completed a long range plan, and developed and recommended various policies to improve the ADP activities at the NIDR.

Technology

RMIST has concentrated on upgrading its equipment and expertise and establishing procedures to improve the transfer of this information to other components of NIDR. For example, all dedicated terminals in RMIST were replaced with personal computers that can be used also for terminal emulation; and preparations for installing a LAN system were made.

Personnel

Section staff members represent NIDR on a number of NIH coordinating groups including the ADP Systems Planning, ADP Systems Security, ADP/ Extramural Programs Committee, the Advisory Committee for Employees with Disabilities, Office Technology Coordinating Committee, and Information Resources Management Committee. A member of the RMIST staff has been appointed a member of a new committee organized by the Division of Research Grants (DRG) to consider how the DRG IMPAC system can be successfully migrated into a Database Management System (DBMS) environment.

Special recognition was given by the PHS ADP/EP Coordination Committee to: Sheldon Fishman for his leadership in bringing database technology to NIH; Carla Flora by the PHS ADP Committee for her development of the S-CRISP program; and Deane Hill by the PHS

ADP Committee for her continuing service on its Executive Committee. In addition, Mary Eileen Lukes received the NIDR Employee of the Month award, and Janet Pomerantz received the 1989 Award for Outstanding Contributions to the NIH-Handicapped Employees Committee.

OFFICE OF COMMUNICATIONS (OC)

In Fiscal Year 1990, a reorganization took place within OD's Office of Planning, Evaluation and Communications (OPEC). The Public Inquiries and Reports Section, formerly a component of OPEC, was elevated to the Office of Communications (OC). This office is charged with the development, coordination and implementation of a communications plan for the Institute. Research advances in the oral health sciences and NIDR program activities are shared with the public, Congress and the dental profession through a variety of communications mechanisms, including the development and distribution of patient and professional education materials, publications, exhibits, scientific reports and audio-visual productions. The Office of Communications also manages media outreach and press relations activities with both the general and trade press. In addition, OC coordinates all information clearance procedures for scientific publications, media interviews, public materials, audio-visuals, printing and graphics. Other responsibilities include coordination of all Privacy Act and Freedom of Information Act functions for the Institute.

Special Projects

Special projects carried out in FY 1990 included the development and coordination of a major media campaign highlighting NIDR-supported research on the health effects of smokeless tobacco in over 1,000 professional baseball players. The campaign featured a press release detailing the findings of the study, NIH radio news service feed used by 412 stations throughout the country, and a video news release seen by 5 million viewers via satellite to television networks nationwide.

In spring 1990, the National Toxicology Program (NTP) completed an animal study showing inconclusive evidence of a link between fluoride and cancer. OC staff prepared and disseminated the Institute's position statement on the effectiveness of water fluoridation, collaborated with the American Dental Association in developing communications strategies about the fluoride issue, and provided information and advice to the NTP and the Public Health Service about the release of the study results. OC also coordinated fluoride information activities within the Institute, and with NIH and the Centers for Disease Control for handling inquiries from the press, outside researchers, public health officials, and the public.

In 1989, staff successfully coordinated the completion of a major documentary film on dental science entitled, "The Changing Faces of Dentistry." During FY 1990, OC arranged for its distribution and airing on public television stations in San Francisco, Buffalo, Phoenix, Anchorage and Bangor, Maine. Staff prepared publicity materials for use by the stations in advertising the program. Copies of the film have also been distributed to the nation's dental

schools, and plans are under way for the production of a half-hour version of the program which will be disseminated to school systems.

Office of Communications staff planned and designed two new exhibits for display at professional meetings. An exhibit entitled "Seal Out Dental Decay" was developed for use at the annual meeting of the American Dental Association. Focusing on prevention, this exhibit pointed out the low use of sealants by practitioners and stressed research confirming sealant safety and effectiveness.

A new institutional exhibit was designed for use at the meeting of the National Dental Association in Houston. Geared to minority dental professionals, the exhibit defines the NIDR and its role as the primary funding agent for oral health research and training in the United States.

An exhibit on Georges Fattet, one of the most significant and colorful figures in dentistry in 19th century Europe, will open in January 1991 at the National Library of Medicine. OC staff is collaborating with NLM on the design and promotion of this exhibit.

OC undertook the initial steps toward establishing a national oral health information clearinghouse. Staff consulted with several clearinghouse project officers at NIH to learn the procedures involved in setting up a resource center and made a presentation of NIDR's plans at the semi-annual meeting of members of the Combined Health Information Database.

OC oversaw the distribution of *Dental Science in a New Age*, an historical perspective of the NIDR, in its limited first printing by Montrose Press. The Office of Communications has recently completed negotiations with State University of Iowa Press for the broad scale publishing and marketing of this reference work.

OC staff collaborated with the NIH audio-visual section to develop a video laboratory report on the NIDR Pain Clinic. This tape, and a shorter version focusing on the use of animals in pain research, are the first entries in the new video library of NIH research, a resource of current stock footage which will be made available to the press.

A member of OC staff served on the steering committee to plan and organize a special 3-day conference in Missoula, Montana, entitled "Choosing the Paths to Tomorrow." Targeted to native American students, the conference focused on career opportunities for minorities in biomedical research. Staff displayed an exhibit, attended meeting sessions, and served as a professional leader during the conference.

Staff is collaborating with the National Library of Medicine to establish fluoridation archives at the NLM. The archives will include the papers of the Institute's first Director, Dr. Trendley Dean, and a wealth of recent and historical materials on this subject from both the NIDR and the Centers for Disease Control.

Public and Professional Education

OC initiated planning efforts in FY 1990 for a 3-day continuing education program for practicing dentists entitled "Scientific Frontiers in Clinical Dentistry: An Update at the National Institute of Dental Research." Staff assembled a program of speakers on research topics relevant to the practitioner and developed strategies for publicizing the availability of the symposium scheduled for spring 1991.

OC planned, coordinated and directed all arrangements for the 1990 Seymour J. Kreshover Lecture, "The Sense of Taste: New Directions for Dentistry," delivered by Dr. Linda Bartoshuk of the Yale University School of Medicine. Staff set up coverage of the lecture by newspaper and television reporters, and arranged for the program to be videotaped and distributed to all U.S. dental schools.

OC arranged for publication of the proceedings from the "Consensus Development Conference on Oral Complications of Cancer Therapies: Diagnosis, Prevention and Treatment" by the National Cancer Institute. Staff oversaw distribution of the monograph to major cancer centers, hospital-based dentists, oncology nurses, and dentists who see cancer patients.

A pamphlet on the availability of DENTALPROJ, a new database developed by the NIDR, was prepared by OC in FY 1990 and published in the *NLM Technical Bulletin.* The article alerts readers to the fact that DENTALPROJ is now online and is accessible through MEDLINE. A companion pamphlet describing "NIDR ONLINE," a program information service of the NIDR, was also printed this year and distributed at professional meetings.

Staff developed and submitted an article on the NIDR Pain Research Clinic for publication in the *Philip Journal*, a professional publication for dentists in West Germany.

Staff prepared two press releases featuring intramural research on cancer metastasis and the development of a monoclonal antibody that neutralizes rabies virus.

Staff continues to assist in the recruitment of patients for the NIDR clinical research program in the areas of pain, herpes, dry mouth and other oral health studies, and coordinates distribution of a slide presentation for professionals on the oral complications of cancer therapies, a program developed by staff of the NIDR Dental Clinic.

OC staff exhibited at the annual scientific sessions of the American Association of Dental Schools, American Association for Dental Research/International Association for Dental Research, National Dental Association, and the American Dental Association.

During this fiscal year, OC also carried out the following activities in the areas of public and professional education: developed press summaries on NIDR-supported research advances for the IADR/AADR general session in Cincinnati; prepared monthly issuances of the "NIDR Research Digest" for inclusion in the IADR newsletter; contributed articles on recent research

advances to the *Journal of the American Dental Association* for the continuing NIDR series; continued periodic publication of "NIDR Research News;" and contributed regularly to the "NIH Record."

In related activities, OC staff provided background material for articles on NIDR research efforts to professional journals and health-oriented publications including *Science News*, *Dentist Magazine*, *Dental Health Advisor*, *Mature Health*, *American Health Magazine*, and *Health Confidential*. Staff also provided slides, photographs and video footage on a variety of dental-related subjects to the American Fund for Dental Health, American Dental Association and Public Health Service.

Publications

OC printed a handbook for new members of the National Advisory Dental Research Council. Also in progress are patient education pamphlets on temporomandibular joint dysfunction and the oral effects of smokeless tobacco.

Staff prepared the following publications for printing during FY 1990: "Dry Mouth (Xerostomia);" "Pain Research: From Laboratory to Clinic;" "Oral Complications of Cancer Therapies;" "Consejos de cuidado dental para diabeticos" (Dental Tips for Diabetics); "Enfermedad periodontal en los diabeticos: Guia para los pacientes" (Periodontal Disease and Diabetes: A Guide for Patients); "Prescription for Sound Teeth;" and "Seal Out Dental Decay."

The following publications were updated and reprinted in FY 1990: "Graduate Training Supported by the National Institute of Dental Research," and the NIDR, Extramural and Intramural brochures.

Media

During FY 1990, OC arranged interviews and provided background information on dental research topics to numerous general circulation magazines such as *Reader's Digest*, *McCall's*, *Women's World*, *Family Circle Magazine*, *Parenting Magazine*, *Working Mother Magazine*, *Fortune*, and *Self*; to the trade press; and to a variety of major metropolitan newspapers, news magazines and wire services, including the *Washington Post*, *Wall Street Journal*, *New York Times*, *Parade Magazine*, *Newsweek*, *Baltimore Sun*, *Los Angeles Times*, Associated Press, and Newhouse News Service. Staff also handled information requests from broadcast media, including ABC's 20/20, ABC Prime Time, Cable Satellite Public Affairs Network, Christian Broadcasting Network, British TV, CBC-Toronto and local stations.

The following interviews were among those arranged with the major media in FY 1990:
Dr. Joyce Reese addressed the mercury toxicity issue on Jack Anderson's "Insider" television program;
Dr. Albert Guckes was interviewed for ABC Prime Time on the subject of dental implants, their use, and required training;

Dr. Matthew Kinnard discussed research findings about smokeless tobacco with Cable News Network;
Dr. Steven Corbin addressed the fluoride issue with WJLA-TV, a local network station.

Reports

OC contributed to eight Special Reports to Congress on scientific activities carried out in FY 1989. These included research advances in the following areas: aging, cystic fibrosis, digestive diseases, diabetes, genetic diseases, arthritis, AIDS and minority health. Staff also reviewed the final galleys for the NIDR Director's Report for the NIH Biennial Report, 1989-1990.

General Communications Activities and Services

In FY 1990, OC responded to over 3,600 telephone calls and approximately 3,000 letters and postcards requesting general information on a broad range of topics from the public, professionals, and the media. Over half a million publications were distributed. OC also prepared numerous responses to Congressional and controlled correspondence for the Director and other DHHS officials.

OC prepared several presidential messages that were delivered at the annual meetings of professional dental organizations. Staff also provided background information and talking points for Secretary Louis Sullivan's keynote address at the general session of the American Association of Dental Schools.

OC helped coordinate an Institute-wide "Town Meeting" in May 1990 to provide employees an opportunity to ask questions and explore issues with Executive Staff. OC published and circulated to NIDR staff the responses to questions raised at that meeting.

Staff provided medical arts, photography, graphics and printing services to the Institute for activities such as the EEO Bulletin and the NIDR Awards booklet. OC also arranged the production and printing of health promotion materials for the Disease Prevention and Health Promotion Branch.

OC continued to oversee a contract with a clipping service to monitor NIDR-related research activities reported in the press. Staff also oversaw a contract with a video monitoring service to retrieve NIDR-related news items that appear on television across the country.

Staff continued to coordinate and conduct tours of the NIDR research facilities as needed.

The Office of Communications coordinated manuscript and abstract clearance through OD; arranged for review and clearance, by Institute experts, of articles prepared by the lay press; directed the NIDR's contract mailing and storage operations with St. Elizabeth's Hospital; and maintained and provided mailing labels to various organizations.

Staff instituted a new contract with CSR to reorganize the Institute's various mailing lists and to develop a database to ensure ready access to specific audiences.

OC prepared the Director's Report for each meeting of the National Advisory Dental Research Council this fiscal year. Staff also arranged a contract for preparation of the minutes of the NADRC and Program Advisory Committee meetings.

OC continued to respond to frequent requests from sister Institutes, other government agencies, and outside organizations for slides and photographs of NIDR laboratories, and clinical and basic research.

Other services included providing resource material for the "NIDR ON-LINE" communications system; coordinating Institute submissions to the *NIH Scientific Directory and Annual Bibliography*; and coordinating exhibit scheduling and arrangements for the Disease Prevention and Health Promotion Branch.

Freedom of Information/Privacy Act

During this fiscal year, the Office of Communications assumed responsibility for coordinating the Institute's Freedom of Information and Privacy Act requests. Numbering approximately 40 in FY 1990, FOI requests were received from individuals, private companies, universities, public interest groups, law offices and the Congress. The broad range of topics included the use of animals in research, children's oral health survey data, water fluoridation and grant information.

Requests are entered into a centralized NIH computer tracking system, then sent to the appropriate program area for response or answered directly by the NIDR FOIA/PA coordinator. The coordinator is responsible for maintaining the paper trail on each request and formally closing out requests through the NIH FOI office and the Division of Financial Management. In FY 1990, the coordinator participated in meetings with the NIH Epidemiology Committee and the Department's legal office and FOI staff to formulate a DHHS opinion paper on the release of data from the children's oral health survey.

The Institute maintains Privacy Act systems of records on participants in epidemiological and clinical center patient care studies and receives over 100 requests for patient records each year.

The FOIA/PA coordinator participates in training workshops and meetings with the NIH FOI Office, the Office of the General Counsel and the NIH Epidemiology Committee on matters of data confidentiality and release of unpublished data. The coordinator also prepares annual reports for the NIH FOI and Privacy Act Offices.

Personnel Activities

In July 1990, Brent Jaquet, Chief of the Office of Communications, was named Special Assistant to the Director. Susan Johnson was appointed Acting Chief, Office of Communications.

While Chief, OC, Brent Jaquet served on the trans-NIH Science Education Subcommittee, the public relations committee for the Children's Inn, and the Information Officers training subcommittee on public affairs forums.

Wayne Little, technical writer/editor, joined OC staff during FY 1990. He also serves as the Institute's Freedom of Information Act/ Privacy Act coordinator.

Peggy Buckler, a local high school teacher with a background in consumer education, performed a variety of assignments as a summer information intern.

Staff participated in Institute-sponsored training during FY 1990, including writing and editing courses, video production, and computer training.

Awards

Sally Wilberding, public affairs specialist, received the NIH Merit Award for sustained exemplary service and commitment to the public while handling information inquiries for the National Institute of Dental Research.

Two publications written by NIDR staff won awards this year for technical merit from the Society for Technical Communication: "American Contributions to the New Age of Dental Research," edited by Susan Johnson in collaboration with NIDR historian Ruth Harris, accompanied a commemorative exhibit at the National Library of Medicine.

"Dental Science for Dental Health," authored by Jody Dove and Mary Daum, was developed in conjunction with a historical exhibit displayed at the Smithsonian Institution's National Museum of American History.

Publications

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OFFICE OF ADMINISTRATIVE MANAGEMENT

FINANCIAL MANAGEMENT SECTION (FMS)

The Financial Management Section coordinates the Institute's financial activities for the Office of the Director, including the development of the Institute's budget and its execution during the fiscal year. The FMS is also the Institute's repository for accounting records, statistical data, and appropriations reports, and serves as principal staff advisor to the Director on financial matters.

NIDR Budget

During FY 1990, the FMS assumed full responsibility for the formulation of the Institute's budget request for FY 1992 by developing the initial budgetary levels; preparing justifications and statistical materials that supported the request; and apprising the Institute Director of changes made through negotiations as the budget was transmitted through NIH, PHS, the Department, and OMB.

Because of the overlap in the budget cycles, the FY 1992 budget was negotiated as the FY 1991 budget request was reviewed by the Congress for legislative enactment. If approved, the FY 1991 request will be incorporated into an appropriation bill. As part of this budget process, FMS staff accompanied the Director to the formal congressional appropriations hearings and provided additional justification materials to the Committee as needed. After action on the request by Congress, the FMS prepared statements that summarized the effect of congressional action on the Institute's budgetary operations and program goals for FY 1991.

While the formulation and legislative processes were in progress, FMS staff monitored grant, contract, intramural, and direct operating expenditures for FY 1990 and provided managerial and financial advice regarding the Institute's extramural and intramural programs. When necessary, they worked with Institute administrative and program staff to determine if reprogramming actions would be necessary. The FMS also apportioned funding by quarter to support planned activities and, at the end of the year, prepared the actual obligation reports.

Automation

Several new computerized programs have been developed by FMS staff to facilitate the budget process. These include programs to calculate current/constant dollar comparisons, indirect costs, and status of funds; to display several budget exhibits required by OMB; and to produce apportionment requests, financial plans, and allowances.

Other Activities

The FMS provided special reports and monitored the Institute's trans-NIH activities, including research in diabetes, arthritis, nutrition, disease prevention, and AIDS. The FMS was also responsible for responding to numerous inquiries from the Congress, OMB, and other Federal and non-Federal agencies regarding NIDR program and financial data.

PERSONNEL MANAGEMENT SECTION (PMS)

The Personnel Management Section is the focal point for the Institute's Civil Service and Commissioned Corps personnel management activities, including staffing and recruitment (including merit promotion), classification, pay administration, employee relations, and employee development and training.

Following is a summary of major activities in the following areas during FY 1990:

Reorganizations

Several minor reorganizations/realignments took place during FY 1990. In the Office of the Director, the Public Inquiries and Reports Section was renamed the Office of Communications. The Office of Planning, Evaluation and Communications was disbanded and in its place the Office of Planning, Evaluation and Data Systems was established. In the Intramural Research Program, several sections were created.

Staffing and Recruitment

This area of personnel management received much attention again this year. A Senior Executive Search for a Director of the Epidemiology and Oral Disease Prevention Program was conducted and a selection package prepared. SES recruitment for an Intramural Branch Chief also was initiated. In addition, a number of research, administrative, staff, and clerical support positions were recruited for and filled.

NIH continued its efforts to gain increased authorities for recruitment of a wide variety of positions. A major recruitment effort was undertaken with the first NIH Job Fair in May, and PMS staff participated fully. The Job Fair produced six new employees for NIDR, with several recruitments still in process. PMS staff also provided input into other staffing issues such as the revised NIH announcement for Health Scientist Administrators and Grants Associates, the delegated examining authority for Chemists, Biologists and Microbiologists, and the Summer Intramural Research Training Award (IRTA) Program.

In addition, PMS staff assumed responsibility for Institute coordination of the AIDS Research Loan Repayment Program, a new program designed to attract researchers to NIH to perform AIDS research.

Classification and Pay Administration

The PMS staff continued to provide classification advice to both managers and employees, with several major classification actions being resolved. Other special activities in this area involved continued interest in the Physician's Comparability Act, with particular interest in the inclusion of dentists, the proposed Health Research Service, special salary rates for dental hygienists and for clerical/technical staff.

Employee Relations

With the Administration's continued attention on the ethical conduct of Federal employees, PMS staff were called upon to increase their involvement in this area. Staff coordinated a mandatory Ethics Briefing which was attended by most NIDR employees subject to the strictest ethical requirements. This increased emphasis particularly affects certain outside activities, and PMS staff have provided information and responses to both Institute employees and NIH staffers on this subject.

Several other issues received considerable attention this year. Some of those involved Random Drug Testing of persons in certain designated positions, Flexible Workplace, Alternative Work Schedules, and Internal Controls. PMS staff again were involved in reviewing preliminary materials, identifying affected positions and employees, and/or providing input to senior management staff on the implications of the new or revised policies in these areas.

Awards

The Institute continues to have an active awards program, as its managers and supervisors nominate staff for recognition for both NIDR and other incentive and honor awards. As a result, some 72 staff members were recognized at the Annual NIDR Awards Ceremony in October. Staff were recognized for special acts or service, high quality work performance, and for their special or professional contribution to the mission of the NIDR, NIH, PHS or DHHS.

With the Secretary's initiation of the Employee of the Month Award this year, the opportunity now exists for another form of recognition for NIDR employees. PMS staff worked with Program Directors and administrative staff to outline operating procedures for this new award, and several employees have received recognition.

Again this year, PMS staff worked closely with NIDR Budget Managers to implement the Departmental performance award system for EPMS staff. The system gives supervisors the opportunity to consider employees for two types of awards for performance—a quality increase or a performance award, with the quality increase considered NIDR's highest form of recognition for performance. The Budget Managers met in the Spring to consider Institute employees for both types of awards, and to authorize the EPMS performance awards, which were paid in June.

EEO Activities

The PMS staff continues to collaborate with the NIDR EEO Manager and the NIDR EEO Advisory Committee on matters of joint concern. They participate in advisory committee meetings to keep the EEO community informed about Institute personnel policies and procedures. They participate with the EEO Manager in the establishment and implementation of new or revised EEO policies, as appropriate. The staff also works closely with the EEO Manager and with program managers to assure the feasibility and legality of personnel activities related to affirmative action and equal opportunity.

Professional Activities

PMS staff continued their participation in a number of activities, both within and outside the NIDR, related to human resources management. They continued active participation in regular and special Institute meetings such as those held by the IRP Laboratory and Branch Chiefs, the EEO Advisory Committee, the Administrative Staff, the Executive Staff, and in seminars such as the newly established Administrative Staff Seminar Series. They participated in trans-NIH activities such as career and job fairs, personnel operations workshops, the Personnel Officers Retreat, qualifications review boards, and on personnel workgroups to improve personnel management operations. Continued membership and active participation in the International Personnel Management Association and the NIH Human Resources Conference also provided increased knowledge and broader perspectives into this field. Finally, staff shared their knowledge of the field by providing a training opportunity for a personnel management intern and through participation in the evaluation of a local university's graduate program in human resource management.

EQUAL EMPLOYMENT OPPORTUNITY PROGRAM (EEO)

Public Law 92-261, the Equal Opportunity Act of 1972, requires that all Federal personnel actions be free from discrimination and that affirmative action programs be developed to carry out the purpose and intent of the Public Law. The National Institute of Dental Research affirmative action and civil rights programs are centered in the Institute's Equal Employment Opportunity Office. This office serves as the principal source of information for and advisor to the Institute Director and to senior management on matters of equal employment opportunity, affirmative action, Federal Equal Employment Opportunity Recruitment Plan, civil rights and contract compliance. In addition, the EEO office is responsible for the special emphasis programs for Hispanics, minorities, women and the handicapped.

The EEO program continues to be involved in numerous activities with minority schools, prepares reports and analyses of the Institute's profile and arranges seminars which are designed to increase the awareness of minorities, women and the handicapped about career opportunities.

Discrimination Complaints

The Institute had one formal discrimination complaint outstanding in 1990. The EEO manager and counselor continue to provide, on an as needed basis, career counseling, guidance on employment applications, training opportunities and problem-solving in supervisor/employee relations.

EEO Advisory Committee

The NIDR EEO Advisory Committee serves as a liaison between NIDR employees and management. Its purpose is to define and make recommendations on Institute employee problems wherever they may exist and to advise the Director and his staff of these concerns. The committee seeks to achieve equal opportunity through career development, education and training, and related activities without regard to race, color, religion, sex, age, national origin or handicap. Also serving as members of the committee are representatives to the NIH Federal Women's Program, the NIH Handicapped Employees Advisory Committee, the NIH Hispanic American Advisory Committee, the NIH Asian Pacific Islander American Advisory Committee, the Black Employees Advisory Committee, and the NIDR EEO counselor.

During 1990, the advisory committee and the Institute Safety Committee sponsored a seminar on Radiation, Biological and Chemical Safety. The seminar provided new laboratory technicians with required laboratory training and alleviated a measure of anxiety in NIDR staff who are not required to take safety courses. Approximately 40 employees attended the seminar.

In recognizing the month of February as Dental Health Month, the EEO Advisory Committee held a special forum for employees. The theme for this celebration was "Tips for Dental Care." Scientists shared with the 55 participants current procedures for treating and preventing oral disease.

The committee also nominated to the Director one deserving individual for the NIDR EEO Special Achievement Award. This person will be recognized for invaluable contributions to equal employment opportunity at the Institute's award ceremony in October 1990.

The EEO office also sponsored a 2-day training session for the NIDR EEO Advisory Committee on the Federal equal employment opportunity laws and regulations which prohibit discrimination in employment. The Handicapped Individuals and Disabled Veterans Program was highlighted during this session.

Recruitment and Awareness

The EEO manager continues to identify and communicate with minority, handicapped and women's organizations concerning our mission and activities. This office, in conjunction with NIDR Handicapped Advisory Committee representatives, participated in the National Symposium on Perspectives on Employment of Persons with Disabilities and the annual

meeting of the President's Committee on Employment of the Handicapped for networking and for discussing dental research.

NIDR provides reasonable accommodation for disabled employees during inclement weather. This past year, five employees established agreements under the "Inclement Weather Policy."

Staff exhibited at the 47th joint annual meeting of the National Institute of Science Beta Kappa Chi Scientific Honor Society and the Brookhaven Semester Program in Jackson, Mississippi. The meeting provided an opportunity for networking and discussing research training opportunities at NIDR.

The EEO manager represented NIDR at a Symposium on Career Opportunities in Biomedical Sciences in Houston, Texas. The symposium was sponsored by Morehouse Medical College and the Association of Minority Health Professional Schools. These groups are attempting to reverse the trends of underrepresentation of minorities in biochemical research.

Staff participated in a national symposium sponsored by the NIH and the University of Texas Health Science Center in San Antonio. The purpose of the conference was to familiarize high school, college and graduate students, particularly Hispanics, with the various career paths and programs of support in biomedical disciplines.

Staff exhibited at the 77th annual convention of the National Dental Association in Houston. The convention provided an opportunity for networking and disseminating information on NIDR. Three disabled employees accepted positions in the intramural program during this fiscal year.

In cooperation with other NIH EEO offices, the NIDR EEO manager conducted tours of the Institute's research facilities for groups of minority, women and handicapped students.

Minority Access to Research Careers Program
Minority Biomedical Research Support Program

Through cooperative agreements with the National Institute of General Medical Sciences and the Division of Research Resources, the NIDR supports components of the Minority Access to Research Careers (MARC) and the Minority Biomedical Research Support (MBRS) Programs that relate to the overall mission of the Institute. Staff participated in the annual MBRS symposium held in Houston and the annual MARC conference in Washington, D.C. The two events provided an opportunity for staff to discuss NIDR research training opportunities with faculty and students. In FY 1990, the Institute sponsored four MARC students for a summer research training experience in NIDR laboratories.

Community Outreach

The EEO office continues to provide 27 minority institutions with the Institute's surplus scientific materials. This service was expanded to include a high school in Montgomery County with a significant minority population.

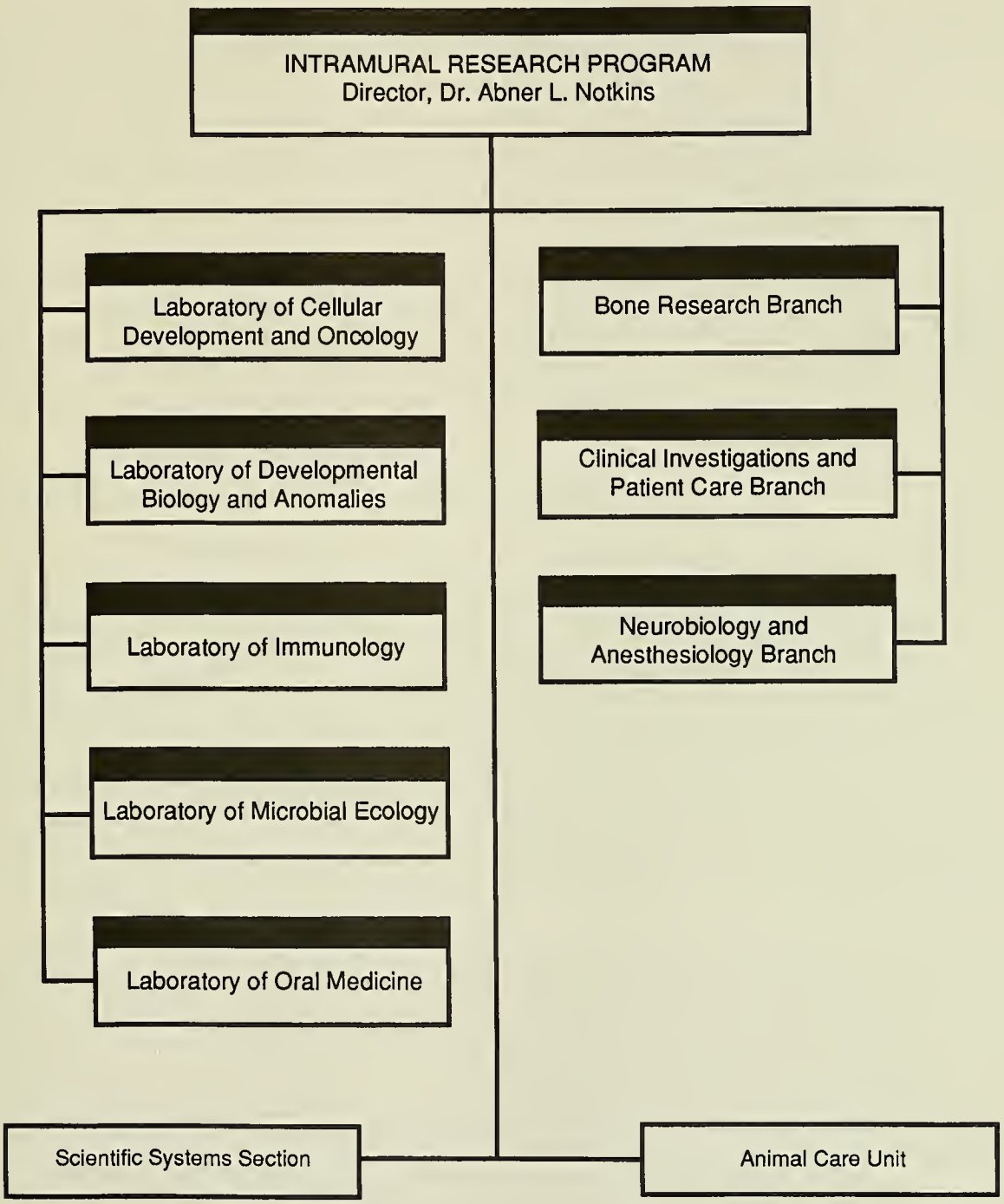
Two employees from the intramural program and the EEO manager represented the NIDR as special judges in the District of Columbia Annual Science Fair. Four outstanding students were recognized for their excellent projects.

Civil Rights

The EEO manager continues to serve as the Institute's Federal Contract Compliance Coordinator. New contracting and project officers in the Institute completed training on contract compliance and administered the EEO check list for nonconstruction contracts in accordance with Executive Order 11246. The Institute continues to participate in the NIH Consultant File on Committees/Advisory Groups, the NIH Visiting Professor Program, the Small and Disadvantaged Business Program, and the Small Grants Program.

Intramural Research Program

NATIONAL INSTITUTE OF DENTAL RESEARCH



LABORATORY OF CELLULAR DEVELOPMENT AND ONCOLOGY

The Laboratory of Cellular Development and Oncology conducts programs of basic research on normal structure and function of cellular and extracellular components and on their alterations in disease states. Emphasis is placed on factors that govern normal and abnormal differentiation, growth, metabolism, and repair of cells and tissues and on the role of posttranslational changes in biological regulation. Unity in this laboratory, which is composed of three sections and one unit, each of which possesses its own specialized interest and expertise, is achieved through the search for structure-function relationships.

A number of significant research advances were made this year. These, which are summarized below according to section, were made possible through the scientific proficiency of the staff members and the multiformity of their knowledge, the many collaborations with scientists within and outside of the laboratory, the development and applications of powerful technologies, and the interdisciplinary nature of the laboratory.

MOLECULAR AND CELLULAR BIOLOGY SECTION

The continuing aim of this section is to elucidate molecular mechanisms that are responsible for conversion of normal cells to a malignant state. Under the leadership of Dr. Keith Robbins, attention has been focused on the oncogenic sequences within the genomes of certain tumorigenic retroviruses. The *fgr* oncogene was identified as the transforming component of Gardner-Rasheed feline sarcoma virus, and the *fyn* gene was isolated from normal human fibroblasts. In addition, the cDNA molecules that represent *fgr* and *fyn* proto-oncogene transcripts were isolated and sequenced. This work provided a basis for development of the immunologic reagents needed for the identification of the translational products of these proto-oncogenes. Both gene products are protein-tyrosine kinases with conserved catalytic domains and unique amino-terminal regions. Current efforts are aimed at determining their normal physiologic roles as well as their mechanisms as oncogenic agents.

In a search for sites of c-*fgr* proto-oncogene action, it was found that expression of c-*fgr* proto-oncogene mRNA is limited to normal peripheral blood granulocytes, monocytes, and alveolar macrophages, all of which contain 50 to 100 copies of c-*fgr* mRNA per cell. Examination of the expression of $p55^{c\text{-}fgr}$ in normal human neutrophils (PMN) with the use of peptide antibodies against the amino- or carboxyl-terminal regions of $p55^{c\text{-}fgr}$ revealed a protein of 55 kDa in lysates of PMN, but not in other cells, thus establishing the presence of this protein in normal neutrophils. Neutrophil-derived $p55^{c\text{-}fgr}$ was found to be enzymatically active by immune complex kinase assays and to contain phosphotyrosine as the sole

phosphoamino acid. These findings established neutrophil $p55^{c\text{-}fgr}$ as a protein-tyrosine kinase.

In an effort to define possible cellular locations in which the tyrosine kinase activity of $p55^{c\text{-}fgr}$ is exerted, human PMN were fractionated into cytosol and particulate membrane compartments. Abundant $p55^{c\text{-}fgr}$ was detected in the plasma membrane-enriched fractions as well as in fractions containing secondary and tertiary, but not primary, granules. When secondary granule secretion was induced with the chemoattractant peptide formyl-Met-Leu-Phe, a marked decrease in $p55^{c\text{-}fgr}$ and c-*fgr* kinase was observed in fractions depleted of secondary granules. A concomitant increase in the concentration of $p55^{c\text{-}fgr}$ and in its enzymatic activity was observed in fractions containing plasma membrane. From these findings it is concluded that $p55^{c\text{-}fgr}$ is associated with functional secretory granules and is redistributed within normal neutrophils in response to their activation.

In order to investigate the possible involvement of tyrosine phosphorylation in signaling exocytosis through the high-affinity IgE receptor, rat basophilic leukemia cells were tested for tyrosine phosphorylation upon IgE receptor activation. Tyrosine phosphorylation was rapid, detectable 1 min after stimulation, and correlated with both the time course and antigen dose for histamine release. Reversal of FcεRI cross-linking prevented continuation of the degranulation process and resulted in rapid loss of tyrosine phosphorylation. The receptor-mediated tyrosine phosphorylation was still induced in the absence of calcium in the medium, whereas the calcium ionophore A23187 induced histamine release in the absence of a perceptible increase in protein tyrosine phosphorylation. Thus, tyrosine phosphorylation is an early signal following FcεRI aggregation and is independent of the exocytotic process itself. Taken together, these findings functionally link protein phosphorylation on tyrosine residues to FcεRI-mediated signal transduction leading to histamine release.

Recent studies showed that ligand-activated growth factor receptors, as well as transforming versions of nonreceptor protein-tyrosine kinases, associate noncovalently with phosphatidylinositol-3 kinase (PI-3 kinase). Reasoning that PI-3 kinase might also play a role in the normal functions of nonreceptor kinases, section members sought to determine whether association with PI-3 kinase might serve as a measure of nonreceptor protein-tyrosine kinase activation under physiological conditions. It was found that $p60^{c\text{-}src}$ as well as $p59^{fyn}$, the product of another member of the *src* family of proto-oncogenes, physically associated with a PI kinase activity within 5 s after exposure to thrombin. Furthermore, PI kinase reaction products generated in $p60^{v\text{-}src}$-, $p60^{c\text{-}src}$-, or $p59^{fyn}$-containing immunoprecipitates were indistinguishable, demonstrating the identity of the associated enzyme as PI-3 kinase. These observations point up a thrombin-dependent interaction between $p60^{c\text{-}src}$ or $p59^{fyn}$ and PI-3 kinase and suggest a role for nonreceptor protein-tyrosine kinases in human platelet signal transduction.

Implications regarding the mechanism of transformation by nonreceptor protein-tyrosine kinases were also forthcoming from these findings. When activated by polyomavirus middle T antigen (20-22) or by transformation mutations, the *src* translational product associates with PI-3 kinase activity. In addition, PI-3 kinase associates with activated forms of the *fyn*

translational product. Thus, PI-3 kinase association with normal $p60^{c\text{-}src}$ and $p59^{fyn}$ under physiological conditions represents a property shared with transforming versions of these proteins, suggesting the possibility that transformation by $p60^{v\text{-}src}$ and related proteins involves constitutive activation of a pathway normally mediated by nonreceptor kinases in postmitotic cells.

PEPTIDE AND IMMUNOCHEMISTRY UNIT

Work in the Peptide and Immunochemistry Unit, headed by Dr. Frank Robey, is directed toward obtaining an understanding of the role of primary structure in protein-protein interactions and toward application of this knowledge in cell attachment studies. It was discovered this year that C-reactive protein (CRP), a human serum component, immobilizes cells to solid supports, e.g., plastics, through a tight association with fibronectin, which is bound to the cells. A 12-amino acid sequence of CRP was identified as the part of this protein responsible for binding with fibronectin. Plastics incubated with a dilute solution of a synthetic peptide modeled on this fibronectin-binding region of CRP bind enough peptide to support attachment of those cells to which fibronectin is bound. When the single free sulfhydryl group of the synthetic peptide is oxidized to form peptide dimer, significantly lower levels of peptide are needed for cell attachment. That fibronectin *per se* is bound to the synthetic peptide was demonstrated with the use of affinity chromatography, dot blot analysis, and ELISA.

HIV-1 is the causative agent of AIDS. The binding of gp120, the envelope protein from HIV-1, to CD-4, the HIV-1 receptor on cells, has been a primary focus of studies on AIDS. Members of this section synthesized a 14-member peptide corresponding to a portion of the primary neutralizing epitope (PNE) from a gp120 isolate termed IIIB. This peptide was polymerized using a technique described below to yield a mixture of molecules of molecular masses approaching 50 kilodaltons. Serum from rabbits immunized with this polymer mixture displayed antibody titres against recombinant gp120 IIIB (Genentech) as high as $1{:}10^6$. These values, obtained with ELISA plates coated with 10 µg/well of the gp120, exceed by more than two orders of magnitude the values reported by others who immunized with protein conjugates of the 14-member parent peptide. Tests of the ability of antisera to peptide polymer to prevent infection of cells by HIV-1 IIIB are currently underway. If, as expected, these tests are positive, the polymer will be evaluated as a vaccine against HIV-1 infection in monkeys. The potential value of peptide polymer as a vaccine is enhanced by its totally synthetic nature and its ease of production.

A recent observation that the 14-member gp120-derived peptide is capable of immobilizing CD-4-containing cells on plastic tissue culture plates and that some cells attach and spread on plates coated with peptide polymers has stimulated much interest. Tests of response of T cells to peptide polymers are underway, and consideration is being given to the possible usefulness of these polymers as drug targeting agents.

The key compound used for preparation of the peptide polymers described above was developed this year in collaboration with Dr. John Inman, NIAID, NIH. This compound, N-α-t-butyloxycarbonyl-β-alanyl-ε-bromoacetyl-L-lysine, allows specific placement of the reactive bromoacetyl group in a synthetic peptide chain by automated insertion in place of an amino acid residue. Polymerization is induced under mild conditions between the bromoacetyl group and a peptide cysteine -SH group. Specific placement of the groups directs the type of polymerization, e.g., head-to-tail, head-to-head, or tail-to-tail polymerization.

As a consequence of the basic cell attachment findings, together with the new methods of peptide polymerization, Dr. Robey established a cooperative research and development agreement (CRADA) with Dentsply International, Inc., of York, PA. This CRADA is directed toward production of novel dental implant materials.

BONE CELL BIOLOGY SECTION

A central interest of the Bone Cell Biology Section, headed by Dr. Hari Reddi, is the control of bone differentiation and repair by endogenous growth and differentiation factors. Osteogenin, a bone morphogenetic protein, was cloned with the use of recombinant DNA techniques. The clone was isolated from the library of small cell carcinoma line NCI H69. Plasmids were constructed for expression in mammalian cells and osteogenin was expressed in 193 cells. It was partially purified by heparin-sepharose affinity chromatography. The isolated protein retained osteogenic activity. Its deduced amino acid sequence revealed considerable homology in the carboxy-terminal 110 amino acids to two developmentally important genes in Xenopus Vg-1 (49%). It is also noteworthy that osteogenin has sequence homology to several regulatory factors: transforming growth factor β types 1 and 2 (34%), inhibin β chains (37%), inhibin α chains (30%), and Millerian inhibitory substance (32%). This important advance sets the stage for expression of human osteogenin in various expression systems. The availability of human osteogenin with attendant data on efficacy, safety, and toxicity will permit clinical applications in periodontal, craniofacial, and orthopaedic surgery.

During the course of systematic studies on the interaction of osteogenin with various macromolecules and cells, an unexpected discovery was made that it binds to type IV collagen. Basement membranes of blood vessels consist of type IV collagen and laminin. It is well known that vascular invasion is a prerequisite for new bone formation. Thus, the fact that osteogenin binds to invading blood vessel basal laminar components such as type IV collagen provides the first molecular evidence for a role of vascularization in bone differentiation. The interaction of osteogenin with type IV collagen is not affected by fibronectin and laminin. This fact shows that the binding site for osteogenin is unique. Current experiments focus on the molecular site of this interaction.

In a related development, reconstituted basement membrane gels were found to promote terminal differentiation of osteoblasts with cell processes reminiscent of canalicular processes.

This differentiation was dependent upon intact cytoskeleton and new protein synthesis and was stimulated by laminin. Antibodies to laminin and to laminin receptor blocked this phenomenon. In addition, the laminin-derived synthetic peptides, Tyr-Ile-Gly-Ser-Arg-NH_2 and Cys-Ser-Arg-Ala-Arg-Lys-Gln-Ala-Ala-Lys-Ile-Val-Ala-Val-Ser-Ala-Glu-Arg-NH_2, altered the morphology of osteoblastic cells. These results imply that basement membrane derived from invading vascular endothelium during early bone development is critical for the terminal differentiation of osteoblasts.

In order to gain further insights into the developmental role of osteogenin during embryonic cartilage and bone morphogenesis, the binding and localization of iodinated osteogenin was examined. Rat embryos at different stages of development from 11 to 20 days were sectioned, and the specific binding of osteogenin was determined by whole embryo autoradiography. During days 11 to 15, maximal binding was restricted to perichondrium during limb and vertebral morphogenesis. Osteogenin was also localized in developing calvarium and craniofacial bones. This observed pattern of binding during skeletal morphogenesis implies a developmental role for osteogenin.

In recognition of these continuing outstanding studies, Dr. Reddi was presented the NIH Director's Award for 1990.

ENZYME CHEMISTRY SECTION

Research in this section, headed by Dr. John Folk, is directed toward the enzymology of posttranslational modification reactions, cellular and systemic regulation of the enzymes, and the alterations of these reactions in disease states.

Part of the efforts of the section continue to be directed toward the highly conserved eukaryotic protein synthesis initiation factor 4D (eIF-4D). eIF-4D is the only protein known to contain the unusual amino acid hypusine [N^{ε}-(4-amino-2-hydroxybutyl-)lysine]. Two precursors of eIF-4D that contain lysine in place of hypusine were previously isolated and shown to have no eIF-4D activity in stimulating methionyl-puromycin synthesis. This year, an eIF-4D precursor prepared by expression of human eIF-4D cDNA in *E. coli* was also found to be inactive in this assay. However, when this protein was incubated *in vitro* with spermidine and the enzyme deoxyhypusine synthase, under which regime lysine-50 is converted to the hypusine precursor deoxyhypusine [N^{ε}-(4-aminobutyl)lysine], it was found to provide significant methionyl-puromycin synthesis stimulation. Thus, this simple posttranslational chemical change is sufficient to bestow biological activity. At the same time this finding raises a question as to the role of hydroxylation in maturation of eIF-4D.

As an extension of these structure-function studies, a variant form of eIF-4D-containing homodeoxyhypusine [N^{ε}-(5-aminopentyl)lysine] was prepared. When spermidine-deficient cells are incubated with cadaverine, a spermidine analog, aminopropylcadaverine, is formed. This analog substitutes for spermidine in modification of eIF-4D precursor. The homodeoxyhypusine-containing eIF-4D variant was prepared in a cell-free system by treating

the cloned eIF-4D precursor with aminopropylcadaverine and the enzyme deoxyhypusine synthase. This variant showed no effect in the methionyl-puromycin synthesis assay, suggesting a specific requirement for the 4-carbon length chain addition to the precursor lysine residue.

For the last few years, studies with the enzymes termed transglutaminases have been focused primarily on their physiological roles and their regulation. Using a synthetic oligonucleotide modeled on the consensual active site of the transglutaminases, it was found that human newborn foreskin epidermis expresses three different mRNA species, whereas cultured normal epidermal keratinocytes express only two of these three species. This result is consistent with earlier biochemical and immunological evidence that protransglutaminase E, a zymogen which upon activation provides two-thirds of the total transglutaminase activity of skin, is expressed only in terminally differentiating epidermal cells of skin.

In collaboration with Dr. Peter Steiner, NIAMSD, a cDNA clone encoding for the membrane-bound enzyme, transglutaminase K, was isolated and completely sequenced. The deduced sequence has 813 amino acid residues. The protein is of pI 5.7 and is of globular nature. It shares 49-53% sequence homology with other transglutaminases and contains additional sequences that appear to be responsible for its unique property of association with membrane.

It is well known that fibrinogen binds to various cells in culture. The site of cellular binding appears to be distinct from the integrin super-family of extracellular matrix protein receptors. The fibrinogen receptor isolated by affinity purification possesses transglutaminase catalytic activity and cross-reacts with antiserum to the membrane-bound enzyme, suggesting that indeed this enzyme is a receptor for fibrinogen.

Certain salivary proteins interact with oral epithelial cells to form what is called the mucosal pellicle. This pellicle plays a role in providing a protective barrier to the mucosal epithelium. It was found that an acidic proline-rich protein of saliva is an excellent substrate for an oral membrane-bound transglutaminase and is cross-linked by this enzyme *in vitro* to desquamated buccal epithelial cells. The enzyme differs from the well-characterized transglutaminase K in certain properties, e.g., isoelectric point, and is readily released into salivary fluid where it rapidly loses enzymatic activity. Because fibrinogen competes with the salivary proline-rich protein for binding and cross-linking sites on epithelial cells, it is suggested that the membrane-bound transglutaminase serves as a receptor for salivary proteins, as well as the enzyme for cross-linking to form mucosal pellicle.

A method for immortalizing gingival fibroblasts and epithelial cells was developed using transfection techniques. These cell lines should serve as valuable tools for *in vitro* investigations of pellicle formation.

This brief review shows the laboratory to have been active and productive in all of its programs, although without major changes in program direction. In some cases, new results have led to new projects. Emphasis, however, continues to be on growth and cellular regulation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00001-38LCDO

PERIOD COVERED: October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Transglutaminases: Specificity and Control.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Folk, J.E.	Acting Chief	LCDO NIDR
OTHERS:	Chen, G.	Guest Researcher	LCDO NIDR

COOPERATING UNITS (if any): Dr. J. Gorman, CSIRO, Australian National Health Laboratory, Geelong, Australia; Dr. M. Fink, Baylor University, Waco, Texas; Dr. L. Fesus, University School of Medicine, Debrecen, Hungary

LAB/BRANCH: Laboratory of Cellular Development and Oncology

SECTION: Enzyme Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.80	1.50	.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒X (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transglutaminases are enzymes that occur ubiquitously in eukaryotic cells, as well as in many extracellular regions. Although they vary significantly in molecular form, they catalyze a single covalent modification reaction, the outcome of which is the permanent attachment of certain protein molecules to one another subsequent to the assembly of their polypeptide chains. The importance of this post-translational event, which occurs through so called ϵ(γ-glutamyl)lysine or bis-(γ-glutamyl)polyamine cross-links is evident in fibrin clot stabilization in hemastasis, vaginal plug formation as a result of postejaculatory clotting of seminal plasma, and production of the cell envelope of the stratum corneum during terminal differentiation of keratinocytes in the epidermis. Each of these reactions is catalyzed by a different transglutaminase and the characteristics of each reflects the individual specificity of the enzyme involved. The purposes of this project are to gain understanding of the molecular basis for specificity differences among the transglutaminases, to construct specific inhibitors for the various enzymes based on this knowledge of specificity differences, and to apply these inhibitors as a means of determining further biological roles for the transglutaminases. Methods have been developed for detecting specificity differences for lysine residues and preliminary tests are encouraging. A number of inhibitors for transglutaminases are under study and isothiocyanates seem applicable.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00049-19LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological Function of Transglutaminases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chung, S.I.	Research Chemist	LCDO NIDR
OTHERS:	Han, J.H.	Visiting Fellow	LCDO NIDR
	Chung, H.J.	Fogarty Fellow	LCDO NIDR
	Choi, J.K.	Guest Researcher	LCDO NIDR
	Termine, J.D.	Chief, BRB	BRB NIDR

COOPERATING UNITS *(if any)*
Peter Steinert, Laboratory of Skin Biology, NIAMSD

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Enzyme Chemistry Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.20	4.00	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The physiological function and mode of regulation of transglutaminases are being studied as to their role in the formation of "provisional stroma" (fibrin or fibrin-connective tissue) during tissue or bone fracture repair, in the cross-linking of extracellular matrix proteins during cell-cell interaction, and in the modulation of specific cellular processes. The coagulant layer formed at injury sites is one of the vital elements of hemostasis and diathesis. The major constitutent of this coagulant gel is fibrin. A number of proteins, e.g., fibronectin, thrombospondin, vitronectin, etc., that are known to be involved in cell attachment reactions are cross-linked to fibrin. It was found that fibrin(ogen) binds to specific cellular receptors of B16/F10 melanoma cells. These fibrinogen receptors are distinct from the well-characterized integrin super family of ECM receptors. Cellular transglutaminases in terminally differentiated epidermis catalyze the cross-linking of several proteins to form cornified envelope. The majority of cytosol granular layers of epidermis appears to be activated during the apoptotic process in terminally differentiated epidermis to form corneum layers. A membrane-associated transglutaminase catalyzes the cross-linking of salivary proteins to oral epithelium to form the mucosal pellicle which serves as a protective barrier.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00204-14LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Growth and Differentiation Factors in Bone and Cartilage.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	Reddi, A.H.	Chief, BCBS	LCDO NIDR
OTHERS:	Chen, P.	Visiting Fellow	LCDO NIDR
	Cunningham, N.	Staff Fellow	LCDO NIDR
	Harrison, E.	Research Chemist	LCDO NIDR
	Luyten, F.	Visiting Associate	LCDO NIDR
	Paralkar, V.	Guest Researcher	LCDO NIDR

COOPERATING UNITS *(if any)*
Dr. Glenn Hammonds, Genentech, San Francisco, CA; Dr. Jeffrey Hollinger, U.S. Army Institute for Dental Research, Washington, DC

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Bone Cell Biology Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.20	6.90	.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the project is to investigate the role of growth and differentiation factors in bone and cartilage. Special emphasis is placed on initiation factors in bone development. Osteogenin is a prototypic example of a bone development initiation factor. The projects currently under investigation with the salient findings are: 1) Cloning and expression of osteogenin genes; 2) Production and characterization of antibodies to synthetic peptides to "active sites" in osteogenin; 3) Interaction of osteogenin with various extracellular matrix macromoles. Osteogenin binds with high affinity to type IV collagen. This finding has important developmental implications; 4) Expression of tissue specific genes in response to osteogenin; 5) Autoradiographic localization of binding sites to osteogenin in developing embryos; 6) The differentiation of osteoblastic cell processes on type IV collagen and other substrata, and 7) Application of osteogenin in craniofacial defects in primates. Recent work has demonstrated the utility of osteogenin in correction of massive craniofacial defects.

PHS 6040 (Rev. 1/84)

GPO 914-918

OTHERS:	Yu, Yu	Guest Researcher	LCDO NIDR
	Kleinman, H	Research Chemist	LDBA NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00311-10LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hypusine in eIF-4D: Biosynthesis and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Park, M.H.	Research Chemist	LCDO, NIDR
OTHERS:	Folk, J.E.	Acting Chief	LCDO, NIDR
	Wolff, E.C.	Chemist	LCDO, NIDR

COOPERATING UNITS *(if any)*
Dr. Hanauske-Abel, Rush Medical Center, Chicago, IL; Dr. A. Abbruzzese, 1st Medical School, University of Naples, Italy; Dr. John W.B. Hershey, University of California, Davis, CA

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Enzyme Chemistry Section

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.70	2.50	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eukaryotic protein translation initiation factor 4D (eIF-4D) contains one residue of hypusine and appears to be the only cellular protein with this one unique amino acid. Hypusine is produced post-translationally by transfer of the butylamine portion of the polyamine spermidine to a lysine residue in the eIF-4D precursor and subsequent hydroxylation. These findings reveal a novel cellular metabolic pathway. Comparison of activities of mature eIF-4D, eIF-4D precursors that contain unmodified lysine in place of hypusine and the deoxyhypusine containing eIF-4D prepared by in vitro modification of cloned eIF-4D precursor by deoxyhypusine synthase, in methionyl-puromycin synthesis indicates that deoxyhypusine and hypusine are essential for the activity of eIF-4D in this model protein synthesis initiation system. Studies are underway to relate the structure of hypusine to the physiological function of eIF-4D and to its mode of action in eukaryotic protein synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00433-04LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Aspects of C-reactive protein.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Chief, PIU	LCDO NIDR
OTHERS:	Batinic, D.	Visiting Fellow	LCDO NIDR
	Harris, T.	Staff Fellow	LCDO NIDR
	Nguyen, A.	IRTA Fellow	LCDO NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.74	1.63	.11

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

C-Reactive protein (CRP) is an acute phase protein. Its serum levels can of CRP increase by as much as 2000-fold during the first twenty-four hours following the onset of inflammation. A serum protein closely related to CRP, SAP, has no known function. A common property shared by CRP and SAP is their ability to bind fibronectin in a calcium-dependent manner. This property may lead to a clearer understanding of functions for both proteins.

Using techniques, including cell attachment assays, tissue culture, peptide synthesis and immunoassay, a peptide from the primary sequence of SAP was found to support attachment of cells to plastic. This attachment occurs through binding to fibronectin on cells. Incubation of low levels of peptide with plastic results in enough peptide binding to support attachment of fibronectin-containing cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00434-04LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on HIV-1 Targeted Drug Delivery Systems.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Chief, PIU	LCDO NIDR
OTHERS:	Batinic, D.	Visiting Fellow	LCDO NIDR
	Harris, T.	Staff Fellow	LCDO NIDR
	Kolodny, N.	Visiting Scientist	LCDO NIDR

COOPERATING UNITS *(if any)*
Dr. O. Shapira-Nahor, NIH, NIAID

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.49	1.40	.09

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

HIV-1 is the causative agent of AIDS. CD-4 is the cellular receptor for HIV-1 and its amino acid sequence is known.

A small peptide of CD4 was identified as responsible, at least in part, for the binding of the HIV-1 envelope protein to cells. Studies are underway to develop agents capable of recognizing this part of CD-4 in the hope that anti HIV-1 drugs may be delivered to this part of the CD4 molecule. In addition, a 14-member synthetic peptide corresponding to a portion of the primary neutralizing epitope from the HIV-1 gp120 was found to align very closely with this CD4 region. Binding studies are currently underway to test at the interaction of polymers of this peptide and antibody to the polymer with CD-4-bearing cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00437-04LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Peptide Polymers as Vaccine Candidates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frank A. Robey	Chief, PIU	LCDO NIDR
Others:	Nelly Kolodny	Visiting Fellow	LCDO NIDR

COOPERATING UNITS *(if any)*

John Inman	Research Chemist	LI NIAID

LAB/BRANCH
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Peptide and Immunochemistry Unit

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.40	1.30	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a few instances, researchers have found that synthetic peptides act as immunogens to provide protection against viruses. To function in this manner, a peptide must be coupled to a carrier protein in order to remain in the host long enough for an immune response. Attempts are underway to improve the peptide to carrier protein conjugation strategy by developing vaccines composed of peptide polymers without protein. Such vaccines of highly defined chemical composition may be specific for a certain pathogen, without the side effects that often occur in the peptide-carrier protein approach.

New methods for conjugation and methods to evaluate the degree of conjugation are being developed in this laboratory. Such methods will be useful in the quality control of peptide polymers and are needed to define the reproducibility of the syntheses.

A compound, N-α-tBOC-ξ-bromoacetyl-B-alanyl-L-lysine, prepared in this laboratory is designed to place a bromoacetyl moiety in any position along a synthetic peptide chain. The bromo group is displaced by sulfhydryl-containing nucleophiles to give a stable thioether linkage. Synthetic peptide polymers with linkages from head-to-head, head-to-tail and tail-to-tail were prepared using this approach. These polymers are under being study for their immunogenic properties in rabbits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00479-02LCDO

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms Responsible for Oncogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robbins, K.C.	Chief, MCBS	LCDO NIDR
OTHERS:	Sartor, O.	Senior Staff Fellow	LCDO NIDR
	Cardinali, M.	Visiting Associate	LCDO NIDR
	Agarwal, A.	Visiting Fellow	LCDO NIDR

COOPERATING UNITS *(if any)*
Joseph B. Bolen, LTVB, NCI
William J. LaRochelle, LCMB, NCI
Stuart A. Aaronson, LCMB, NCI

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Molecular and Cellular Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.10	2.95	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unraduced type. Do not exceed the space provided.)*

Three approaches were taken to address the mechanism of cellular transformation induced by nonreceptor protein-tyrosine kinases. One involves overexpression of fgr genes specifying normal or aberrant kinases in NIH/3T3 cells. The findings document a rare malignant transformation by high levels of $p55^{c\text{-}fgr}$. Furthermore, it was shown that by mutation of sequences encoding the carboxyl terminus of $p55^{c\text{-}fgr}$, the c-fgr gene is converted into a potent, dominant acting oncogene. A search for identify of substrates for these activated tyrosine kinases shows substrate criteria to include physical association with the enzyme in vivo, as well as tyrosine phosphorylation by the enzyme both in vivo and in vitro. Using these criteria molecules of 135 kd and 70 kd that preferentially interact with and are tyrosine phosphorylated by transforming as compared to normal versions of src, fyn, and fgr kinases were identified. The third approach involves searching for evidence of activated tyrosine kinases in naturally occurring human neoplasia, especially squamous cell carcinomas of the head and neck. Sensitive assays are being developed for activation, including PI-3 kinase association and tyrosine phosphorylation of certain substrates in vitro and in vivo in order to enhance the probability of identifying such lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00480-02LCDO

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Normal Physiologic Roles for Nonreceptor Protein-Tyrosine Kinases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robbins, K.C.	Chief, MCBS	LCDO NIDR
OTHERS:	J.S. Gutkind	Visiting Associate	LCDO NIDR
	Lacal, P.	Guest Researcher	LCDO NIDR
	Xu, N.	Visiting Fellow	LCDO NIDR
	Siraganian, R.P.	Chief, CIS	LI NIDR

COOPERATING UNITS (if any)
Timothey J. Ley, Washington University, St. Louis, Missouri

LAB/BRANCH
Laboratory of Cellular Development and Oncology

SECTION
Molecular and Cellular Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.65	3.50	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The working hypothesis states that nonreceptor protein-tyrosine kinases transduce environmental signals in fully mature cells. During the current reporting period important evidence has been obtained supporting this hypothesis. In an effort to define possible subcellular locations where the tyrosine kinase activity of $p55^{c\text{-}fgr}$ might be exerted, human PMN was fractionated and assayed for the enzyme. The findings demonstrated that $p55^{c\text{-}fgr}$ is associated with plasma membrane as well as functional secretory granules and is redistributed within normal neutrophils in response to their activation. It was also sought to determine whether platelets, a rich source for p59 association with PI-3 kinase, might serve as a measure of nonreceptor protein-tyrosine kinase activation under physiologic conditions. p60, as well as p59, the product of another member of the src family of proto-oncogenes, was found to physically associate with PI-3 kinase within 5 s after exposure to thrombin. The possible involvement of tyrosine phosphorylation in signalling degranulation through the high-affinity IgE receptor (FcεRI) was examined. Tyrosine phosphorylation was shown to be an early signal following FcεRI aggregation, independent of the exocytotic process itself. These findings functionally link protein phosphorylation on tyrosine residues to FcεRI-mediated signal transduction leading to degranulation.

LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

The Laboratory of Developmental Biology and Anomalies (LDBA) continues an innovative and dynamic research program employing molecular and cellular approaches to study the structure of the extracellular matrix and its role in development and in disease. Dr. Kenneth M. Yamada has been selected Chief of LDBA and will bring with him a cell and molecular biology group. His expertise in fibronectin and integrin receptors and their role in cell migration and development will complement on-going studies of the LDBA staff.

LDBA continues to make significant advances in understanding the roles, molecular mechanisms of action, and regulation of the extracellular matrix and its receptors. Using both molecular and cellular approaches, new research initiatives have been developed and substantial progress has been made. Specifically, progress has been made in our studies on tumor cell invasion and metastases, AIDS, structure and gene regulation of basement membrane and cartilage, and the biological functions of the extracellular matrix.

Tumor Studies

Studies on the growth of tumors and their invasive and metastatic activity have continued as a major focus of LDBA. Previously, we found that tumor cells grow well and invade a basement membrane substratum in vitro. Recently, we found that when tumor cells are premixed with basement membrane prior to subcutaneous injection in mice, rapidly growing tumors are observed within days with both malignant and non-malignant cells. Ten different types of tumor cell lines have been tested (including breast cancer, submandibular cancer, small cell lung carcinoma, prostate cancer, renal cell carcinoma, etc.) and found to yield tumors almost 100% of the time. Small numbers of cells (25,000/mouse) yield tumors suggesting the feasibility of growing tumors from needle biopsies. Since less than 5% of all tumors can be grown in mice, this method has potentially wide application and allows for new models to study tumor growth and spread. We have started to define the factor(s) in the basement membrane responsible for this activity using selective depletion of the basement membrane, as well as synthetic peptides of known active sites on constituent molecules. One peptide (YIGSR) from the laminin B1 chain inhibits tumor growth, whereas another peptide (IKVAV) from the A chain of laminin stimulates tumor growth. Laminin alone will promote tumor growth but not as well as the complete basement matrix.

The activity of collagenase IV is important during tumor metastasis to distant sites. Using three approaches: (1) activation of the enzyme by laminin peptides, (2) transfection of cDNA for collagenase IV, and (3) inhibition of the enzyme by inducing synthesis of an endogenous inhibitor, we demonstrate key mechanisms for altering the metastatic behavior of cells.

Previously, it had been shown that the activity of collagenase IV was increased by laminin. We identified a specific site on laminin containing the sequence IKVAV which promotes this activity using synthetic peptides. When co-injected into mice with tumor cells, a five-fold increase in metastases is observed. This peptide is active when injected up to 6 hours after the tumor cells. Non-metastatic tumor cells are also found to have increased invasive activity when exposed to this peptide. This peptide is being used to generate new models of tumor metastases. Transfection of collagenase IV as well as other proteases into tumor cells is being used to assess the role of these enzymes in the metastatic cascade. Together with Dr. Greg Goldberg (Washington Univ., St. Louis), we find that transfection of fibrosarcoma cells with the genes for collagenase IV and stromelysin, induce tumor metastases and growth. TGF-ß reduces tumor metastases by increasing the synthesis of the endogenous collagenase inhibitor TIMP (tissue inhibitor of metalloproteinase). Such data demonstrate that the phenotype can be modulated by regulating the activity of collagenase IV and new therapeutic or diagnostic approaches may be developed.

Several cDNA clones which reduce invasiveness of malignant tumor cells have been isolated from an expression cDNA library of human fibroblasts. After transfection of the cDNA library into highly invasive human fibrosarcoma HT1080 cells and mouse melanoma F10 cells, G418-resistant cells were selected for low invasiveness by an *in vitro* chemoinvasion assay using matrigel, or for slow proliferation by colchicine treatment. Plasmids were recovered from low invasive cells by Cos cell fusion. Plasmids containing 2.2 kb and 1.2 kb cDNA isolated from HT1080 and F10 cells respectively, are shown to be active for reducing invasiveness of parental cells in the second-round transfection. The 1.2 kb insert has been sequenced and found to encode a unique protein. Characterization of these proteins will be useful in understanding of molecular basis of the metastatic process, as well as for potential diagnostic and therapeutic applications.

AIDS

The transactivator of transcription of the AIDS virus HIV1 is an 86 amino acid protein termed tat. Since transgenic mice with this gene develop Kaposi's sarcoma, a skin and oral cavity tumor, we have focused on the in vitro functional changes of cells either transfected with this gene, infected with HIV1, or exposed to synthetic tat protein. Our data in collaboration with Dr. Mary Klotman (NCI), demonstrated that HIV1-infected CD4+ human T- lymphocytes show increased adhesion to fibronectin and the adherent cells develop large filopodia. An integrin receptor ($\alpha 5 \beta 1$) appears to be involved based on antibody and peptide competition assays, and the synthesis of this receptor is increased in the presence of the virus. In addition, in collaboration with Dr. Maurice Green (Univ. St. Louis), we find that the tat protein itself is biologically active and increases neural cell adhesion, but reduces process outgrowth on laminin. Such data demonstrate that the HIV1 virus and TAT protein can modify cell behavior, and may initiate certain pathologies such as Kaposi's sarcoma and the neurological degeneration associated with AIDS disease.

Type IV Collagen

The major form of type IV collagen consists of two α1 [IV] and one α2 [IV] chains, forming a triple-helical structure. Two genes for the α1 [IV] and α2 [IV] are closely mapped on the same chromosome, separated by 130 bp in a head-to-head arrangement. Transfection of constructs containing the regulatory elements of both genes reveals that the α1 [IV] and α2 [IV] genes are regulated by a bidirectional promoter and a shared common enhancer. The enhancer is located in the first intron of the α1(IV) gene and determines the cell type-specific expression of both genes. Developmental regulation of the type IV collagen genes has been investigated using F9 mouse teratocarcinoma cells. These studies suggest that a low level of expression of the collagen IV gene in undifferentiated F9 cells is regulated by chromatin structure and/or by DNA methylation. *In vitro* transcription data indicate that the undifferentiated F9 cells already have nuclear factors necessary for transcribing the gene and thus support this notion. The minimum size of the enhancer which is required for the high level of enhancer activity is 210 bp. DNase-footprint analysis reveals that two regions within the 210 bp enhancer sequence interact with nuclear factors from F9 and EHS tumor cells. Two nuclear factors that interact with one of the sequences were purified by a series of chromatographic steps. One of the nuclear factors has a molecular weight of 92 kDa and its N-terminal sequence, as determined by micro-sequencing, indicates a protein unique from previously reported factors.

Full length cDNA from the mouse α1(IV) and α2(IV) chains has been prepared and placed under the control of a strong heterologous promoter to study the structure and function of type IV collagen. These plasmids are transfected into eukaryotic cells to determine whether homodimers can be formed and function, and how heterodimers are assembled and secreted. Several mutations have also been generated including deletions and point mutations in the major triple-helical region.

Laminin

The basement membrane glycoprotein laminin is very active with cultured cells and promotes cell adhesion, migration, neurite outgrowth, tumor metastases, and collagenase IV production. We have continued our studies using synthetic peptides to identify active sites for the various biological activities. To date, five active sites have been defined with synthetic peptides and their corresponding antibodies. An 18-mer containing IKVAV is an active site from the A chain of laminin near the carboxyl end of the long arm, and has been found to promote cell adhesion, migration, tumor metastases, collagenase IV activity, and neurite outgrowth. A 5-mer containing YIGSR from the B1 chain is active for cell adhesion and migration, and it inhibits tumor growth and metastases. This peptide also blocks blood vessel formation (angiogenesis) in vitro, in vivo in the chick CAM assay, and in the rabbit eye model. Another B1 chain peptide containing PDSGR has activity similar to YIGSR but it is much weaker. An RGD-containing site on the A chain is active for cell adhesion and spreading, but only with certain cells. More recently, a 17-mer peptide from the C-terminal globular domain of the A chain has been identified as active for cell attachment, heparan binding, and promoting neurite outgrowth. We have also used another approach for expressing cDNA in

order to identify active sites, and to study function and assembly of each chain of laminin. A series of expression vectors with full length cDNA for the B1 and B2 chains, and a part of the A chain, have been constructed and introduced into various cells.

Several genomic clones have been isolated from a mouse genomic library by screening the most 5′ portion of the mouse laminin A chain cDNA. Two of them contain exons encoding the N-terminal part of the A chain. The other two contain an exon encoding an 82 amino-acid sequence with 75% identity to the N-terminal globular domain of the A chain. In addition to five previously identified laminin chains, the clones could code for a new type of laminin chain.

Although laminin has been shown to bind a number of different cell surface receptors, it is not clear which active sites of laminin interact with a specific receptor. We have identified a receptor for the IKVAV sequence of the A chain by cross-linking cell surface proteins with ^{125}I-labeled ligand. The receptor with a molecular weight of 70 kDa specifically interacts with an IKVAV containing synthetic peptide, and has been purified from the brain of newborn mice by several steps of affinity chromatography and gel electrophoresis. The N-terminal sequence of the receptor has been determined and shown to be unique.

Differentiation

The basement membrane is biologically active as demonstrated from in vitro studies using reconstituted matrigel. We find that various cells differentiate when cultured on it, including kidney tubular cells which form branching tubules with a lumen, glandular cells such as those from the submandibular gland which form gland-like structures, endothelial cells which form capillary-like structures, and bone cells which form canaliculi and deposit minerals. We are investigating the mechanisms for the differentiated response using antibodies to cell surface and to basement membrane molecules, synthetic laminin-derived peptides, removal and resupplementation of growth factors, and kinase stimulators and inhibitors. Our data demonstrate cell-type specific interactions. For example, endothelial cells use multiple receptors (integrin and 67/32K) and at least two sites (RGD and YIGSR) on laminin to attach and align during tube formation.

Although the basement membrane matrix is enriched in growth factors, these are not required for endothelial cell tube formation. Kidney tubule cells, however, will not form tubules if the growth factors are removed. When EGF is restored, tubule formation proceeds rapidly on the matrix. Kinase stimulators appear to increase endothelial cell tubule formation, whereas inhibitors reduce the differentiation, suggesting a role for phosphorylation in endothelial cell tube formation. Knowledge about endogenous factors which promote and/or inhibit angiogenesis is important in therapeutic approaches to certain pathologies.

Using a differential screening of a cDNA library, we have identified several gene products whose expression is induced by laminin and appears to be important for differentiation of neural cells in response to laminin. Some of them are genes involved in dephosphorylation. In addition, a gene coding for a protein with leucine zipper motif characteristic for a DNA

binding protein has been identified. Although this putative DNA binding protein is not specific for neural cells, its synthesis is increased several-fold by laminin.

The levels of c-fos and c-jun are also increased about 10-fold in PC-12 cells by laminin. Laminin induces dephosphorylation of serine and threonine residues of c-jun. Gel-shift assays and DNA transfection experiments suggest that the dephosphorylation of c-jun turns on its own transcription as well as that of c-fos. We believe that the increased levels of c-jun and c-fos complex is crucial to switch on other genes needed for the differentiation of PC-12 cells.

The intracellular signalling events involved in the neuronal response to laminin have also been characterized. Laminin causes a marked decrease in phosphate incorporation into total proteins. Using kinase inhibitors and stimulators, phosphatase inhibitors, and various second messenger analogues, we find that both phosphorylation and dephosphorylation events are required for axonal process formation. Such approaches are being employed with endothelial cells, tumor cells, and glandular cells and the data indicate that different intracellular mechanisms are used by each cell type in response to laminin.

Basement Membrane Heparan-Sulfate Proteoglycan: Perlecan

Basement membrane heparan-sulfate proteoglycan (HSPG) plays an important role in filtration by regulating protein passage in capillary and glomeruli. HSPG is one of the largest single polypeptides with a molecular weight of about 400 kDa. Since this proteoglycan resembles beads of pearls in the electron microscope, we have termed it perlecan. Although we are still missing the C-terminus, we have sequenced about 90% of the protein. Perlecan consists of a series of distinct domains. The most N-terminal domain contains three potential heparan-sulfate attachment sites. The next domain is homologous to the LDL-receptor followed by the sequence similar to the short arm of the laminin A chain. The laminin A chain-like domain is then followed by 10 repeats similar to repeats in the neural cell adhesion molecule, N-CAM. The newly sequenced domain consists of at least two repeats similar to repeats found in the C-terminal globular domain of the laminin A chain. We have been identifying the heparan binding sites and cell attachment domains by expressing cDNA, encoding different segments of the protein core.

Cartilage Components

The characteristic feature of cartilage is its extensive extracellular matrix which functions to resist compression in the joint. The major components of the matrix are cartilage collagen fibrils and proteoglycan aggregates. The collagen fibrils contain type II collagen as the primary collagen, but also contain types IX and XI collagen. The proteoglycan aggregate is composed of a variety of molecules including the major proteoglycan of cartilage (we named aggrecan), link protein, and hyaluronic acid. We find that the expression of the rat type II collagen gene is regulated by several DNA elements. These are an enhancer in the first intron and a short DNA sequence in the promoter. Both elements are required for the high levels of expression of the type II collagen gene. In addition to these positive elements, two negative regulatory elements (silencers) are present in the 5′-flanking region which suppress

the expression of type II collagen gene in fibroblasts. The silencers also function with a heterologous promoter. The enhancer overrides the activity of the silencers in chondrocytes. We have identified several nuclear factors which bind to the enhancer. We previously established immortalized rat chondrocytes (IRC cells) by infection with a retrovirus vector containing c-myc. IRC cells show a chondrocytic phenotype except for a very low level of type II collagen. Co-transfection with c-myc and collagen II promoter constructs reveals that expression of c-myc preferentially inhibits promoter activity of the type II collagen gene. We have also identified a binding site of c-myc to be around -660 in the 5′-flanking region of the type II collagen gene.

Link protein is involved in stabilizing the interactions between hyaluronic acid and aggrecan. Link protein genes from rat and human have been isolated and characterized. In contrast to the promoter of the type II collagen gene, the link protein promoter shows significant activity in chondrocytes as well as in other types of cells which do not express link protein. Sequences in the first intron of the gene increase the promoter activity several fold. These studies indicate that the regulation of link protein and type II collagen genes are different, although both are major proteins synthesized by chondrocytes in cartilage.

Retinoic acid is known to down-regulate the expression of cartilage components in chondrocytes. The link protein gene has an AP-1 site which is important for transcriptional activation by interacting with a complex of c-jun and c-fos. Retinoic acid treatment of chondrocytes reduces the binding activity of c-jun to the AP-1 site of the gene, although the synthesis of c-jun does not change. The reduced binding of c-jun appears to be responsible for the decreased synthesis of link protein in retinoic acid-treated chondrocytes.

Aggrecan consists of a protein core of a 220 kDa which is modified with more than 100 glycosaminoglycan side chains. We previously determined a primary structure of aggrecan from a rat chondrosarcoma. We have now completed the sequence of human aggrecan by sequencing cDNA and genomic clones. The complete sequence is 7137 nucleotides long and encodes 2336 amino acids. The human aggrecan consists of a number of distinct domains. The N-terminal globular domains, G1 and G2, show homology to the link protein. These domains are followed by the keratan-sulfate and chondroitin-sulfate attachment domains. They contain numerous ser-gly repeats; a potential attachment site for glycosaminoglycan side chains. Another globular domain, G3, makes up the C-terminus. G3 is a complex structure which was first noted to share sequence homology with a family of vertebrate lectins, but possesses two additional disulfide-containing domains, one with homology to the EGF repeat, and one related to complement regulatory proteins. The EGF-like domain is an alternatively spliced sequence (Baldwin et al., Jefferson Medical School). There are several features of the human aggrecan distinct from the rat-derived molecule. Two insertions in the human sequence are found in the putative keratan-sulfate attachment domain and in the chondroitin-sulfate attachment domain. These are 11 repeats of a six amino-acid sequence, and 19 repeats of a 19 amino-acid sequence. In addition to these insertions, there are two alternatively spliced sequences located on the C-terminal globular domain.

Transgenic Mice

We have a number of genes to be studied for gene regulation and protein function in transgenic mice. We have employed several approaches: (1) expressing a reporter gene construct containing regulatory elements of a gene, (2) expressing cDNA and/or a mini gene, (3) expressing mutant genes, and (4) suppressing expression of a functional protein by homologous recombination.

We have generated transgenic mice lines containing a reporter construct with either type II collagen or type IV collagen promoter, and an enhancer. These studies show that the enhancer is required for cell-type specific expression of these collagen genes. We have also made a construct containing a segment of the A chain gene with positive and negative selectable marker genes. This construct will be used to select embryonic stem cells lacking expression of the A chain by homologous recombination and will subsequently produce transgenic mice defective in the A chain gene. We have been creating transgenic mice carrying osteogenin, a bone morphogenetic factor under the control of either metallothionien or type II collagen promoter-enhancer.

Matrix Expression and Disease

We are studying the molecular mechanisms by which matrix proteins and their receptors are regulated in diseases. Many renal, pulmonary, and hepatic diseases are characterized by abnormal matrix deposition, which leads to impaired function. Using molecular and cellular approaches, we are evaluating changes in the extracellular matrix proteins and their receptors in renal diseases such as polycystic kidney disease, kidney cancer, diabetes, cyclosporine toxicity, and retroviral infection. For example, we find increased thromboxane A2 production in the diabetic kidney; and a thromboxane A2 analog, U46619, stimulates increased transcription of type IV collagen and laminin in mouse teratocarcinoma cells. These data suggest an important role for the eicosanoid pathway in regulating the production of extracellular matrix components, and may explain in part the increased basement membrane thickening observed in diabetes. We are also studying a murine model for polycystic kidney disease. The kidneys from these animals show increased collagen and laminin production. We find considerable EGF in the cyst fluid although mRNA levels are reduced. Whether the EGF has a role in initiating the formation of the cysts or is a consequence is not yet clear. These and related studies on cyclosporine toxicity (where fibrosis is observed) are aimed at determining how the expression of certain genes is altered during abnormal cell differentiation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00230-14 DB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Proteins in Tissue Architecture and Cell Function

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

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Grant, D.	Vis. Fel.	DB NIDR	Dym, M.	Guest Res.	DB NIDR
Cid, M.	Guest Res	NIAID	Shiraishi, N.	Vis. Sci.	DB NIDR
Yamada, Y.	Chief, MB	DB NIDR	Klotman, P	Spec. Exp.	DB NIDR

COOPERATING UNITS *(if any)*: NIA,NCI, NIAID, NINCDS, Univ. of VA, Harvard Univ., Georgetown Univ., Monsanto, John Hopkins Univ, Univ. MN (Duluth), Pasteur Inst., France

LAB/BRANCH: Laboratory of Developmental Biology and Anomalies

SECTION: Cell Biology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.7	6.1	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spaca provided.)*

Cell Matrix interactions are important regulatory events during embryogenesis and repair. From in vitro studies using purified components, a better understanding of how cells adhere, migrate, proliferate, and differentiate in response to tissue and cell-specific matrix molecules has been established. We have found that the basement membrane, the extracellular which underlies all epithelial cells and endothelial cells and surrounds muscle cells, promotes cell differentiation in vitro. Endothelial cells form capillary-like structures with a lumen, Sertoli cells form cord-like structures, bone cells form canaliculi, glandular cells form glands, etc. Our goal is to define the molecular and cellular events involved in this process. Our approach has been to identify the (1) biologically active matrix components, (2) localize active sites on the matrix component with site specific antibodies and synthetic peptides, (3) identify and characterize cellular receptors, and (4) gain an understanding of the intracellular events involved in the biological response. Specifically, we have identified five active sites on the basement membrane glycoprotein laminin. These peptides are active for cell adhesion, migration, neurite outgrowth, collagen IV production, promotion, and inhibition of tumor metastases. Such peptides have potential as therapeutic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00481-02 DB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Connective Tissue Gene Expression in Development and Disease

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Ray, P.	Vis. Sci.	DB NIDR	Yamada, Y.	Chief, MBS	DB NIDR
Ye, J.	Vis. Sci.	DB NIDR	Kopp, J.	Staff Fel.	DB NIDR
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.1	5.3	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The spatial and temporal expression of extracellular matrix proteins and their receptors are critical for normal tissue organization, development, and growth. Conversely, abnormal expression of these extracellular proteins and receptors as a result of either genetic or acquired diseases often represents the pathologic basis for clinical illness. For example, many renal, pulmonary, hepatic, and skin diseases are characterized by abnormal deposition of extracellular matrix proteins leading to impaired function and healing. The purpose of these studies is to understand the molecular mechanisms by which genes for the extracellular matrix proteins and their receptors are regulated during normal development and in disease. Using a combination of molecular, cellular, and physiologic techniques, we are evaluating normal and pathologic conditions associated with changes in the expression of extracellular matrix proteins and their receptors including normal fetal development, disordered renal growth typical of polycystic kidney disease and cancers of the kidney, and the increase in extracellular matrix proteins observed in diabetic nephropathy, cyclosporine toxicity, and with retroviral infections. We are currently evaluating matrix gene expression in an attempt to identify important transcriptional factors during development and disease; we are exploring the specific peptide sequences of matrix proteins which are necessary for renal cell attachment, differentiation, and cell function; and we are creating transgenic mice in order to evaluate the importance of tissue-specific gene expression which may contribute to the clinical manifestations of disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00482-02 DB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Tumor Growth and Metastases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*: G.S. Searle C., Skokie, IL; Washington Univ., St. Louis, MO; NCI; Georgetown Univ., DC

LAB/BRANCH: Laboratory of Developmental Biology and Anomalies

SECTION: Cell Biology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	4.7	1.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are conducted to better define the mechanisms involved in tumor growth and spread (i.e. metastases). Most human tumors cannot be studied in vitro because they grow poorly in culture and in nude mice (less than 5% grow). Previously we found that tumor cells grow well in vitro when exposed to a basement membrane substratum. When tumor cells are premixed with a basement membrane mixture (matrigel) and injected subcutaneously, rapid growth of all tumors studied to date is observed. Tumor metastasis requires that the cells adhere to the vessel basement membrane, degrade it and migrate through. Laminin is a potent inducer of collagenase, and we find that a synthetic peptide from laminin of 18 amino acids promotes collagenase IV activity and tumor metastases. This peptide also induces collagenase IV activity in non malignant cells and makes them have an invasive phenotype. Likewise, transfection of tumor cells with this enzyme results in increased tumor spread. In related studies, we find that TGF-ß reduces the invasive phenotype by increasing the synthesis of a collagenase inhibitor, TIMP (tissue inhibitor of metaloproteinase). Our goal is to better understand the malignant phenotype to devise diagnostic and therapeutic strategies to reduce tumor growth and spread.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00483-02 DB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 chereclers or less. Title must fit on one line between the borders.): Gene Regulation and Function of Cartilage

PRINCIPAL INVESTIGATOR (List other professionel personnel below the Principel Investigetor.) (Neme, title, leboratory, and institute affiliation)

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Rhodes, C.	Biologist	DB NIDR	Harris, G.	Secretary	DB NIDR
Becvar, R.	Vis. Fel.	DB NIDR	Luyten, F.	Vis. Assoc.	LDCO
Line, S.	Vis. Fel.	DB NIDR	Kimata, K.	Guest Res.	Japan

COOPERATING UNITS (if any): John Hopkins Univ., Shriner's Hosp., Univ. of Texas, Univ. Montreal, Canada, Nagoya Univ., Japan.

LAB/BRANCH: Laboratory of Developmental Biology and Anomalies

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.05	6.75	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cartilage is a highly specialized tissue which functions to resist compression and to absorb shock. The purpose of this project is to understand molecular mechanisms by which genes for cartilage components are regulated and expressed during normal development and in disease states. The alteration of cartilage matrix protein are likely to be associated with human diseases such as osteoporosis, osteoarthritis, and rheumatoid arthritis.

We have determined the primary structure of some of the cartilage components. We have also isolated and characterized genes for these proteins. DNA elements which regulate these genes have been identified and nuclear protein factors bound to them have been characterized. Structure and function relationship has been studied using expression vectors and synthetic peptide approaches. DNA prepared from patients with chondrodysplasia has been screened to examine their linkage to cartilage genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00484-02 DB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Animal Models of Connective Tissue Diseases in Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Yamada, Y.	Chief, MBS	DB NIDR
Bruggeman, L.	Vis. Sci.	DB NIDR
Mosley, G.	Bio.Lab.Tech.	DB NIDR
Strong, D.	Bio.Lab.Tech.	DB NIDR
Gabriel, V.	Bio.Lab.Tech.	DB NIDR
Harris, G.	Secretary	DB NIDR

COOPERATING UNITS *(if any)*: Eli Lilly, Shriner's Hosp., Univ. of Texas, Osaka Univ.

LAB/BRANCH: Laboratory of Developmental Biology and Anomalies

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	.75	2.95

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to understand the molecular basis of connective tissue components as well as their gene regulation in normal development and in disease state using transgenic mice as models. Transgenic mice created by injection of DNA into mouse embryos have been exploited for the elucidation of factors which determine tissue specificity of gene expression. Phenotypic changes due to expression of foreign gene center the control of tissue specific heterologous promoters have also been studied. Creation of transgenic animals which carry mutated exogenous genes as models for human genetic diseases of cartilage and basement membrane have been exploited. Recently developed technique of targeted homologous recombination makes it possible to suppress the function of a specific function. We are establishing embryonic stem (ES) cell lines carrying a gene targeted by homologous recombination of exogenous DNA construct and introducing recipient blastogenesis in an effort to obtain chimeric mice that contain the altered genetic information in their germ line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00485-02 DB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Gene Regulation and Function of Basement Membrane

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Yamada, Y.	Chief,MBS	DB NIDR	Fukuda, K.	Vis. Fel.	DB NIDR
Burbelo, P.	Staff Fel.	DB NIDR	Kleinman, H.	Chief,CBS	DB NIDR
Bruggeman, L.	Vis. Sci.	DB NIDR	Shiraishi,N.	Vis. Fel.	DB NIDR
Klotman, P.	Spec. Exp.	DB NIDR	Gabriel, G.	Biologist	DB NIDR
Polistina, C.	Guest Res.	DB NIDR	Kubota, S.	Vis. Asc.	DB NIDR
Noonan, D.	Guest Res.	Italy	Dudek, D.	Edit.Asst.	DB NIDR

COOPERATING UNITS *(if any)*: John Hopkins, Max-Planck Inst., Asahi Kasei Chem. Corp., NCI, Inst. Naz. Ricerca sul Cancro, Italy, Univ. of VA, and Walter Reed Army Hosp.

LAB/BRANCH: Laboratory of Developmental Biology and Anomalies

SECTION: Molecular Biology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.7	7.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basement membrane is the first extracellular matrix produced during development and provides a physical support of a variety of cells. Basement membrane consists of a unique set of proteins which have various biological activities including cell migration, adhesion, differentiation, and growth. We have been studying molecular basis of the expression and function of basement membrane components as well as their receptors.

Recombinant DNA techniques have been used to prepare molecular clones for various basement membrane components. The primary structure of most of these components has been determined by cDNA sequencing. We have identified some of regulatory DNA elements of basement membrane genes. Several nuclear protein factors have been identified and characterized. Relationships between the structure and function of the proteins have been studied using synthetic peptides and expressing exogenous genes in a variety of cell cultures.

PHS 6040 (Rev. 1/84) GPO 914-918

Mori, T.	Guest Res.	DB NIDR
Penington, C.	Guest Res.	Univ. of VA
Chiang, P.	Guest Res.	Walter Reed Army Hosp.
Sawada, M.	Vis. Fel.	DB NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00508-01

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Pathogenesis of Human Immunodeficiency Virus 1 (HIV 1)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Klotman, P.	Spec. Exp.	DB NIDR	Horigan, E.	Biologist	DB NIDR
Kleinman, H.	Res. Chem.	DB NIDR	Dudek, D.	Edit. Asst.	DB NIDR
Yamada, K.	Chief	NCI			
Kibbey, M.	Vis. Sci.	DB NIDR			
Weeks, B.	Biologist	DB NIDR			
Cannon, F.	Biologist	DB NIDR			

COOPERATING UNITS *(if any)*: NCI; Univ. St. Louis, MO; Univ. Calif., San Diego, CA.

LAB/BRANCH: Laboratory of Developmental Biology and Anomalies

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	.9	1.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project is to determine how HIV-1 alters the behavior of host cells. Infected patients suffer recurrent infections due to reduced CD4+ T cells, Kaposi's sarcoma, B-cell lymphomas and neurological changes. We find that CD4+ T cells infected with the HIV-1 are several-fold more adhesive to fibronectin undergo spreading with increased expression of cell surface filopodia. The interaction with fibronectin is mediated by the α5β1 integrin. We likewise find that tat protein is biologically active with neural cells, but the cellular receptor does not appear to be an integrin. Renal disease is common in AIDS patients and in mice made transgenic with the tat gene. We are investigating the regulation of extracellular matrix gene expression in the kidney of such transgenic mice. Our goal is to obtain a better understanding of the mechanisms involved in the pathology of this virus infection.

PHS 6040 (Rev. 1/84)

GPO 914-918

LABORATORY OF IMMUNOLOGY

The Laboratory of Immunology is comprised of some 26 professional scientists and 15 support staff. With a significant reduction in FTE positions over the past few years, there has been an increased necessity to recruit scientists with their own financial support. In this regard, our senior investigators have been highly successful in attracting a number of talented guest researchers from this country and abroad.

The laboratory continues to conduct basic and applied research on mechanisms of acute and chronic inflammatory diseases. Our studies focus on understanding the pathways by which various stimuli activate basophils and mast cells to secrete inflammatory mediators such as biogenic amines and arachidonic acid metabolites. Considerable emphasis is placed on the molecular biology of cell receptors for immunoglobulins and on signal transduction pathways that are involved in secretion. Research is also aimed at an understanding of the role of lymphocyte-monocyte interactions in chronic inflammation and in the Acquired Immune Deficiency Syndrome. The biological and biochemical effects and molecular characteristics of polypeptide hormones/cytokines, released by inflammatory cells, are being investigated. During the past year, the laboratory has made a number of significant research advancements in all of its program areas. These are documented below in the section reports and are emphasized in the listing of manuscripts published or in press during 1989-90.

CELLULAR IMMUNOLOGY SECTION

Cellular immune mechanisms are central to host defense against a variety of antigens, microorganisms and tumor cells. Both lymphocytes and monocytes are responsible for the initiation, perpetuation and resolution of cellular immune responses. Characterization of the contributions of these cells and how their functions are altered in immunodeficiency diseases may enable modulation of potentially pathologic cellular immune sequelae. It is towards this objective that Cellular Immunology Section (CIS) scientists continue to focus their research efforts in spite of decreased budget and personnel. In addition to our objectives in fundamental immunologic research, the Section has received supplemental funds to carry out an AIDS-related research program for the past 4 years. Although our AIDS program has been highly successful, funding has not been adequate to sustain all of our activities in this area. For this reason, certain of our projects will have to be curtailed or terminated.

In continuing studies, CIS research efforts during the past year have expanded our knowledge concerning monocyte physiology and pathobiology. For example, monocyte recruitment is pivotal in the evolution of inflammatory diseases and preliminary evidence suggests that when leukocytes, newly recruited at a source of chemotactic stimuli, are not subsequently activated,

they undergo a process of programmed cell death (PCD). Referred to as apoptosis, this process serves to minimize unneeded leukocyte accumulation. Apoptosis or "cell suicide" is characterized by fragmentation of DNA, altered cell size, membrane blebbing and other features distinct from necrotic cell death. However, if exposed to relevant activation signals, such as LPS and γIFN, the cells escape PCD and undergo differentiation. One manifestation of this differentiation process is a loss in their ability to respond to subsequent chemotactic stimuli, thereby promoting monocyte accumulation and persistence in chronic inflammatory lesions. Specifically, monocytes obtained from synovial fluid of patients with rheumatoid arthritis exhibited significantly decreased chemotactic activity to C5a compared to monocytes from peripheral blood. In contrast, their chemotactic responsiveness to the synthetic peptide, FMLP, was nearly normal. Whereas FMLP-receptor (FMLP-R) expression, determined by dual parameter FACS analysis, was similar on both blood and inflammatory monocytes, C5aR expression was markedly reduced on cells isolated from synovial fluids. The inflammatory cytokines, γIFN and GM-CSF, but not TNF-α, selectively decreased C5aR in parallel with decreased *in vitro* chemotactic activity to C5a. Thus, these data indicate that 1) synovial effusions and their constituent inflammatory mediators modulate phenotypic and functional changes in monocytes, 2) chemotactic ligand receptors are independently regulated following activation, and 3) decreased C5aR and chemotactic potential following activation likely provide a mechanism whereby monocyte-macrophages persist within the inflamed synovium.

Additional efforts to dissect cellular and molecular mechanisms of inflammatory cell regulation document that mononuclear phagocytes isolated directly from an inflammatory lesion, in contrast to blood monocytes, express cell surface CD16 (FcγRIII). This is important because occupancy of CD16 receptors triggers immunophagocytosis and the release of reactive oxygen intermediates. Since we recently showed that TGF-β induces CD16 on blood monocytes *in vitro*, and the levels of TGF-β in synovial fluid may exceed 25 pg/ml, CIS studies demonstrated a causative relationship between these events and TGF-β. Although the TGF-β enhancement of FcγR activity might be considered an important microbicidal mechanism, it may also contribute to the destructive potential of inflammatory lesions, since ligand-CD16 interaction triggers not only phagocytosis, but also generation of reactive oxygen intermediates (ROI). The extracellular release of these toxic oxygen species clearly contributes to tissue damage. Consequently, this mechanism of host defense which has evolved for the benefit of the host may contribute to the pathology associated with chronic inflammation.

The potential contribution of ROI to chronic destructive inflammatory lesions could be directly tested in an experimental animal model of erosive arthritis. After systemic administration of Group A streptococcal cell walls (SCW), rats develop acute and chronic erosive polyarthritis. A single intraarticular injection of superoxide dismutase and to a lesser extent, catalase, significantly reduced the SCW-induced inflammatory response and evolution of erosive arthritis in the treated animals. These data indicate that ROI play a pivotal role in synovitis and furthermore, that suppression of these inflammatory mediators is of benefit in curtailing both acute and chronic inflammation.

In addition to ROI, tissue damage in chronic inflammatory lesions, such as periodontal disease and rheumatoid arthritis, is associated with excessive production of neutral proteases including collagenase. The production of collagenase by activated monocytes has been shown to be modulated by guanine nucleotide binding proteins (G proteins) which are heterotrimers composed of α, β and γ chains. Since G proteins are associated with modulation in phospholipase activity and adenylate cyclase, both of which regulate collagenase production in agonist stimulated monocytes, it is important to explore these interrelationships. Whereas pretreatment of activated monocytes with cholera toxin, which ADP ribosylates Gsα, significantly increased release of arachidonic acid metabolites and collagenase production; pertussis toxin, an inducer of ADP ribosylation of Giα, inhibited eicosanoid and enzyme synthesis. Western blot analysis of monocyte G proteins revealed that control and activated cells expressed equal amounts of the same G proteins. However, only the 46 kDa Gsα was ribosylated in the activated monocytes. Since cholera toxin elevates cAMP in control and activated monocytes, but only enhances eicosanoid synthesis in activated cells, it appears that the 46 kDa Gsα is linked to phospholipase activity. Ongoing studies will determine whether the altered ADP ribosylation results from a phosphorylation and/or conformational change of Gsα and if similar changes are observed in monocytes isolated from sites of chronic inflammation.

A major effort is underway to characterize the potential immunosuppressive effects of TGF-β. Not only is TGF-β a potent pro-inflammatory mediator, but it also has profound immunosuppressive activity. The immunosuppressive role of TGF-β was dramatically illustrated in chronic arthritic lesions. In 20 of 22 synovial fluids and 12 of 13 synovial tissue culture supernatants, TGF-β completely suppressed interleukin-1 (IL-1) bioactivity. Numerous studies have demonstrated an inhibitor of IL-1 in synovial fluid, but this study provided the first known identification of the putative inhibitor.

Based on these observations, additional studies were initiated in the experimental animal system to administer TGF-β systemically (intraperitoneally) in arthritic animals and monitor the course of the disease. Treatment of the animals which had received an arthritis inducing dose of SCW with TGF-β markedly reduced both the acute and chronic inflammatory responses. At day 5, the articular index (AI), based on the degree of erythema, edema and distortion was reduced >80% in the TGF-β treated animals. Moreover, during the chronic mononuclear cell-mediated phase, the arthritis was suppressed even further in the animals receiving TGF-β. Histopathological analysis revealed a marked reduction in inflammatory cell recruitment into the synovial tissues consistent with reversal of SCW-induced leukocytosis by TGF-β. These studies suggest that this pleiotropic cytokine may interrupt, at several sites, the interdependent inflammatory process, and therefore may be useful as a therapeutic agent in chronic, inflammatory disease.

In another new initiative to define mechanisms of immunosuppression, studies were undertaken with collaborators in NCI to define a role for IL-4 based on its current use as a chemotherapeutic modality. Similar to TGF-β, IL-4 appears to play a bifunctional role in the immune response: both augmenting certain cellular responses and inhibiting others. Specifically, IL-4 has been shown to inhibit monocyte arachidonic acid metabolism and

collagenase production, down-regulate the production of IL-1, TNF-α, PGE_2 and superoxide anion, and block the expression of TGF-β-induced FcγRIII. This cytokine may prove crucial in modulating the monocyte portion of the inflammatory and tissue repair response. The mechanism by which this inhibition occurs is not known, but evidence suggests the existence of an IL-4-inducible protein(s) which controls the transcription and/or stability of certain relevant mRNA species.

Since the monocyte first encounters many microorganisms and antigens in the gastrointestinal tract, CIS scientists have expanded their studies on mononuclear phagocytes, in keeping with the mission of NIDR, to focus on the function of mucosal macrophages. The goal of these studies is to elucidate the mechanism(s) by which monocytes/macrophages contribute to the immunopathology of mucosal diseases in order to facilitate the design of immunomodulatory agents directed to regulating monocyte/macrophage function.

In the gastrointestinal tract, chronic infection of the gastric antrum by *Helicobacter pylori*, a non-invasive bacterium, is associated with type B (non-immune) gastritis and duodenal ulcers. *H. pylori* surface proteins and their major component, urease, were identified in the lamina propria of infected, but not control subjects. These products have potent chemotactic activity for monocytes and neutrophils, stimulate monocyte production of IL-1, TNF-α, and O_2^-. These studies are consistent with the hypothesis that during colonization of the gastric antrum, *H. pylori* shed products are absorbed across the epithelium and serve as chemoattractants, recruiting and activating leukocytes to the inflammatory site.

The gastrointestinal tract is also a major route of entry for HIV-1, implicating the mucosa in the translocation of virus from the lumen to underlying lymphoid tissues. Moreover, the oral cavity-gastrointestinal tract is the organ system most frequently symptomatic with the clinical manifestations of HIV disease. Consequently, investigators in CIS have initiated a series of investigations into the immunologic and clinical manifestations of HIV-1 infection in these tissues in parallel with their studies on blood monocytes. Candidiasis of the oral cavity in HIV-infected patients is a marker of impending infection with life-threatening opportunistic pathogens. To determine whether *Candida albicans* in patients with AIDS represents a unique strain(s), *C. albicans* isolated from 24 patients with AIDS was compared with *Candida* isolated from 23 healthy adults. By a variety of microbiologic criteria and by molecular analysis of *Eco*RI restriction fragments of DNA, *C. albicans* isolated from patients and controls were not distinguishable, suggesting that the candidiasis associated with AIDS is not due to the presence of a unique or particularly virulent strain(s), but is likely the consequence of a defect in host defense mechanisms. Moreover, in a recently completed prospective study of the etiologies of oral and esophageal symptoms in patients with AIDS, a correlation between the presence of HIV in the mucosa and mucosal candidiasis was demonstrated.

Studies directed at investigating the function of monocytes at the mucosal level required development of new methodologies for isolating and purifying resident mucosal macrophages. Having isolated these cells, investigators are attempting to infect lamina propria macrophages with HIV-1 to establish a connection between the virus and mucosal abnormalities characteristic of AIDS. Moreover, it is anticipated that these studies will determine whether

the virus can enter mucosal M cells to be transported to the lymphocytes and macrophages within the underlying lymphoid follicle.

Because aberrant or inappropriate expression of cytokines and cell surface antigens in mucosal and blood monocytes may play an important role in disease conditions, research activities in this Section continue to focus on characterizing these intricate pathways. Previous studies revealed differences in growth factor expression, most notably TNF-α, by monocytes following exposure to HIV-1 with enhanced cytokine gene expression occurring during the period of increasing viral production. These studies implicated TNF-α in the upregulation of HIV-1 expression and preliminary new studies suggest that the TNF-binding nuclear protein is upregulated during the infection process. To further define the molecular sequelae of HIV-infection, THP-1 cells were transfected to study the involvement of transactivating factors in HIV-I and TNF-α gene expression. Increased CAT activity was observed in cells cotransfected with the HIV-LTR CAT construct and a SV40-driven construct containing the TAT sequence, a known transactivator for HIV-1. The HIV-1 LTR was also upregulated when the THP-1 cells were stimulated with LPS, an inducer of TNF, supporting data demonstrating TNF activation of HIV-1 LTR, but not a role for HIV-1 transactivating factors or NF-κb in activation of the TNF promoter. This TNF-α-driven upregulation of HIV-1 expression coupled with HIV-1 driven upregulation of TNF-α expression would provide a positive regulatory circuit favoring viral replication, likely at the expense of host gene expression.

Because monocytes are emerging as a pivotal cell in HIV infection and in the evolution of AIDS, CIS investigators have directed efforts at targeting this population for anti-viral therapy. Given the success of liposomes in targeting agents to monocyte/macrophages for treatment of other diseases, it seemed plausible that this capability and perhaps other pharmacokinetic-modulating properties of liposomes could be used to selectively treat the reservoir of HIV-infected macrophages. Encapsulated 2′, 3′ dideoxycytidine-5′-triphosphate (ddCTP) was compared with free ddCTP and 2′, 3′-dideoxycytidine (ddC) in HIV-1 infected human monocyte/macrophage cultures. These treatments inhibited virus replication at nanomolar drug levels. The response to liposome-(ddCTP) suggests that the capability of liposomes for targeting drugs to macrophages *in vivo* could potentially be exploited to improve the therapeutic index of dideoxynucleoside drugs in the therapy of AIDS.

Another unique aspect of monocyte phenotype and function following HIV-1 infection is the expression of cell surface (FcγRIII) CD16 which may also provide a selective target for anti-viral therapies. Whereas monocytes in the circulation of normal individuals express only two receptors for the constant region of immunoglobulin, FcγRI and FcγRII, AIDS monocytes express significant levels of a third FcγR, FcγRIII (CD16), which is normally associated with activation or maturation of the monocyte population. By dual fluorescence analysis using a monoclonal antibody specific for FcγRIII (mAb 3G8), a greater than 3-fold increase in FcγRIII positive monocytes was observed in AIDS patients. AIDS monocytes also expressed FcγRIII specific mRNA which is expressed minimally or not at all in control monocytes. Moreover, as the only cytokine known to modulate FcγRIII expression on blood monocytes, transforming growth factor-beta (TGF-β) was found to be elevated in the serum of AIDS

patients. Thus, the increased CD16 expression on peripheral blood monocytes in AIDS patients may be the consequence of elevated circulating levels of the polypeptide hormone TGF-β, and TGF-β may also contribute to the profound immunosuppression in AIDS. These observations provide new insight into the immunopathogenesis of this disease and suggest novel therapeutic approaches.

CLINICAL IMMUNOLOGY SECTION

The Clinical Immunology Section studies the mechanisms of secretion from mast cells, basophils and pancreatic acinar cells. Cultured basophilic leukemia cells (RBL-2H3) divide rapidly in culture, have granules containing histamine and serotonin, grow attached to plastic and have surface IgG and IgE receptors. Therefore, they are very useful for biochemical and morphological studies to understand the mechanisms involved in the activation of the cell for the release of cellular granules. The cells can be activated to release their granules either by crosslinking the immunoglobulin surface receptors or by a calcium ionophore. These cells secrete two types of mediators; the preformed type that include histamine and serotonin and the secondary mediators that are synthesized following cell activation and include the metabolites of arachidonic acid. A number of different biochemical steps have been described that occur during the release process; these include receptor crosslinking, activation of phospholipase C and A2 enzymes, the hydrolysis of phosphatidyl inositol, increased cytoplasmic Ca^{2+} due to an influx of extracellular calcium, and the phosphorylation of cytoplasmic proteins. The release is accompanied by distinctive morphological changes. A series of variants of the rat basophilic leukemia cell have also been selected that do not release histamine. The studies during the past year have continued the use of monoclonal antibodies that have been produced in this laboratory to probe the secretory events in these cell lines.

A number of monoclonal antibodies (mAb) have been isolated that bind to the high affinity IgE receptor (FcεRI) on rat basophilic leukemia cells. One of these antibodies was used for affinity purification of the receptor and the associated proteins. The amino acid sequence from the purified protein was then used to isolate the cDNA for both the rat and human high affinity receptors. Presently, affinity purification of other receptor proteins are under way to further define the role of the different components in signal transduction.

Monoclonal antibodies were raised to the beta subunit of the FcεRI. The components of the receptor were isolated from rat basophilic cell line (RBL-2H3) by immunoaffinity purification on anti-receptor mAb. The 3 different subunits were further separated by HPLC chromatography. Mice were immunized with the homogenous β component and mAb selected. These antibodies were of the IgG_1, IgG_{2a} and IgG_{2b} class and bound to the β component on immunoblots of purified FcεRI or RBL-2H3 membrane preparations. The mAb also precipitated the α subunit from ^{125}I-surface labeled cells. Cross-inhibition assays defined at least 4 different epitopes, 2 of these on the cell surface and accessible for mAb binding in intact cells. None of these mAb inhibited ^{125}I-IgE binding or the binding of other anti-FcεRI antibodies. The anti-β mAb did not activate the cells for histamine release.

Therefore, the crosslinking of the β component does not result in histamine release although it is closely associated with the α subunit of the FcεRI.

Studies are continuing with mAb that recognize other proteins close to or associated with the FcεRI. The mAb AD1 was isolated that recognized a cell surface protein on rat basophilic leukemia cells (RBL-2H3). At high concentration this antibody inhibited IgE-mediated (49% inhibition at 100 μg/ml) but not calcium ionophore-induced histamine release. The mAb AD1 did not inhibit the binding of IgE nor of several antibodies directed to the FcεRI. Likewise, IgE did not inhibit mAb AD1 binding. However, several intact anti-FcεRI antibodies inhibited mAb AD1 binding but not as Fab fragments. Therefore, the sites on the cell surface to which mAb AD1 binds are close to FcεRI. The mAb AD1 immunoprecipitated a broad 50-60 kDa band from ^{125}I-surface labelled RBL-2H3 cells that upon peptide:N-glycosidase F treatment was transformed into a sharp M_r 27 kDa band. A similar 27 kDa protein was immunoprecipitated from surface radio-labelled cells after tunicamycin treatment. Thus, the protein recognized by mAb AD1 is highly glycosylated with predominantly N-linked oligosaccharides. The N-terminal sequence of 43 amino acids was found to be different from any subunits of FcεRI but very similar to that of the human melanoma-associated antigen ME491. Therefore, mAb AD1 binds to a surface glycoprotein on RBL-2H3 cells sterically close to the FcεRI but distinct from the recognized subunits of the receptor.

Several monoclonal antibodies have been isolated that inhibit the binding of IgE to its high affinity receptor on the rat basophilic leukemia cell. One of these antibodies, mAb AA4, inhibits histamine release, but does not block the binding of other anti-IgE receptors to the cell surface. The number of mAb AA4 molecules bound per cell is 14 times the number of IgE receptors. The mAb AA4 binds to two disialogangliosides (antigen I and antigen II) that are present on the RBL-2H3 cell line. The two antigens had been characterized previously and found to be α-galactosyl derivatives of the ganglioside GD_{1b}. Antigen I has 1 whereas antigen II has 2 galactosyl groups attached to GD_{1b}. Therefore, this mAb binds to surface glycolipids that are associated with the receptor, thereby inhibiting the binding of IgE. These gangliosides were present in the lipid extracts from different tissues. Immunofluorescence and immunohistological methods were used to investigate the distribution of the gangliosides on cells in different tissues. A survey of 19 different tissues from adult male rats revealed that mAb AA4 bound only to mast cells. In sections of brain there was no staining with mAb AA4, and no identifiable mast cells. The staining of mAb AA4 was specific for mast cells, and, with the exception of bone marrow, no cells other than mast cells stained with mAb AA4. In bone marrow, a population of large, poorly differentiated cells also stained with mAb AA4. The morphology of the cells indicates that they are most likely immature mast cells. Therefore, the gangliosides recognized by mAb AA4 are markers for rat mast cells.

We investigated the presence of the ganglioside antigens I and antigen II in several variants of the rat basophilic leukemia cell line. In contrast to the parental RBL-2H3, 2 variant cell lines had very low (0.3% and 1.0%) and 2 others had intermediate levels (9% and 17%) of ^{125}I-mAb AA4 binding, with corresponding amounts of antigen I and antigen II in the lipid extract. The mAb AA4 inhibited ^{125}I-IgE binding in the parental RBL-2H3 cells and in only one variant with intermediate amounts of the ganglioside. The mAb AA4 immunoprecipitated

a broad 40-60 kDa ^{125}I-surface labeled cells different from the α-subunit of FcεRI. There was correlation between the amount of antigen I and antigen II in cell extracts, the number of mAb AA4 binding sites on the cell surface and the amount of this 40-60 kDa protein precipitated from the different cell lines. Thus, this membrane protein appears to be associated with antigen I and antigen II and may play a role in the mAb AA4-induced modulation of IgE-mediated histamine release. Furthermore, the ganglioside distribution on the cell surface was not random and in only some of the cell lines was it close to the high affinity IgE receptor.

In the past year emphasis has also been placed on further characterizing the effects of monoclonal antibody (AA4) on RBL-2H3 cells. As described above, mAb AA4 binds to a glycolipid on the cell surface of the RBL-2H3 cells which is associated with, but not part of the FcεRI. Immediately after the binding of mAb AA4, the RBL-2H3 cells undergo striking morphological changes which appear identical to the changes seen when the FcεRI receptor is activated. However, the changes produced by AA4 are not accompanied by histamine release. When the cells are exposed to mAb AA4, the surface begins to ruffle, and with time, the cells lose their normal spindle-shaped appearance and begin to spread. The mAb AA4 binding has a profound effect on the cytoskeleton as determined both biochemically and morphologically. There is an increase in the amount of polymerized actin within the cells, which is associated with the surface ruffles. Binding of mAb AA4 also produces a redistribution of two other cytoskeletal components, tubulin and vimentin. However, there is increase in the amount of either of these cytoskeletal elements and they do not associate directly with the plasma membrane.

The biochemical pathway by which the binding of mAb AA4 to gangliosides on the cell surface bring about these morphological changes are being investigated. When mAb AA4 binds to the cell surface there is a modest yet significant increase in phosphatidyl inositol breakdown. This increase in phosphatidyl inositol turnover is dependent upon the concentration of mAb AA4, with a maximum response occurring at 10 µg/ml. There is also an increase in intracellular calcium concentration from 109 nM to 149 nM. In contrast, when the FcεRI receptor is activated under the same conditions, the intracellular calcium increases to 409 nM. Protein kinase C (PKC) is also activated by mAb AA4 as demonstrated by a fluorescent phorbol ester derivative. In control RBL-2H3 cells the majority of the fluorescent dye is concentrated in the cell body in association with secretory granules. Thirty minutes following IgE-receptor mediated stimulation, the average fluorescence intensity per cell is reduced to 44% ± 7.7% of control values. In comparison, when the cells are exposed to mAb AA4 for 30 minutes, the average fluorescence intensity per cell drops to 27% ± 2.5% of controls. Staurosporine, an inhibitor of PKC, will prevent the mAb AA4-induced changes in the RBL-2H3 cells. These studies indicate that binding of the mAb AA4 to the cell surface produces the same morphological and biochemical effects as those seen after activation of the FcεRI. However, the biochemical changes are attenuated in comparison to those produced by activation of the IgE receptor.

The preceding studies focused on the early changes (less than 30 minutes) seen after mAb AA4 binding to the gangliosides on RBL-2H3 cells. Another aspect under investigation is the

mechanism of inhibition of histamine release seen after mAb AA4 binding. When mAb AA4 (10 µg/ml) binds to the surface of RBL-2H3 cells that have been sensitized with IgE, there is a slow reduction in histamine release with approximately 50% inhibition by about 10 hours after exposure to mAb AA4. Results of immunofluorescence examination suggest that this inhibition of histamine release may be due to a removal by capping of the glycolipid antigen for mAb AA4. Further studies will define the changes in the FcεRI and determine if it continues to be associated with the mAb AA4 binding gangliosides.

The changes in the cytoskeleton of RBL-2H3 cells following antigen- and ionophore-induced histamine release have also been examined. After exposure of the cells to either secretagogue, the cells spread, and there is a rearrangement of the cytoskeleton. In addition, by scanning electron microscopy, deep ruffles developed on the surface of the cells undergoing IgE-mediated release. The surface changes were not as pronounced with calcium ionophore. In unstimulated cells actin was localized at the cell periphery, just under the plasma membrane. After stimulation, it was associated with the cell periphery and concentrated in the surface ruffles. As the stimulated cells spread, intermediate filaments and microtubules became distributed throughout the cell body, but there was no obvious association with the membrane ruffles. These morphological changes were dependent on the presence of extracellular calcium as well as on the concentration of ionophore or antigen. The changes were also correlated with the amount of histamine released. Additionally, IgE mediated stimulation resulted in increased uptake of the soluble phase tracer lucifer yellow only in the receptor activated cells. The observed differences may be due to the involvement of the FcεRI receptor in IgE mediated secretion.

Since the RBL-2H3 cells undergo striking morphological changes after IgE-mediated secretion, it was of interest to examine if these changes were dependent on activation of the FcεRI. Cells were stimulated to release histamine by two different mechanisms: activation of the FcεRI receptor by antigen and treatment with the calcium ionophore A23187. After exposure of the cells to either secretagogue, the cells spread over the surface of the culture dish and underwent rearrangement of the cytoskeleton. In addition, scanning electron microscopy revealed that deep ruffles developed on the surface of the cells undergoing IgE-mediated release. The surface changes were not as pronounced with the ionophore. The distribution of the cytoskeletal elements was examined by immunofluorescence using FITC-phalloidin and antibodies against vimentin and tubulin. In unstimulated cells actin was localized at the cell periphery, just under the plasma membrane. In the stimulated cells it was associated with the cell periphery and concentrated in the surface ruffles. As the stimulated cells spread, intermediate filaments and microtubules became distributed throughout the cell body, but there was no obvious association with the membrane ruffles. These morphological changes were dependent on the presence of extracellular calcium and on the concentration of ionophore or antigen, and were also correlated with the amount of histamine released. Additionally, IgE-mediated stimulation led to increased uptake of the soluble-phase tracer Lucifer yellow, whereas stimulation with the ionophore A23187 showed no increase in Lucifer yellow internalization. Ionophore A23187 produced changes similar to but not identical to those seen in the RBL-2H3 cells after IgE-mediated histamine release. The differences may be due to the involvement of the FcεRI receptor in IgE-mediated secretion.

Experiments during this year also investigated the biochemical pathways in signal transduction. The very interesting observation was made that there was tyrosine phosphorylation coupled to FcεRI signalling in the RBL-2H3 cells. As has been studied extensively for a number of years, the crosslinking by antigen of IgE bound to FcεRI on basophils and mast cells initiates a number of metabolic events culminating in histamine release. We investigated the possible involvement of tyrosine phosphorylation in FcεRI-mediated signalling in rat basophilic leukemia (RBL-2H3) cells. Cell lysates were analyzed by immunoblotting using phosphotyrosine-specific antibodies. Tyrosine-phosphorylated proteins were observed following FcεRI crosslinking, the most prominent having a M.W. of 72 kDa. Tyrosine phosphorylation was already detected 1 min after stimulation and correlated with both the time-course and antigen -dose for histamine release. Reversal of the receptor crosslinking resulted in rapid loss of both the tyrosine phosphorylation and continued degranulation. The calcium-ionophore A23187 induced histamine release without any obvious increase in tyrosine phosphorylation. The FcεRI tyrosine phosphorylations were still induced in the absence of calcium in the medium and after protein kinase C-depletion. Therefore, in RBL-2H3 cells, our findings link for the first time protein-tyrosine phosphorylation to FcεRI aggregation and receptor signal transduction.

Further evidence for a role of tyrosine phosphorylation in signal transduction in basophils was obtained with human basophils. The leukocyte common antigen (CD45) is a family of proteins on the cells that were recently recognized to be tyrosine phosphatase. In experiments during this year we discovered that monoclonal antibodies that react with the leukocyte common antigen (CD45) inhibit IgE-receptor mediated histamine release from human basophils. Hybridomas were selected that inhibited IgE-mediated histamine release from human basophils. Human peripheral leukocytes were incubated with the mAb, washed and challenged with anti-human IgE to release histamine. Two mAb were selected (HB 9AB6 and HB 10AB2). Both are of the IgG_1 subclass and were purified from mouse ascites. The 50% inhibitory concentration of HB 9AB6 is 0.8 µg/ml and for HB 10AB2 is 0.7 µg/ml. Neither mAb directly released histamine from human basophils nor did they inhibit histamine release induced by fMetLeuPhe, calcium ionophore A23187 or PMA. There was little inhibition of IgE-mediated release when the preincubation was performed at 4°C or reduced to 1 hr. By FACS analysis, the mAb bound to all peripheral blood leukocytes and immunoprecipitated a ~200 kD protein from leukocytes and human cell lines. In binding studies, the 2 mAb and a known anti-CD45 bound to the same protein. However, one of the mAb recognized a different epitope. Therefore, the CD45 surface antigen, a membrane protein tyrosine phosphatase, plays an important role in IgE-receptor mediated histamine release from human basophils.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00034-22 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Histamine Release

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	LI NIDR
Hook, William A.	Research Microbiologist	LI NIDR
Berenstein, Elsa H.	Microbiologist	LI NIDR
Benhamou, Mark	Visiting Fellow	LI NIDR
Volker, Stephan	Visiting Fellow	LI NIDR
Swieter, Mark	NRC Research Associate	LI NIDR
Hamawy, Majed	IRTA Fellow	LI NIDR

COOPERATING UNITS *(if any)*

NICHD ODCPR, NIH (M. Karten)

LAB/BRANCH

Laboratory of Immunology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.35	5.60	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody and other secretagogues including LHRH peptides and the Ca^{2+} ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and of biochemical changes during cell activation. Large numbers of these cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.

PHS 6040 (Rev. 1/84) GPO 914-918

Other

Kitani, Seiichi	Visiting Associate	LI NIDR
Oliver, Constance	Research Biologist	LI NIDR
Koh, Crystal	Microbiologist	LI NIDR
Bader, Greta	Biologist	LI NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00046-19 LI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Normal and Pathologic Mechanisms of Inflammation and Repair

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sharon M. Wahl	LI, NIDR
Henry Wong	LI, NIDR
Dennis Mangan	LI, NIDR
Mary Brandes	LI, NIDR
Glenn Welch	LI, NIDR
Sue Dougherty	LI, NIDR

COOPERATING UNITS *(if any)*
Lalage Wakefield, NCI; Mike Lotze, NCI; Ildy Katona, USUHS

LAB/BRANCH
Laboratory of Immunology

SECTION
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INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.60	1.90

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Host defense to pathogens and other antigens is dependent upon mononuclear cell recruitment, activation and finally, downregulation. This research project focuses on delineating the cellular and molecular mechanisms controlling these events. Whereas blood monocytes readily respond to inflammatory chemotactic stimuli, mononuclear phagocytes from within inflammatory sites exhibit defective chemotactic activity due to loss of chemotactic ligand (C5a) receptors. Increased monocyte maturation as reflected by increased HLA-DR was found to be associated with decreased chemotaxis receptor expression providing a mechanism for macrophage accumulation within an inflammatory site. Once within the inflammatory site, macrophages are induced to express CD16 (FcγR111) by the inflammatory cytokine, TGF-β. Signal transduction through this receptor regulates immunophagocytosis and can promote tissue destruction through the release of reactive oxygen intermediates (ROI). The ability of TGF-β to stimulate monocyte phenotypic and functional changes is transient since TGF-β receptors are lost during differentiation. Additional mechanisms of immune suppression may occur through the release of IL-4 which can suppress TGF-β-induced FcγR111 expression, inhibit ROI, and downregulate monokine production. Thus, immune cell-derived cytokines provide a series of intercellular signals which initiate, augment and ultimately, suppress the immune response.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00199-14 LI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Oliver, Constance	Research Biologist	LI NIDR
Siraganian, Reuben P.	Chief, Clinical Immunology	LI NIDR
Waters, Judith F	Biologist	LI NIDR
Weedon, Lynda L	Biologist	LI NIDR
Fujimura, Akira	Visiting Fellow	LI NIDR
Jamur, Celia	Visiting Fellow	LI NIDR

COOPERATING UNITS *(if any)*
Dr. A. Robbins, LBM, NIDDK

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Laboratory of Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	2.6	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Secretory and endocytic process in several cell types are currently under investigation. A rat basophilic leukemia cell line (RBL-2H3), short term cultures of isolated exocrine acinar cells, a pancreatic acinar cell line (AR42J), and other cultured cells are being used to study various aspects of endocytic and secretory processes. Emphasis is placed on morphological, cytochemical and biochemical characterization of these processes in the cultured cells. Events involved in receptor activation, signal transduction and endocytic mechanisms are under investigation. The lysosomal system and its role in endocytic and secretory pathways is also under study.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00290-11 LI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief Clinical Immunology	LI NIDR
Hook, William A.	Research Microbiologist	LI NIDR
Berenstein, Elsa H	Microbiologist	LI NIDR
Fischler, Cynthia	Bio. Lab. Technician	LI NIDR
Kitani, Seiichi	Visiting Fellow	LI NIDR
Mergenhagen, Stephan E	Chief, Lab. Immunology	LI NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

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Clinical Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	1.4	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (☒) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the high affinity IgE (FcεRI) receptor subunits of rat mast cells, to human IgE, and several that modulate histamine release from human or rat mast cells. These monoclonal antibodies are being used for biochemical and biological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00392-07 LI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Macrophage Responses to Mucosal Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Phillip D. Smith, M.D.	Senior Investigator	LI, NIDR
Uwe E.H. Mai, M.D.	Guest Researcher	LI, NIDR
Nancy Ogihara, B.S.	Biologist	LI, NIDR
Larry M. Wahl, Ph.D.	Microbiologist	LI, NIDR
Sharon M. Wahl, Ph.D.	Chief, Cellular Immunology Section	LI, NIDR

COOPERATING UNITS *(if any)*

J.M. Orenstein, M.D., Ph.D., G.W. University Medical Center; M.S. Chernick, M.D. NIDDK, NIH

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.05	3.35	2.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mononuclear phagocyte, as both circulating monocyte and tissue macrophage, plays a critical role in host immunologic, inflammatory and defense reactions to infectious microorganisms. Accordingly, the focus of this laboratory has been to elucidate monocytes/macrophage function in three mucosal diseases: type B gastritis, cystic fibrosis, and cytomegalovirus colitis. Our studies suggest that the monocyte/macrophage plays an important role in mediating the immunopathologic response to the pathogens associated with these diseases through the upregulation of cytokine production.

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GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00424-05 LI

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Molecular Analysis of Monocyte Phenotype and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

McCartney-Francis, Nancy	Senior Staff Fellow	LI, NIDR
Wahl, Sharon M.	Chief, CIS	LI, NIDR
Wahl, Larry M.	Biologist	LI, NIDR
Mizel, Diane	Chemist	LI, NIDR
Dougherty, Suanne	Microbiologist	LI, NIDR
Wong, Henry	Staff Fellow	LI, NIDR

COOPERATING UNITS *(if any)*: M. Norcross, FDA

LAB/BRANCH: Laboratory of Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.2	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this research program is to define the cytokines elaborated by monocytes and the molecular mechanisms that regulate their expression. Exposure to TGF-β influences the responsiveness of monocytes to secondary stimuli such as lipopolysaccharide (LPS). Pretreatment with TGF-β results in a dramatic increase in the level of LPS-induced GM-CSF RNA and protein. However, similar treatment results in a 50% reduction in the level of IL-1 and TNF-α RNA. While LPS alone induces the expression of a variety of monokines, pretreatment with TGF-β appears to prime the cells to the effects of LPS, influencing not only the magnitude of the response but also the kinetics. This interplay between inflammatory cells and cytokines may be important in the regulation of the immune response. Moreover, modulation of cytokine expression may influence the outcome of diseases such as AIDS. Exposure of monocytes to HIV-1, the etiological agent of AIDS, results in a biphasic induction pattern of cytokine expression, most notably TNF-α. The close association of HIV-1 and monocyte gene expression suggests that they may share a common regulatory mechanism. HIV-1 gene expression in transfected THP-1 cells (a mature monocyte cell line) is increased after stimulation with LPS, suggesting that cytokine induction may influence viral expression. However, cotransfection of the TNF-α promoter with TAT, a transactivator protein for HIV-1, does not lead to increased TNF expression. Analysis of nuclear proteins demonstrated that resting monocytes constitutively express NF-κB binding proteins and the level of expression is relatively unchanged by the state of activation or by infection with HIV-1. However, preliminary studies suggest that a TNF-binding protein is upregulated in HIV-1-infected monocytes and may be important in the induction of TNF gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00441-04 LI

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Immunoregulation of Experimentally Induced Immune Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen, Janice B.	Chemist	LI, NIDR
Wahl, Sharon M.	Chief, CIS	LI, NIDR
Manthey, Carl P.	PRAT	LI, NIDR
Brandes, Mary	IRTA	LI, NIDR
Costa, Gina	Biologist	LI, NIDR
Kossmann, Thomas	Guest Researcher	LI, NIDR
Mergenhagen, Stephan E.	Chief, LI	LI, NIDR

COOPERATING UNITS *(if any)*: E. Amento, Genentech; A. Hand, CIPCB, NIDR; L. Ellingsworth and Y. Ogawa, Collagen Corp.; R. Simmons, Syntex; U. Heine, NCI; K. Ohura, Osaka Dental University, Japan

LAB/BRANCH: Laboratory of Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.13	1.53	3.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Systemic injection of streptococcal cell walls (SCW) initiates acute and chronic inflammation in liver, spleen and peripheral joints of susceptible rats. Associated with the host response is a marked elevation in circulating leukocytes (WBC), within 3 to 5 days after SCW administration, > 3 fold above controls. The persistence of leukocytosis parallels development of chronic inflammation in target tissues. In studies to identify the cellular and molecular events leading to these chronic inflammatory lesions, we have utilized site specific agents in the evaluation of this pathophysiological process. A potent immunomodulatory cytokine, transforming growth factor-beta (TGFβ), was identified in the inflamed synovium and granulomas of SCW-injected rats. Extracellular TGFβ1 was found to be associated exclusively with microfibrils of elastin present in the extracellular matrix of the inflamed joint. In parallel, TGFβ is expressed throughout granuloma development, and appears to mediate the recruitment and activation of monocytes and fibroblasts. Daily i.p. dosing of recombinant TGFβ suppressed both the acute and chronic phases of the disease. Leukocytosis was reversed in the treated rats. Histological analysis of the joints revealed a marked reduction in inflammatory cell recruitment, less synovial hyperplasia and erosions than the SCW controls. The chronic phase was suppressed when TGFβ was started between the phases. Cartilage and bone destruction could not be reversed when TGFβ was given during chronic disease. In additional studies, daily oral dosing of the antiinflammatory ethyl ester of mycophenolic acid (RS-61443) resulted in a dose-dependent suppression of the chronic arthritis with no differences in the acute phase. Histological evaluation demonstrated that RS-61443-treated animals had fewer inflammatory cells in the synovial tissue than the SCW controls. These studies reflect the contributions and regulation of cell-cell interactions in inflammatory lesions, and the potential for therapeutic intervention.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00456-03 LI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Signal transduction in the monocyte/macrophage.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wahl, Larry M.	Research Biologist	LI, NIDR
Corcoran, Marta L.	Chemist	LI, NIDR

COOPERATING UNITS *(if any)*
D.S. Finbloom, FDA; I. Katona, USUHS; W.L. Farrar, NCI; A. Spiegel, NIDDK; R. Kahn, NCL, J. Weinstein and J. Szebeni, NCI

LAB/BRANCH
Laboratory of Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.22	.82	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the biochemical pathways involved in signal transduction in the monocyte. Recent studies have examined the role of guanine nucleotide binding proteins (G proteins) in the prostaglandin-cyclic AMP dependent pathway of collagenase production by monocytes. Western blot analysis of the G proteins revealed that the same G proteins were present in the membrane of control and stimulated monocytes. However, ADP-ribosylation assays revealed that cholera toxin (CT) ADP-ribosylated a 46-kDa Gsα protein only in the membranes of monocytes that had been stimulated with agents such as Con A or LPS. This correlated with the ability of CT to enhance eicosanoid synthesis of activated but not control monocytes, whereas CT elevated cAMP levels in both control and stimulated monocytes. These results suggest that the 46-kDa Gsα protein may be involved in the regulation of phospholipase activity. Thus, G proteins regulate both phospholipase and adenylyl cyclase in monocytes, events that are an integral part of the activation sequence leading to collagenase production by these cells.

In additional studies, since IL-4 has been shown to inhibit PGE2 synthesis by monocytes, this cytokine was examined for its effect on the biochemical events leading to collagenase production. Nanogram amounts of IL-4 were shown to not only inhibit PGE_2, but to also block the release of arachidonic acid and the subsequent synthesis of metabolites from both the cyclooxygenase and lipoxygenase pathway as well as collagenase. These findings indicated that IL-4 is a potent suppressor of phospholipase activity of the monocyte. Evidence for the suppression of phospholipase by IL-4, and therefore the subsequent PGE_2 production, as the mechanism for inhibition of collagenase was demonstrated by the ability of exogenous PGE_2 to restore collagenase production in IL-4 treated monocyte cultures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00513-01 LI

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Role of Monocytes in AIDS and as Targets for Antiviral Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sharon M. Wahl	LI, NIDR
Janice B. Allen	LI, NIDR
Cristina Morganti-Kossmann	LI, NIDR
C.N. Venkateshan	LI, NIDR

COOPERATING UNITS (if any)
Jean Nichols, Seragen, Boston; Frank Robey, NIDR; John Weinstein, NCI; Janos Szebeni, NCI, Jan Orenstein, GWU

LAB/BRANCH: Laboratory of Immunology

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monocyte-macrophages are important hosts for HIV-1, playing key roles in the dissemination of the virus and the pathogenesis of AIDS. These studies focus on defining altered functions of mononuclear phagocytes from AIDS patients and following HIV-1 infection in vitro. Numerous phenotypic and functional abnormalities have been defined. For example, monocytes in the circulation of normal individuals do not express FcγR111, whereas many AIDS monocytes are FcγRIII positive. Upregulation of this receptor which modulates immunophagocytosis and release of toxic oxygen species may be related to increased circulating levels of TGF-β. TGF-β is also a potent immunosuppressive agent and is produced by HIV infected monocytes. Based on the emerging evidence that monocytes are central to the evolution of AIDS, studies are also directed at exploring potential antiviral therapies targeted at the monocyte population.

In addition to circulating monocytes, HIV is found in high frequency in tissue macrophages. The gastrointestinal tract is a major route of entry for HIV and a series of studies are focusing on the immunologic and clinical manifestations of HIV infection of the oral cavity and gastrointestinal tract.

LABORATORY OF MICROBIAL ECOLOGY

The Laboratory of Microbial Ecology (LME) was established in 1988 by the division of personnel and research programs from the Laboratory of Microbiology and Immunology, which has now been redesignated the Laboratory of Immunology.

In June of 1989, Dr. Jerry M. Keith transferred from the National Institute of Allergy and Infectious Diseases and was appointed Chief of the Laboratory. Priorities over the past year were to expand the laboratory into research areas complementary to the already existing programs and to upgrade the laboratory facilities. An active molecular genetics program in bacterial virulence factors established by Dr. Keith was subsequently integrated into the laboratory. This experienced, well-established new staff brought a scientific expertise in molecular genetics and the "new biology" to LME and has enhanced the intellectual environment of the laboratory. A reorganization of the laboratory has established new goals for existing and future research programs. The laboratory is now formally divided into four research units reflecting specific functional research disciplines: 1) Pathogenic Mechanisms; 2) Microbial Interactions; 3) Microbial Physiology and Biochemistry; and 4) Virulence Factors and Molecular Biology. Although the laboratory had been initially ordered in this direction, this formal reorganization and the addition of a new research discipline brought a more aggressive focus to existing research programs in the laboratory and defined the goals for the development of future research. These changes established the basis for a strong laboratory charter that will emphasize and reflect the mission of NIDR and NIH.

The primary mission of LME is to understand the etiology of diseases related to oral health. It was considered important that the area of "microbial ecology" be pursued in order to identify and characterize microorganisms involved in these diseases because many of these etiologic agents and their mechanisms of pathogenesis have remained elusive. To accomplish the mission of LME, it is important to identify oral pathogens, characterize cell-to-cell interactions among oral microorganisms, study the interactions between oral microorganisms and host tissue, and analyze host responses to the oral flora. LME scientists study the biochemistry, molecular biology, physiology, and genetics of microbial organisms. To further understand microbial colonization, the surface structures on bacteria that mediate attachment to other microbes and to host tissues and the receptors for these microbial adhesins are being characterized. In parallel investigations, LME scientists study the means by which certain organisms evade host defense systems and the chain of events that lead to inflammation and consequent tissue destruction. In addition, sugar and amino acid transport systems, as well as key regulatory metabolic enzymes of bacteria, are studied in order to understand complex intermicrobial interactions and nutrient competition.

After the reorganization, regularly scheduled meetings with the LME section leaders were established to discuss research programs and laboratory management. Senior researchers can now coordinate their research efforts and goals. A weekly laboratory "work-in-progress" seminar was also established in which LME investigators present their current research results. These seminars are presented by each research group, and this has resulted in a dynamic interaction among laboratory members. The entire laboratory is functioning with a common focus and mission.

Some of the physical facilities have now been remodeled to meet safety guidelines concerning biological level containment as well as utilizing space more efficiently. One double module has been completed as well as minor renovations to several single modules. More remodeling is scheduled. Dr. London's laboratory will be converted to a double module and a new general equipment room is scheduled for this summer. Dr. Sandberg's laboratory will be renovated in the fall. The transfer of approximately $200,000 of capital equipment from my old laboratory to NIDR and the procurement of new major pieces of equipment have increased our capabilities. In addition, the laboratory can now conveniently utilize biotechnology such as hybridoma production and oligonucleotide syntheses. Facilities for animal studies such as aerosol challenge are under construction. New computer accounts have been established, and the laboratory computers have been networked to allow more efficient use of equipment and better data management. These changes have resulted in an exciting cohesive new laboratory whose research activities will continue to be significant and productive in the future.

In June 1990, LME underwent a very successful review by the Board of Scientific Counselors.

MICROBIAL INTERACTIONS SECTION

The major effort of the group this past year focused on cloning and sequencing adhesive proteins found on the surface of both gram-positive and gram-negative oral bacteria. The gene encoding the fimbria-associated GalNAc-specific adhesin of *Prevotella (Bacteroides) loeschei* is currently being sequenced. The gene appears to be transcribed in a rather complex fashion that may involve a frameshift of inversion of a segment of genome. Recent proteolytic studies indicate that the GalNAc-specific (*S. oralis*-specific) adhesin and the GalNAc-insensitive (*A. israelii*-specific) adhesin are structurally related. The former can be cleaved to yield a 55-kD fragment that reacts strongly with monoclonal antibodies prepared against the actinomyces-specific adhesin. The gene encoding an adhesin located on the surface of *Streptococcus gordonii* has recently been cloned and sequenced. The 38-kD protein appears to be bifunctional, permitting the bacterium to recognize and bind to a salivary protein that adheres to hydroxyapatite surfaces and to coaggregate with strains of *Actinomyces naeslundii* in a sugar noninhibitable interaction. Now that the gene has been sequenced, studies have been initiated to determine whether the protein has a single or multiple binding sites for the two diverse interactions. Work is also continuing to identify the *Fusobacterium nucleatum* adhesin that mediates coaggregation with a number of oral gram-

negative bacteria. Similarly, the *Veillonella atypica* adhesin that mediates coaggregation with *S. oralis* is being isolated using affinity matrices to selectively remove the protein.

Understanding of the initial colonization process has been advanced by studies describing intrageneric coaggregations that occur almost exclusively among stains of *Streptococcus sanguis, Streptococcus oralis*, and *Streptococcus gordonii*. The oral actinomyces exhibit this trait to a very limited extent. These studies are helping to clarify the early dynamics of plaque formation since these microorganisms represent the initial colonizing flora.

An AIDS-related research project has recently been initiated. In collaboration with the Department of Anaerobic Bacteriology at the Virginia Polytechnical Institute and State College, this section is studying the effect of HIV-1 infection of the oral microflora. The bacterial content of plaque samples taken from HIV-seropositive and ARC-AIDS subjects is being enumerated and identified to determine whether gross or subtle alterations result from the viral infection. Thus far, 12 of a total of 40 patients have been screened; however, complete analyses cannot be performed until a larger segment of the cohort has been examined.

PATHOGENIC MECHANISMS SECTION

The research activities of the Pathogenic Mechanisms Section continue to focus on the molecular basis of the interactions of oral bacteria with bacterial and host cell receptors and the biological consequences of these attachment processes, including colonization, inflammation of oral tissues, and the potential destruction of the microorganisms.

The adherence of *Actinomyces viscosus* T14V to the tooth surface is dependent on the recognition of proline-rich proteins in the acquired pellicle by the type 1 fimbriae, whereas the type 2 fimbriae of this bacterium and *A. naeslundii* WVU45 mediate their interactions with certain strains of oral streptococci, epithelial cells, and polymorphonuclear leukocytes (PMNs). The genes for the structural subunit of each different fimbria have been cloned, and they encode proteins of 533 to 535 amino acid residues beginning with cleavable signal sequences of 30 or 32 residues. Each mature subunit ($M_r \approx 54{,}000$) was predominantly hydrophilic except for a carboxy terminal segment that was identified as a potential membrane-spanning domain. The different *Actinomyces* spp. fimbrial subunits showed no significant global homologies with other proteins including the pilins of gram-negative bacteria, but high homology was noted between the type 2 subunits of strains T14V and WVU45 (79% amino acid sequence similarity) and lower homology between either type 2 subunit and the type 1 subunit of strain T14V (49% amino acid sequence similarity). Whereas specific inhibition of fimbriae-mediated adherence to SHA or *Streptococcus oralis* 34 was observed with Fab fragments prepared from certain rabbit anti-fimbriae antibodies, different monoclonal or polyclonal antibodies directed against the 54-kD subunits of type 1 or type 2 fimbriae lacked these activities, suggesting that the adhesins may be distinct from the fimbrial subunit.

Considerable information has been acquired concerning the receptors for the actinomyces fimbrial lectin on certain strains of oral streptococci. These receptors were detected on all twelve strains of *S. oralis* examined but less frequently or not at all on other viridans streptococci. Antigenically distinct linear polysaccharides, each composed of a different phosphodiester-linked repeating unit, were identified as the receptor structures on *S. oralis* strains 34, 10557, and C104 and *S. mitis* J22. Significantly, antibodies formed against the receptor polysaccharide of strain 34,

$$[\rightarrow 6GalNAc\alpha 1\rightarrow 3Rha\beta 1\rightarrow 4Glc\beta 1\rightarrow 6Gal_f\beta 1\rightarrow 6GalNAc\beta 1\rightarrow 3Gal\alpha 1\text{-}PO^-_3\text{-}]_n,$$

were directed at the α-linked GalNAc end of the hexasaccharide unit, while lectin recognition involved the GalNAcβ1→3Gal at the other end of the chain. A similar separation of antigenic and receptor regions was apparent from the structure of the J22 receptor,

$$[\rightarrow 6GalNAc\alpha 1\rightarrow 3Rha\beta 1\rightarrow 4Glc\beta 1\rightarrow 6Gal_f\beta 1\rightarrow 6Gal\beta 1\rightarrow 3GalNAc\alpha 1\text{-}PO^-_3\text{-}]_n$$
$$Rha\alpha 1\rightarrow 2$$

A structural difference between the J22 and 34 polysaccharides was the presence of Galβ1→3GalNAc in the former and GalNAcβ1→3Gal in the latter. Structural studies of the antigenically distinct receptor polysaccharides of strains 10557 and C104 have shown that their oligosaccharide repeating units also have Galβ1→3GalNAc and GalNAcβ1→3Gal, respectively, at the proposed site of lectin recognition.

Receptors containing Galβ1→3GalNAc on PMNs have also been implicated in interactions of the actinomyces with these phagocytic cells. A putative 110-kD PMN glycoprotein receptor for the fimbrial lectin was identified by certain Gal/GalNAc and Galβ1→3GalNAc-reactive plant lectins on sialidase-treated Western blots of PMN extracts separated by SDS-PAGE. The 110-kD glycoprotein was also present in extracts of HL-60 cells induced to differentiate toward PMNs by incubation with DMSO for 3 or 7 days. This band was absent in extracts of undifferentiated HL-60 cells that did, however, contain a slightly heavier (approximately 120 kD) glycoprotein detected by the plant lectins. These findings suggest that either different receptors are expressed at different stages of differentiation or that the receptors undergo differentiation-induced processing. Although radiolabeled actinomyces bound to undifferentiated cells, their binding increased during the differentiation process. Potential glycolipid receptors were also detected by direct binding of the actinomyces to PMN gangliosides separated by thin layer chromatography.

The recognition of these glycoconjugate receptors on sialidase-treated PMNs by the actinomyces fimbrial lectin resulted in phagocytosis and destruction of the bacteria. Bactericidal activity was inhibited by β-linked galactosides, and mutants lacking the type 2 fimbriae were not killed. *A. viscosus* T14V, but not a mutant lacking fimbriae, also stimulated the production of superoxide anions and the release of the contents of secondary PMN granules.

The interaction of a sialic acid-reactive lectin on *S. gordonii* DL1 with PMNs resulted in N-acetylneuramin-lactose inhibitable ingestion of the bacteria, production of superoxide anions, and release of secondary granule contents. However, the streptococci were not killed. *S. gondonii* DL1 functioned as a vehicle for the initiation of killing of *A. viscosus* T14V with which it coaggregates. Neither bacterium was destroyed when added together at low

concentrations to PMNs. However, coaggregation of the bacteria at high concentrations, dilution, and incubation with PMNs resulted in killing of the actinomyces. This effect was not inhibited by lactose but was significantly decreased by N-acetylneuramin-lactose.

IgG and fragments of the third component of complement (C3) deposited on bacteria also interact with specific receptors on PMNs. Anti-cell surface or anti-type 1 fimbriae IgGs initiated killing of *A. viscosus* T14V by PMNs. An anti-type 2 fimbriae IgG was only minimally effective. These findings did not correlate with the affinities of the IgGs or the numbers of IgG binding sites per bacterium. Complexes containing *A. viscosus* T14V and each of the IgGs initiated similar binding of ^{125}I-C3 fragments and the IgG specificity determined the site of binding of the C3 fragments. Deposition of C3 fragments by the anti-cell surface IgG significantly enhanced bactericidal activity. Thus, effective opsonization was strictly dependent on IgG and C3 localization. Cooperativity between the actinomyces lectin and C3 fragments also occurred. Complement deposited on the bacterial surface by IgM initiated killing of *A. viscosus* T14V by non-sialidase-treated PMNs to approximately the same extent as the interaction of the lectin with sialidase-treated PMNs. The bactericidal activity was enhanced more than 50 fold if the bacteria were opsonized with C3 fragments and, in addition, were incubated with PMNs in the presence of sialidase to expose the receptors for the bacterial lectin. Collectively, these findings define a number of mechanisms by which oral bacteria could be destroyed and/or participate in inflammation.

VIRULENCE FACTORS AND MOLECULAR BIOLOGY SECTION

The new research program brought into NIDR by Dr. Keith focuses on human infectious diseases and the development of host immunity and complements the mission of LME. Research is focused on studies that define pathogenic or virulence mechanisms of human microbial pathogens and establish the molecular interactions between these organisms and their human host. Modification of organisms by natural and artificial means of DNA exchange is used to define pathogenic mechanisms. Genetically altered bacteria are used in virulence studies and in experiments designed to determine the effect of genetic changes on host defense mechanisms. In addition, investigators are studying the molecular structure of pathogens, the role of gene products in pathogenic mechanisms, and the development of synthetic antigens that elicit protection against disease. Several models of bacterial pathogenesis have been developed in the laboratory, including *Bordetella pertussis* (the causative agent of whooping cough), *Bacillus anthracis* (the etiologic agent of anthrax), and *Vibrio cholerae* (the agent of cholera). In addition, oral pathogens as well as intracellular microbial agents will be developed as models for the study of molecular pathogenesis.

The Virulence Factors and Molecular Biology research group will soon begin a comprehensive research program to identify pathogenic virulence factors in oral bacteria associated with periodontal disease. This program will utilize the expertise of the research staffs of both Drs. Leppla and Keith. Oral bacteria will be screened for well-established virulence factors, including adenylate cyclase and ADP-ribosylation activities, hemolysins,

hemagglutinins, cytotoxins, mitogens, and cellular invasion. Several new post-doctoral fellows and summer students have been recruited to assist in this research effort.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00043-20 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Physiological and genetic studies on pathogenic oral microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR
Harr, Robert J.	Bio Lab Tech (Micro)	LME, NIDR
Cisar, John O.	Research Microbiologist	LME, NIDR
Thompson, John	Visiting Scientist	LME, NIDR

COOPERATING UNITS *(if any)*
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A.L. Delisle, University of Maryland Dental School, Baltimore, MD

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

N(5)-(carboxyethyl)ornithine synthase (CeOS), an enzyme discovered in *Streptococcus lactis*, catalyzes the NADPH-dependent condensation between pyruvic acid and the terminal amino group of ornithine to yield N(5)-(carboxyethyl) ornithine (CeO). Polyclonal antibodies against CeOS were used to examine (by Western blot analysis) the dissemination and size of the enzyme in various lactic acid bacteria. Of the three genera and six species surveyed, CeOS was detected only in about 50% of the *S. lactis* strains examined. CeOS is constitutive; in several strains (e.g., K1) it exists primarily as a tetramer of 38-kDa subunits, but in other strains (e.g., 133) the subunit mass is 35-kDa. Tracer studies indicate that CeO is biosynthetically derived from exogenous arginine which, upon uptake into the cell, is rapidly converted to ornithine. Once synthesized from ornithine and pyruvate, CeO is relatively stable, which explains why it accumulates to relatively high levels (10 mM) intracellularly. As a result of experiments to determine whether CeOS was encoded on one of the plasmids in strain K1, a spontaneous derivative (K1-42) was isolated which lacked CeOS as well as the ability to ferment sucrose and synthesize the polypeptide antibiotic nisin. Hybridizations with a nisin gene probe indicated that these linked traits are encoded on the chromosome. Because the ability to ferment sucrose (and synthesize nisin) is conjugally transferable, we have proposed that these traits are located on a conjugative transposon. To further localize and clone the CeOS gene, a 39-mer based on a peptide within the amino-terminus of CeOS was synthesized. Comparison of Southern blots of the parent (K1) and the mutant (K1-42) revealed the absence of a 7-kbp *EcoRI-XhoI* fragment that hybridized to the labeled 39-mer. Using this probe for detection, recombinant phages were obtained which contained this latter fragment inserted into the vector Lambda ZAPII, and several of them reacted with CeOS specific antibody.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00061-19 LME

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Complement activation and inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandberg Ann L.	Chief, Pathogenic Mechanisms Section	LME, NIDR
Lyman, Caron A.	Staff Fellow	LME, NIDR
Mudrick, Linda L.	Microbiologist	LME, NIDR
Cisar, John O.	Research Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*: Dr. Albert Vatter, University of Colorado

LAB/BRANCH: Laboratory of Microbial Ecology

SECTION: Pathogenic Mechanisms Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.30	2.30	1.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The recognition of polymorphonuclear leukocyte (PMN) asialo- or sialoglycoconjugate receptors by the Gal/GalNAc reactive fimbrial lectin of *A. viscosus* T14V or the sialic acid reactive lectin of *S. gordonii* DL1, respectively, resulted in phagocytosis, production of superoxide anions and release of the contents of secondary PMN granules. However, only phagocytosis initiated by the actinomyces lectin was accompanied by stimulation of PMN chemiluminescence and destruction of the bacteria. Putative PMN glycoprotein and glycolipid receptors for the actinomyces lectin were identified. Studies utilizing the HL60 cell line indicated that the expression of receptors for the actinomyces lectin was enhanced and processing of a glycoprotein receptor occurred during differentiation of the cells towards PMNs. Although *S. gordonii* DL1 remained viable following lectin mediated ingestion by PMNs, these bacteria served as vehicles for the initiation of killing of *A. viscosus* T14V if the two bacteria were coaggregated prior to incubation with the phagocytic cells. IgG and fragments of the third component of complement (C3) deposited on bacteria also initiated PMN dependent killing of *A. viscosus* T14V, but only when localized to certain bacterial surface structures. Anti-cell surface or anti-type 1 fimbriae IgGs initiated killing by PMNs but anti-type 2 fimbriae IgG was minimally effective. When IgG sensitized bacteria were incubated with complement, the localization of C3 fragments on the actinomyces was dependent on the IgG specificity. Only C3 fragments bound to the cell surface enhanced bactericidal activity. Cooperativity between the actinomyces lectin and C3 fragments was also demonstrated. Although the destruction of *A. viscosus* T14V could be initiated either by C3 fragments bound to the bacterial surface or by the lectin associated with the type 2 fimbriae, the bactericidal activity was enhanced more than 50 fold if the bacteria were opsonized with C3 fragments and, in addition, were incubated with PMNs in the presence of sialidase to expose the receptors for the bacterial lectin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00254-13 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Microbial antigens associated with specific adherence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cisar, John O.	Research Microbiologist	LME, NIDR
Cherry, Gail J.	NRSA Fellow	LME, NIDR
Hsu, S. Dana	Microbiologist	LME, NIDR
Sandberg, Ann L.	Chief, Pathogenic Mechanisms Section	LME, NIDR
Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*
University of Florida; University of Maryland; Royal Dental College Aarhus, Denmark

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Pathogenic Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.10	1.10	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Type 1 fimbriae-mediated adherence of *Actinomyces viscosus* T14V to saliva-treated hydroxyapatite (SHA) involves the recognition of proline rich proteins in the acquired pellicle whereas the type 2 fimbriae-mediated coaggregations of strain T14V or *A. naeslundii* WVU45 with *Streptococcus oralis* 34 depends on a Gal/GalNAc-reactive fimbrial lectin. The gene for the structural subunit of each different fimbria encoded a protein of 533 to 535 amino acid residues beginning with a cleavable signal sequence of 30 or 32 residues. Each mature subunit ($M_r \approx 54,000$) was predominantly hydrophilic except for a carboxy terminal segment that was identified as a potential membrane spanning domain. The different *Actinomyces* spp. fimbrial subunits showed no significant global homologies with other proteins including the pilins of gram negative bacteria, but high homology was noted between the type 2 subunits of strains T14V and WVU45 (79% amino acid sequence similarity) and lower homology between either type 2 subunit and the type 1 subunit of strain T14V (49% amino acid sequence similarity). Receptors for the type 2 fimbrial lectin of *Actinomyces* spp. were detected on all strains of *S. oralis* (12/12) but less frequently or not at all on other viridans streptococci. Antigenically distinct linear polysaccharides, each composed of a different, phosphodiester linked repeating unit, were identified as the receptor structures on *S. oralis* strains 34, 10557 and C104 and *S. mitis* J22. As with strains 34 and J22, structural studies of the antigenically distinct receptor polysaccharides of strains 10557 and C104 have shown that their oligosaccharide repeating units have Galβ1→3GalNAc and GalNAcβ1→3Gal, respectively, at the proposed site of lectin recognition. Each of these structures has been associated with the receptor activity of specific mammalian cell surface glycoproteins and glycoplipids, and the apparent mimicry between bacterial and host cell receptors provides a possible explanation for the failure of antibodies to be directed toward the receptor regions of these bacterial polysaccharides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00273-12 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the bordars.)*
Cell-cell interactions between oral actinomyces and other bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kolenbrander, Paul E.	Research Microbiologist	LME, NIDR
Andersen, Roxanna	Microbiologist	LME, NIDR
Ganeshkumar, Nadarajah	Visiting Fellow	LME, NIDR
Hughes, Christopher V.	NRSA Fellow	LME, NIDR
London, Jack P.	Research Microbiologist	LME, NIDR
Roseberry, Christopher	Biologist	LME, NIDR

COOPERATING UNITS *(if any)*
Dr. L.V.H. Moore, VPI and SU, Blacksburg, VA; Dr. B. McBride, U of British Columbia, Canada; Dr. E. Weiss, Tel Aviv University, Israel; Dr. Gary Pearson, Georgetown University, Washington, DC; A. Kagermeier, Erlangen, West Germany

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	2.00	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Coaggregation between *S. gordonii* PK488 and *A. naeslundii* PK606 is mediated by an adhesin of 38 kDa, which also may recognize salivary receptors. All streptococci that coaggregate with this actinomyces express an immunologically cross-reactive protein to this adhesin. From one of these streptococci, *S. sanguis* 12, the *Ssa*B gene which encodes this adhesin has been cloned into *E. coli* and has been sequenced. It is 927 basepairs long, and encodes for a hydrophilic protein of molecular weight of 34,684 which has a hydrophobic signal peptide of 19 amino acids. Antisera against the cloned protein cross reacts with the 38 kDa protein from *S. gordonii* PK488. The ability of these streptococcal surface proteins to recognize both salivary receptors and actinomyces receptors provides the first example of a dual adhesive function of a surface protein recognizing both cellular and noncellular receptors. Coaggregation between *V. atypica* PK1910 and *Streptococcus* spp. appears to occur by two distinct adhesins, and one is galactoside-sensitive. Coaggregation-defective mutants that have lost the galactoside-sensitive coaggregation with streptococci also do not possess a protein of about 47 kDa. A protein of this size has been eluted from an N-acetylgalactosamine-agarose affinity column and is presumed to be the lactose-sensitive adhesin. While intergeneric coaggregation occurs with over 90% of the more than 700 human oral bacterial strains that we have examined, intrageneric coaggregation appears to be considerably more restricted. Only members of the genus *Streptococcus* and a few strains of *Actinomyces* exhibited intrageneric coaggregation. Both of these genera are initial colonizers of a clean tooth surface and intrageneric coaggregation may be of considerable importance in establishing the pioneer microbial community on a nascent surface. The results of each of these investigative approaches are focused on understanding the molecular basis of cell surface recognitions among oral bacteria and their role in microbial ecology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00341-09 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of sugar transport and metabolism in lactic acid and oral bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thompson, John	Visiting Scientist	LME, NIDR
Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. The N(5)-(L-1-Carboxyethyl)-L-ornithine:NADP oxidoreductase gene has been cloned in the λZAPII vector. This enzyme, (also called N(5)carboxyethyl-ornithine synthase, EC 1.5.1.24), which mediates the biosynthesis of a family of previously unknown amino acids, has been expressed in *Escherichia coli*.

2. The gene for N(5)-carboxyethylornithine synthase has been sequenced, and the amino acid sequence of the enzyme has been determined.

3. It has been found that the N(5)-carboxyethylornithine synthase gene, and the genes encoding nisin synthesis and sucrose metabolism are linked and localized to a chromosomal transposon.

4. Polyclonal antibodies prepared against the purified enzyme have been used to survey for the distribution and constitutivity of the enzyme in lactic acid bacteria.

5. The mechanism(s) involved in the transport and dissimilation of fructose by the oral pathogen, *Fusobacterium nucleatum* have been elucidated. Mutants deficient in sugar transport systems have been isolated by UV mutagenesis, and the biochemical lesions have been identified by radiotracer techniques.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00382-07 LME

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Growth and interaction of oral microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robrish, Stanley A.	Research Microbiologist	LME, NIDR
Thompson, John	Visiting Scientist	LME, NIDR
Gomez, Irma M.	Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Microbial Ecology

SECTION: Microbiology Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.20	1.20	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fructose use by *F. nucleatum* (ATCC 10953) proved to be different than other sugar use by this organism and was studied in detail. In contrast to previous studies with glucose or galactose, good growth related to fructose use was found without additional amino acid supplementation to the medium. Anaerobic conditions and glutamate use were necessary for substantial incorporation of radiolabeled fructose into washed cell suspensions. The glutamate dependent radiolabeled fructose retained by washed cell suspensions was recovered as a polyglucose storage product whose properties were identical to that resulting from glucose or galactose use by this organism. In the absence of glutamate, fructose was fermented to D(-) lactate, acetate, and butyrate. The glutamate independent fructose metabolism could be aerobic, however, acetate was the only recoverable product. Analysis of the phosphorylated intermediates of the fructose fermentation revealed an Embden-Meyerhoff pathway but there was an accumulation of a hexose monophosphate intermediate by glutamate grown cells which proved to be fructose-1-phosphate (F1P). The presence of F1P and its associated kinase suggests that fructose enters *F. nucleatum* by a phosphorylation mechanism rather than as the free sugar. Sugar use by other fusobacteria: *F. mortiferum* (ATCC 25557) grew independently of glutamate but required sugar addition. Several other sugars, in addition to glucose could be used by this organism and inhibition of growth and glucose use was found by adding 2-fluoroglucose to cultures or washed suspensions of the organism. Accumulation of radiolabel from glucose could be demonstrated for this organism, however, the sugar accumulation was glutamate independent. *F. russii*, *F. varium*, and *F. gonidaformans* appear to have glutamate stimulated glucose accumulation similar to that demonstrated for *F. nucleatum*. No accumulation of glucose was found by washed cells of *F. perfoetens*, however, there is some evidence that glucose will stimulate growth of the organism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00454-04 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of surface molecules in metabolism and ecology of oral bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

London, Jack P.	Research Microbiologist	LME, NIDR
Allen, Janet	Microbiologist	LME, NIDR
Cavedon, Katherine	IRTA Fellow	LME, NIDR
Citron, Jean	NRSA Fellow	LME, NIDR
Kolenbrander, Paul E.	Research Microbiologist	LME, NIDR
Riley, Chiara	NRSA Fellow	LME, NIDR

COOPERATING UNITS *(if any)*
LC, NHLBI, NIH; LI, NIDR, NIH; University of Connecticut and Tel Aviv University, Tel-Aviv, Israel

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	3.25	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The binding specificities of the adhesive protein associated with the fimbriae of *Bacteroides loeschei* (now *Prevotella loeschei*) that mediates coaggregation with *Streptococcus oralis* and agglutination of RBCs has been further characterized. Studies with 125I-labeled adhesin established that its interaction with the streptococcal cell receptor is indeed a lectin-carbohydrate interaction readily reversed by amino sugars. Interaction with the carbohydrate receptor on *S. oralis*, prevents the streptococcus from coaggregating with its other partner cells, i.e., *Actinomyces naeslundii*, *Veillonella atypica* and *Streptococcus sanguis*. However, adhesion to RBCs or RBC ghosts appears to be a protein-protein interaction. The adhesin also binds to a number of eucaryotic structural proteins including laminin and fibronectin. Limited proteolysis studies indicate that the streptococcal-specific adhesin is antigenically related to the second adhesin which mediates coaggregation between *P. loeschei* and *Actinomyces israelii*.

Currently the gene encoding the streptococcal-specific adhesin is being sequenced to determine its structure; this was facilitated by obtaining an internal peptide sequence of the adhesive protein. A mRNA transcript of the gene was identified; from its size, 2.9 kb, it appears that, at least, one other protein is being synthesized concomitantly with the adhesin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00498-01 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Identification & enumeration of oral bacterial flora of HIV-1 infected subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

London, Jack P.	Research Microbiologist	LME, NIDR
Riley, Chiara	NRSA Fellow	LME, NIDR

COOPERATING UNITS *(if any)*
Department of Anaerobic Bacteriology, Virginia Polytechnic Institute and State University

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a). Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

A collaborative project between the Microbial Interactions Section and the Department of Anaerobic Bacteriology at Virginia Polytechnic Institute and State College was initiated April 1990. The subgingival floras of 20 patients exhibiting frank AIDS and 20 HIV seropositive patients suffering from gingivitis are being enumerated and identified to determine whether: (1) any changes in the normal flora occur following the viral infection; and (2) the gingivitis is associated with a typical gram negative flora, respectively. Twelve subjects, six from each category, have been examined thus far. Qualitatively, the microbial flora appears to be much the same as that seen in HIV uninfected individuals, with slightly more Gram positive bacteria present. However, both the Gram positive and Gram negative bacteria present do not exhibit the same growth characteristics observed previously. In general, they are more difficult to cultivate and show abnormally long lag periods, or refuse to grow at all, when transferred from medium to medium. Internal controls reveal that this is a reproducible trait of oral bacteria from HIV-infected individuals.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00512-01 LME

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Genetic analysis of Bordetella avium pathogenicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gentry-Weeks, Claudia R. Senior Staff Fellow LME, NIDR

COOPERATING UNITS *(if any)*: Roy Curtiss III, Washington University, St. Louis, MO

LAB/BRANCH: Laboratory of Microbial Ecology

SECTION: Toxin Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Five *Escherichia coli* recombinant clones have been identified which react with antibody against *Bordetella avium* outer membrane proteins and produce 21 kDa, 38 kDa, 40 kDa, 43 kDa, and 48 kDa proteins. In addition, one *E. coli* clone has been identified which produces a 56 kDa protein which reacts with turkey convalescent sera and three *E. coli* clones have been identified which react with human convalescent sera from patients infected with *Bordetella pertussis*. The 21 kDa outer membrane protein gene has been introduced into an avirulent *Salmonella typhimurium* strain to allow oral immunization of birds and to determine the role of the 21 kDa protein in protection from disease. Spontaneous phase variants of *B. avium* have been isolated which mimic the phase III variants of *B. pertussis* since they have lost the ability to produce dermonecrotic toxin and four outer membrane proteins. Passage of the *B. avium* spontaneous phase variants through birds results in reversion of the *B.avium* phase variants to the wild-type phenotype. Three *E. coli* clones have been identified which contain cosmid DNA which hybridizes to a radiolabelled oligonucleotide probe for *B. avium* dermonecrotic toxin. DNA sequence analysis is currently being performed to determine the DNA sequence of the dermonecrotic toxin gene and to deduce the amino acid sequence of *B. avium* dermonecrotic toxin.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00514-01 LME

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Anthrax toxin - a model for bacterial pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Leppla, Stephen H.	Research Chemist	LME, NIDR
Singh, Yogendra	NRC Research Associate	LME, NIDR
Klimpel, Kurt R.	NRC Research Associate	LME, NIDR
Quinn, Conrad P.	NRC Research Associate	LME, NIDR

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Microbial Ecology

SECTION: Toxin Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project uses biochemical, genetic, and cell culture methods to study the structure and function of protein toxins involved in bacterial pathogenesis. Current studies focus on the three protein components of anthrax toxin. Prior work defined three domains in the Protective Antigen protein, an N-terminal region needed only to maintain solubility, a C-terminal domain involved in receptor binding, and a central domain required for binding of the other two components, lethal factor (LF) and edema factor (EF). We have now used site specific mutagenesis to show that deletion of twelve amino acids from the C-terminus of PA destroys receptor-binding activity. Mutagenesis was also used to replace twelve selected serine or threonine residues in PA with cysteine. One cysteine mutant was found which has normal binding properties, but appears unable to translocate EF or LF to the cytosol of eukaryotic cells. All of these mutants were expressed in Bacillus species, and the secreted mutant proteins were purified from culture supernatants. LF has been mutagenized at 15 different sites with an oligonucleotide linker that inserts two or four amino acids. Insertions that inactivated the protein were clustered in the N-terminal region considered to be involved in binding to PA and in a C-terminal region of 150 amino acids considered to be the putative catalytic domain. The methods developed with anthrax toxin will later be applied to secreted proteins considered to be virulence factors of oral bacteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00518-01 LME

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of detoxified pertussis toxin for acellular whooping cough vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Keith, Jerry M.	Chief, Laboratory of Microbial Ecology	LME, NIDR
Brown, Douglas R.	Senior Staff Fellow	LME, NIDR
Vadheim, Kirsten	Microbiologist	LME, NIDR
Fields, Raymond	Chemist	LME, NIDR
Cureton, Charlette	Secretary	LME, NIDR
Gray, Monica C.	Clerk-Typist	LME, NIDR
Horne, Devonaline	Biological-Aid	LME, NIDR

COOPERATING UNITS *(if any)*
National Institute of Health, Tokyo, Japan

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Toxin Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.6	2.0	4.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Whooping cough is caused by an infection of the respiratory tract with *Bordetella pertussis* bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole *B. pertussis* cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is pertussis toxin. Clinical trials of acellular pertussis products strongly indicate that pertussis toxin will be a necessary and perhaps sufficient component of any new vaccine. Chemically "inactivated" pertussis toxin vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Through our gene expression experiments we discovered a molecular approach for inactivation of pertussis toxin. Using site-specific DNA mutagenesis, the S1 subunit was modified by a single amino acid substitution. This mutation virtually eliminated toxic activity, yet the immunogenic protective epitope was retained. Other double amino acid substitutions are being evaluated. We have devised several methods to transfer these genetic changes into the chromosome of *B. pertussis*. Using these new mutant strains, a genetically detoxified pertussis toxin molecule has been produced. This nontoxic holotoxin has strong immunoprotective properties and can be used as a vaccine antigen without chemical inactivation.

LABORATORY OF ORAL MEDICINE

The Laboratory of Oral Medicine continues to work on: (i) autoantibodies and their properties, with emphasis on the genes that code for them; (ii) the cloning, sequencing, and characterization of autoantigens; (iii) clinical trials on methods for reactivating and preventing the reactivation of herpes simplex virus; and (iv) development of transgenic mice to study the expression of a variety of genes, especially those of human immunodeficiency virus (HIV).

The Laboratory of Oral Medicine is involved in a number of collaborative projects including (1) preparation of human monoclonal antibodies (Cetus Immune Research Laboratories, Palo Alto, CA); (2) characterization of antibodies to human thyroglobulin and microsomes (Mt. Sinai School of Medicine, New York, NY); (3) study of human V_H and V_L genes (University of Texas Southwestern Medical School, Dallas, TX, and University of Washington School of Medicine, Seattle, WA); (4) human monoclonal antibody to viruses (The Wistar Institute, Philadelphia, PA); (5) production of human monoclonal autoantibodies (NIDR); (6) production of human monoclonal antibodies to HIV (NIAID); (7) development of HIV transgenic mice (NIAID); (8) development of transgenic mice containing CR2 cDNA (Washington University School of Medicine, St. Louis, MO); (9) development of transgenic mice with human cDNA for islet-associated polypeptide (University of Chicago, Chicago, IL); (10) characterization of the 70-kDa autoantigen (NIDDK); (11) studies using synthetic peptides (NIDR); (12) detection of HIV proviral sequences (NIDR); (13) clinical studies on factors that trigger and agents that inhibit reactivation of HSV (NIAID); (14) reactivation of HSV-1 by UV light and prevention by sunblocking agents (UCLA School of Medicine, Los Angeles, CA); (16) detection of HSV genome in biopsy specimens from patients with upper GI tract ulceration (Scripps Clinic and Research Foundation, La Jolla, CA, and National Navy Medical Center, Bethesda, MD); and (17) binding of HTLV-1 to various subsets of leukocytes (NCI).

Since last year, a number of new techniques were introduced into the laboratory and existing ones modified. These include: (1) construction of phagemid cDNA libraries; (2) isolation of tissue-specific cDNA by subtraction library; (3) symmetric, asymmetric and boost polymerase chain reaction for detection of HIV; (4) direct sequencing of DNA using Taq polymerase; (5) semi-dry gel electroblotting; (6) cloning of cDNA by PCR amplification; (7) development of PCR for detection of HSV in clinical specimens; (8) cloning and sequencing of human V_H and V_L genes; (9) targeted amplification by PCR of human germline Ig genes; (10) utilization of electrofusion for preparing human hybridomas; (11) maintenance of human PBMCs in SCID mice; (12) S1 nuclease analysis for the detection of CR2-specific transcripts; (13) use of anti-peptide antibodies to map exposed protein domains; (14) ELISA for antibodies reactive with SS-A and SS-B antigens; and (15) transfection of cells with cDNAs containing CMV, heat shock, metallothionine and Ig promoters.

Some of the more important findings since last year's Annual Report are summarized below.

Analysis of the Normal Human B Cell Repertoire

Recently, a fraction of human B lymphocytes from adult peripheral blood, lymph nodes, and tonsils have been shown to express at low density the surface CD5 molecule, the human equivalent of the mouse Ly-1 molecule. Using specific mouse monoclonal antibodies to CD5 and to human B lymphocytes in double fluorescence flow cytometry, we identified and quantitated $CD5^+$ B lymphocytes in peripheral blood and spleens from healthy adult subjects. Using in part the methodology we developed, $CD5^+$ B cells were segregated by cell sorting from their $CD5^-$ counterparts and infected with EBV to secrete Ig in limiting dilution condition. Culture fluids were investigated for Ig content and reactivity. It was found that the lymphocytes capable of producing autoantibodies similar to those found in at least two important human autoimmune diseases, SLE and rheumatoid arthritis (RA), consistently segregated with the $CD5^+$ B cell subset. More recently, we found by transformation with EBV and limiting dilution analysis that the majority of $CD5^+$ B cells from healthy subjects are indeed committed to the production of antibodies with rheumatoid factor-like activity. By fusion of EBV-transformed $CD5^+$ lymphocytes with a human-mouse heteromyeloma cells, we constructed continuous cell lines producing monoclonal antibodies. These antibodies bound not only to the Fc fragment of immunoglobulin G but also to other self-antigens, such as ssDNA, thyroglobulin, and insulin, as well as to biologically relevant exogenous antigens, such as bacterial constituents and products. Broad multireactivity is therefore an inherent property of the antibodies produced by $CD5^+$ B lymphocytes. The antigen-binding sites of these "autoantibodies" are the expression of gene segments in germline configuration. These immunoglobulins appear to be similar to the classical "natural antibodies" and may serve as the first line of defense against invading microorganisms.

Seven different human-human-mouse cell lines constructed using $CD5^+$ B cells and producing polyreactive mAbs (four IgM, one IgG, and two IgA) were characterized for the V_H gene segments utilized. It was found that three of these mAbs utilized V_HIII and three used V_HIV gene segments, respectively. Although limited in scope, these findings suggest a biased usage of selected V_H gene families by natural autoantibodies from $CD5^+$ B cells. Indeed, V_HI, V_HII, and V_HIII family members account for up to 90% of the genomic DNA repertoire and the V_HIV family comprised only nine members. Our studies also showed that the V_H segments utilized by the polyreactive antibodies from $CD5^+$ B cells are in nearly germline (unmutated) configuration.

Study of the D_H segments utilized by these polyreactive mAbs provided the evidence that a high heterogeneity may exist in the expression of these segments in the normal B cell repertoire. Five out of five of the cell clones producing "genuine" polyreactive antibodies expressed D_H, which were twice as long or almost twice as long as the human D_H segments characterized so far. Although we do not yet have any explanation for these findings, a number of combination or duplication mechanisms can be envisaged to account for the assembling of such D_H gene segments. We are at present continuing the characterization of these genes to be able to relate them to the D_H genes utilized by induced specific antibodies

produced during the course of an immune response to exogenous antigens and specific autoantibodies occurring in patients with autoimmune diseases.

Study of the Autoimmune B Cell Repertoire

Our B cell culture limiting dilution experiments have shown that in conditions of enhanced autoimmune response, such as in Hashimoto's disease, IDDM (newly diagnosed subjects) and SLE, the number of B cells capable of producing IgG to Tg, Ins, and ssDNA, respectively, is dramatically increased. Study of the properties of the monoclonal autoantibodies we generated from patients with such autoimmune diseases showed that these autoantibodies display a high specificity for the relevant self-antigens. However, Hashimoto's, IDDM, or SLE patients do not differ from healthy donors in the number of ($CD5^+$) B cells producing (polyreactive) IgM with similar self-antigen reactivities. These "autoantibodies" are not dissimilar to the polyreactive monoclonal autoantibodies generated from healthy subjects.

These findings suggest that at least two mechanisms contribute to the autoantibody production in most autoimmune diseases: (1) a polyclonal B cell activation, responsible for the production of polyreactive autoantibodies, and (2) an antigen-driven process of clonal selection, possibly involving accumulation of a considerable load of somatic point mutations, and yielding monoreactive high affinity autoantibodies. This (self) antigen-driven selection mimics that found in the response to exogenous antigens, e.g., tetanus toxoid, in the same autoimmune patients, as well as healthy subjects. In fact, similar to that found for IgG anti-tetanus toxoid antibodies induced by vaccination, IgG autoantibodies found in patients with Hashimoto's disease, IDDM, or SLE display a high affinity for the relevant antigen, are monoreactive, and are produced by $CD5^-$, not $CD5^+$, B cells (normal in number in these patients).

The characterization of the V_H and V_L genes of three polyreactive low affinity and three monoreactive high affinity IgM and IgA1 mAbs generated using circulating $CD5^+$ B cells from a single rheumatoid arthritis patient showed that RFs displayed a restricted utilization of V_HIV and V_λ, mainly V_λI, gene segments and differed, therefore, from the monoclonal IgM antibodies found in mixed cryoglobulinemia and Waldenstrom's macroglobulinemia, thus far the only available prototypic RFs. The V_H genes of the two polyreactive low affinity IgM RFs were in germline configuration. The V_H-D_H-J_H and V_λ-J_λ nucleotide sequences of the two monoreactive high affinity IgM RFs were identical, suggesting a common clonal progenitor for their producing cells. The V_H genes expressed by these cells displayed a number of amino acid replacements in the CDRs, as determined by the analysis of the corresponding genomic segment, in a way that suggests an Ig-receptor-dependent selection of such mutations, possibly driven by Ag. Due to the unavailability of the corresponding genomic V_H segments, we could not establish whether the two IgA1 RFs utilized V_H genes in a somatically point-mutated configuration, as suggested by the high degree of nucleotide differences in their CDRs when compared to their closest known germline sequences. Thus, our findings show that at least some $CD5^+$ B lymphocytes are antibody-producing cells capable of undergoing somatic point mutation and affinity maturation processes. The sequences of the genes encoding the H chain CDR3 (D_H segments) revealed that all three

polyreactive low affinity RFs displayed a much longer D_H segment than their monoreactive high affinity counterparts, raising the possibility that a long D_H segment may be one of the factors involved in antibody reactivity. These experiments suggest that a restriction in light chain isotype and V_H gene segment utilization may exist in both polyreactive low affinity and monoreactive high affinity RFs in RA patients. These findings suggest that $CD5^+$ B lymphocytes are cells capable of expressing somatically mutated V_H Ig genes and of undergoing at least some "maturative" process in ways similar to that of conventional $CD5^-$ B cells.

Study of a Prototype Antibody Response to Exogenous Antigens in Humans

The study of the immune response to rabies virus in human subjects who had never been previously exposed to this virus provided with a unique prototype model for the dissection of the kinetics of recruitment of specific B lymphocytes by an exogenous antigen. These studies established that: (1) the B cells recruited during a primary antibody response are committed to the production of polyreactive antibodies, mainly of the IgM class; (2) these B cells are surface $CD5^+$; (3) the B cells recruited after a secondary or tertiary immunization belong to the $CD5^-$ compartment and produce antibodies of the IgG or IgA class; (4) the antibodies produced by the "secondary" and "tertiary" B cells are monoreactive and specific to the inducing antigen. The difference in affinity between the primary polyreactive antibodies and secondary monoreactive antibodies can be as much as 1,000 times. The studies of the V gene segments utilized by the polyreactive low affinity and monoreactive high affinity anti-rabies virus monoclonal antibodies suggested that these two antibody populations are the products of two discrete B cell subrepertoires. These findings provide important clues to the understanding of the molecular and cellular mechanisms underlying the human antibody response to exogenous and, possibly, self-antigens.

We cloned and sequenced the genes encoding the V_H segments of two polyreactive low affinity IgM and three monoreactive high affinity IgG mAbs to rabies virus. We found that the two IgM mAbs utilized members of the V_HIIIa family and the three IgG mAbs utilized members of the V_HI, V_HIIIa, and V_HIV families. The expressed V_H gene segments displayed an 89-97% similarity to known genomic F_H sequences. The D_H gene segments of these mAbs were heterogeneous in length and did not allow us to make any conclusive correlation between their length and antibody poly- or monoreactivity. We are now addressing the issue as to whether the V_H gene segments of monoreactive high affinity antibodies display any degree of somatic mutation. To this end we have just begun to establish the condition for the "targeted" PCR amplification of the germline V_H genes putatively corresponding to the expressed V_H segments in the same subjects who provided the B cells used for the generation of the mAbs.

One of the high affinity human monoclonal antibodies to rabies virus we generated efficiently neutralized the virus *in vitro* and *in vivo*. This antibody may have immediate clinical application in the post-exposure treatment of human rabies and open a new perspective on the generation and therapeutical use of human monoclonal antibodies in diseases.

Autoantigens

Recently, we isolated a novel cDNA by screening human expression libraries with autoantibodies. The cDNA we isolated encodes the 70-kDa component of the Ku autoantigen. Ku is a complex of 70-kDa and 86-kDa proteins found in the nucleus of human cells where it binds DNA. While its function is unknown, Ku is the target of autoantibodies in several autoimmune conditions, including systemic lupus erythematosus and Graves' disease. The expression of the 70-kDa Ku protein could help in elucidating its normal cellular function as well as providing an important reagent for testing sera from patients with autoimmune diseases. Attempts to express the 70-kDa Ku component in a number of bacterial and mammalian expression systems have been largely unsuccessful, perhaps due to the presence of amino acid sequences, which result in rapid protein degradation. However, we now have successfully expressed this protein using a recombinant baculovirus vector. Mammalian proteins expressed by this method are very similar or identical to their native counterparts in terms of post-translational modifications (with the exception of complex N-glycosylation), intracellular targeting, antigenicity, and functional capability.

Expression of the 70-kDa protein in the absence of the 86-kDa Ku component reveals that it has a nuclear targeting signal and is associated with the nuclear matrix. The other properties of the expressed protein, notably its high affinity for DNA, are similar to those of the protein purified from human cells. Our study suggests that the baculovirus expression system may be of considerable value for the production and characterization of human autoantigens. We have recently published these experiments.

Cell Surface Expression of the Ku Autoantigen

Ku is a complex of 70-kDa and 86-kDa proteins found in the nucleus of human cells where it binds DNA. Ku is the target of autoantibodies in several autoimmune conditions, including systemic lupus erythematosus and Graves' disease. We have now conclusively demonstrated, using a variety of techniques, that both the 70-kDa and 86-kDa components of Ku are present in small amounts on the cell surface. It is not yet clear if the proteins are present as a complex in the cell membrane. The proteins are not removed by washing cell membranes extensively with buffers containing 0.5 M NaCl, suggesting that they are integral membrane proteins. Extending these studies, we have used antibodies to synthetic peptides, derived from the 70-kDa protein sequence, to analyze the domains of this protein exposed to the cell surface. By indirect immunofluorescence microscopy and fluorescein-activated cell sorting, we demonstrate that this autoantigen is exposed on the cell surface. In addition, we have identified several domains of the protein that are exposed. Our study provides one of the first demonstrations of a eucaryotic, nuclear DNA-binding protein in the cell membrane. These studies suggest that the 70-kDa protein might play a broader role in autoimmunity than previously thought. It is interesting to note that many other human autoantigens (e.g., Ro, La, Sm, PCNA, nuclear RNPs) are nuclear proteins. However, it has not been clear how such nuclear antigens, which are apparently inaccessible to the immune system, could be targets of an autoimmune attack. The demonstration that the 70-kDa protein is on the cell surface

might explain how an autoimmune response to a "nuclear protein" could adversely affect an intact living cell.

Analysis of DNA Binding Domains of the 70-kDa Ku Autoantigen

To identify the domains of the 70-kDa proteins that bind DNA, we prepared 17 different peptides based on the 70-kDa protein sequence and studied their ability to bind human total genomic DNA. The peptides were chosen based on their charge as well as proximity to the putative leucine zipper regions of the protein. By slot blot analysis, one peptide of 15 amino acids (peptide 25A) bound DNA well and with very high affinity. Another peptide (peptide 4) bound DNA weakly. Several other peptides, including some that overlapped part or all of peptide 25A, demonstrated little or no binding. Peptide 25A was able to inhibit the binding of the recombinant 70-kDa protein to DNA on DNA-cellulose.

Peptide 4 is similar to other DNA-binding domains in that it contains a number of basic amino acids and is contiguous with one of the leucine zipper regions on the protein. However, peptide 25A is not like any other previously identified DNA binding domains. The use of short peptides to identify DNA binding domains may have broad applicability to other DNA binding proteins.

New Approach for Treatment of Herpes Simplex Virus

Efforts to manage infection caused by HSV have focused on the use of antiviral agents to treat or suppress established infections and on the development of a vaccine to prevent initial infections. Over the last couple of years, we have focused on a third approach that might be applicable to selected patients with frequent recurrences. If one could identify factors that trigger reactivation of HSV, it might be possible to reduce the frequency of recurrence by avoiding the triggering stimuli, by utilizing specific agents to block the stimuli, or by administering a short course of antiviral agents prior to the onset of the stimuli. Such an approach is most likely to succeed when the specific stimulus is well defined and can be anticipated.

In humans, ultraviolet (UV) B light, a spectral component of sunlight, induces reactivation of HSV-1 and 2 infections in patients with a history of cutaneous recurrences. Reactivation rates of 27-62% occur following experimental UV exposure. We have attempted to extend these findings and determine whether acyclovir, an antiviral drug known to be effective in the treatment and suppression of spontaneous perigenital herpetic recurrences, would prevent UV light-induced reactivation of HSV-2 infection.

The first phase of this work has now been completed and submitted for publication. We found that 54% of patients exposed for the first time to four minimal erythema doses of UV light at areas of previous HSV-2 recurrence develop site-specific reactivations within 7 days, with a mean time to recurrence (±SEM) of 5.0 ± 0.6 days. In contrast, reactivation did not occur in patients given acyclovir, 200 mg PO five times daily for 6 days, beginning 1 day prior to the first UV exposure. It is concluded that the UV-light model is useful for

examining the events leading to herpetic recurrences in humans and for evaluating the efficacy of new therapeutic agents. The data also add support to the idea that a short course of prophylactic acyclovir is useful in preventing sunlight-induced reactivation of HSV.

Human Immunodeficiency Virus in Saliva

HIV-1 is found in most body fluids. Some studies, but not others, have reported the presence of HIV-1 in saliva. Although anecdotal reports of HIV-1 transmission by saliva have appeared, large epidemiologic studies have failed to demonstrate transmission of the virus by the salivary route to health-care workers or household members exposed to HIV-1-infected patients.

HIV-1 exists in cells as a DNA provirus. Over the last year we used the polymerase chain reaction (PCR) to probe for HIV-1 proviral DNA sequences in saliva. Single samples of saliva collected from 20 HIV-1 seropositive patients were tested by PCR for HIV-1 proviral sequences using primers from the LTR, *gag*, and *env* regions of the virus. Proviral sequences were detected in the saliva of 50% of the patients. Sequential samples of saliva collected at four different times from each of six additional patients led to the detection of proviral sequences in 100% of the patients. Since, however, the detection of HIV-1 required not only the highly sensitive polymerase chain reaction but also multiple samples, it appears that under ordinary circumstances, infected cells are present in saliva in low numbers. Although this may explain the lack of transmission of HIV-1 by the salivary route to household members and health-care workers, the presence of infected cells in the saliva of a high percentage of patients argues for avoidance of situations (e.g., deep-mouth kissing) involving prolonged and intimate contact with saliva.

Human Monoclonal Antibodies to HIV

Human monoclonal antibodies might be useful in the diagnosis and treatment of HIV infection. Over the last year, efforts have been made to develop human monoclonal antibodies to HIV gp160 antigen. This has resulted in the establishment of a human hybridoma producing IgG3 antibody-specific to gp160 protein. The epitope is located on the portion of gp160 which corresponds to the gp41 antigen. This monoclonal does not neutralize HIV virus but reacts with the surface of HIV-infected cells. The antibody is highly specific and does not recognize the epitope on MHC class II antigen that is cross-reactive with gp41. These and other antibodies are now being evaluated.

HIV and Transgenic Mice

It is becoming clear that many patients infected with HIV-1 develop a nephropathy and exhibit focal glomerulosclerosis. Over the last year we made transgenic mice that carry a truncated HIV proviral DNA fragment that encodes for several HIV genes. Three of these mouse lines developed renal disease characterized by focal and segmental glomerulosclerosis, proteinuria, and premature death. The mechanism by which the HIV genes cause renal

disease is now under study. The similarities between HIV-associated renal pathology in transgenic mice and in patients makes this an interesting model for further study.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE00421-05 LOM

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Herpes Simplex Virus and Persistent Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James F. Rooney	Special Expert	LOM, NIDR
Others:	Abner L. Notkins	Medical Director	LOM, NIDR

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LAB/BRANCH
Laboratory of Oral Medicine

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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued studies utilizing ultraviolet light (UV) induced reactivation of herpes simplex virus (HSV) infections in humans. Further analysis of data from a study of acyclovir for the prevention of UV light-induced reactivation of HSV-2 infections has revealed that UV is a potent stimulus to reactivation, inducing HSV-2 infections following 54% of first exposures to UV light. The stimulus appeared to induce recurrences primarily at the site of UV exposure with an average time to recurrence of 4.8 ± 0.3 days (mean ± SE). Treatment with acyclovir markedly reduced the rate of reactivation, suggesting that selected patients may benefit from taking acyclovir to prevent HSV recurrences during periods of high risk of reactivation. These results demonstrate the utility of the UV model for studying the pathogenesis of HSV reactivation and assessing antiviral efficacy. Studies to determine the efficacy of sunblocking agents in the prevention of UV light-induced reactivation of herpes labialis have recently been completed and data is presently being analyzed.

Collaborative studies with Dr. Yvonne Bryson at the UCLA Medical School, Los Angeles, have been underway to assess the ability of various epithelial irritants to produce reactivation of perigenital HSV-2 infections. Application of 0.3% anthralin/acetone under occlusion produced an erythema of comparable duration and intensity to that following UV light exposure, but the rate of HSV reactivation was only 10% as compared to a rate of 40% following UV light exposure. However, repeated application of tape to the skin produced mild erythema and a rate of reactivation of 46% demonstrating for the first time that minor trauma to the skin can produce recurrent HSV lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00423-05 LOM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning, Expression and Characterization of Human Autoantigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ku is a complex of 70kDa and 86kDa proteins, found in the nucleus of human cells where it binds DNA and is the target of autoantibodies in several autoimmune conditions. We have now demonstrated that both the 70kDa and 86kDa components of Ku are present in small amounts on the cell surface and that they are integral membrane proteins. By use of antibodies to synthetic peptides, derived from the 70kDa protein sequence and fluorescein-activated cell sorting we demonstrated that several different domains are exposed on the cell surface. Synthetic peptides were used to identify the domains of the 70kDa protein which bind DNA. By slot blot analysis one peptide of 15 amino acids (peptide 25A) bound DNA with very high affinity and was able to inhibit the binding of the recombinant 70kDa protein to DNA. Another peptide (peptide 4) although contains a number of basic amino acids and is contiguous with one of the leucine zipper regions on the protein bound DNA weakly. The use of short peptides to identify DNA binding domains is original and may have broad applicability to other DNA binding proteins. Efforts are underway to understand the normal cellular function of this protein.

Studies are continuing on cloning of autoantigens involved in diabetes mellitus. Two cDNA clones, (which are preferentially expressed in human insulinoma), were isolated from an insulinoma - glucagonoma subtraction library. One clone, IG-3, detected a transcript of 2.4 kb in human insulinoma tissue. Another cDNA clone IG-20 detected a 5.0 kb transcript in human insulinoma, and at a lower level in the brain. Partial DNA sequence analysis of this clone revealed no homology to known gene sequences and thus represents a unique gene. Studies are underway to characterize these genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00467-03 LOM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human B Cell Repertoire and Autoantibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

EBV transformation in conjunction with limiting dilution culture and somatic hybridization techniques have been used to establish cell lines capable of making human mAbs to a number of self-antigens, e.g., ssDNA, thyroglobulin, insulin, IgG, Fc fragment and exogenous antigens, e.g., tetanus toxoid. These technologies have been applied to the study of the human B cell repertoire in patients with SLE, rheumatoid arthritis, Hashimoto's disease and insulin-dependent diabetes mellitus. B lymphocytes producing two groups of autoantibodies were detected in these patients. The first one includes mAbs binding to multiple self and exogenous antigens, in general, with low affinity. These polyreactive antibodies can also be detected in healthy subjects and are produced by $CD5^+$ B cells. The second one includes high affinity monoreactive autoantibodies and is found only in autoimmune patients. In other experiments, we generated human mAbs to rabies virus in subjects previously vaccinated with the appropriate virus-inactivated vacine, as well as monoreactive rheumatoid factor mAbs from a single patient with rheumatoid arthritis. Five of these mAbs displayed a high binding affinity for rabies virus components and one neutralized the virus. The gene segments coding for the V region of polyreactive antibodies have been sequenced and found to be in germline configuration. In contrast, the gene encoding the valuable regions of the monoreactive monoclonal RF autoantibodies, as well as the high affinity mAbs to rabies virus displayed a number of somatic mutations consistent with a process of antigen-driven clonal selection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00471-03 LOM

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Transgenic Mice as a Model for Studies of AIDS and Other Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreducad type. Do not exceed the space provided.)*

Several lines of transgenic mice have been produced which carry a functional HIV-1 envelope gene construct. These lines display a kidney pathology characterized by focal, segmented glomerulosclerosis and proteinuria resulting in premature death. These mice provide an animal model for HIV-associated renal pathology which has been well documented in humans infected with HIV-1.

In order to develop alternative diagnostic and therapeutic approaches for the treatment of HIV, human monoclonal antibodies to the HIV gp-160 antigen have been produced in this laboratory. One specific human hybridoma which produces an IgG3 antibody specific for gp-160 and whose epitope corresponds to the gp-41 antigen has been newly characterized. It binds to the surface of HIV-infected cells, does not neutralize HIV virus, and binds in a specific manner to an epitope distinct from the gp-41 cross-reactive epitope on MHC class II antigens. Further studies on this and other monoclonal antibodies are being pursued.

Ongoing work involving autoimmune reponses in mice transgenic for herpes virus glycoprotein D have indicated the presence of message specific for gD as early as day 8 of gestation in mice negative for anti-gD antibodies upon challenge with recombinant vaccinia gD and partially tolerant to challenge with HSV. The onset and cell type specific expression of gD during development is currently being studied in situ.

We have initiated the development of transgenic mice which carry the gene for the human complement component C3d (the EBV receptor in human B lymphocytes). Three founder mice have been established which carry a single copy of the transgene. These mice will be bred and subsequently infected with EBV in order to obtain a murine model in which to study EBV-mediated transformation in lymphoid cells.

BONE RESEARCH BRANCH

The Bone Research Branch encompasses programs in cell biology, molecular biology, protein biochemistry and molecular biophysics. Its central focus is on the structure, metabolism and pathology of bone, cartilage and related connective tissues. A number of significant research advances were achieved this year. These are detailed below.

MINERAL CHEMISTRY AND STRUCTURE SECTION

In the Mineral Chemistry and Structure Section (Edward D. Eanes, Chief), artificial lipid vesicles (liposomes) continued to be used as *in vitro* models for studying mineral deposition processes in matrix vesicles, the nidi for initial calcification in some skeletal and dental tissues. It was previously found that high levels of cholesterol (>35%) in the lipidic membrane of liposomes could effectively inhibit calcium phosphate precipitation in their aqueous interiors by blocking transmembrane Ca fluxes necessary to initiate and sustain this precipitation. Studies conducted during FY90 showed that this interference with Ca transport was a general cholesterol effect, independent of the types of phospholipid constituents making up the rest of the lipid membrane of the liposomes studied. Likewise, it was found that membrane cholesterol did not mollify the specific effects these phospholipids had on precipitation. For example, the retarding effect of phosphatidic acid and phosphatidylserine on extraliposomal precipitation was not affected by the level of cholesterol in the membrane. These results suggest that it may be possible to infer the general effect of certain classes of lipidic membrane components on matrix vesicle calcification from liposome studies, despite chemical disparities between the two systems.

A collaborative study with V. Hascall of the Proteoglycan Chemistry Section on the role of proteoglycans (PG) and their constituent parts in biomineralization was continued during FY90. The liposome system was used to examine the possible effect PG may have on the expansion of mineral from matrix vesicle nidi in an extracellular matrix rich in this constituent. Previously it was found that the glycosaminoglycan (GAG) component of intact PG was principally responsible for impeding extravesicular apatite formation in the model liposome system. However, from work done during the current FY, it was found that other regions of the PG molecule, in addition to the GAG domains, may posses potential inhibitory activity, and that this activity may not become fully expressed unless the GAG chains are enzymatically removed from the PG molecule. Some of this non-GAG activity appears to be associated with oligosaccharides N-linked to the PG core protein. These results suggest that enzymatic breakdown of PG *in vivo* may not lessen its control on mineral growth unless all constituent parts of the fragmented molecule are removed from the growth sites. Another finding of possible technical importance was that PG prepared from cartilage tissue by

standard extraction and purification procedures often contain impurities which must be removed in order to properly assess the crystal growth inhibitory effect of PG itself. Two interfering substances commonly associated with PG preparations in this regard were found to be cesium ions and ribonucleic acid.

Collaborative studies with investigators from the American Dental Association and Dental/Medical Materials groups at the National Institute of Standards and Technology continue to be an important part of the Section's research effort. Among the collaborative studies initiated during FY90 was an effort to develop new organo-metallic methacrylate monomers for use as radiopacifying resins in dental composite applications. Another new collaborative study was undertaken to chemically and structurally characterize by infrared, Raman and x-ray diffraction methods calcium phosphate coats formed by plasma fusion on titanium implants. Such plasma coated materials are potentially more biocompatible than pure titanium implants, but knowledge of the chemical and physical composition of these implant coatings is important to establish preparative methods that will optimize the *in vivo* bonding between the coatings and the surrounding bone tissue matrix.

PROTEIN BIOPHYSICS SECTION

Work during the past year in this Section, headed by Dr. Dennis A. Torchia, continues to focus upon studies of protein structure and dynamics using NMR, with the purpose of attaining a better understanding of function. Two proteins, staphylococcal nuclease and III^{Glc}, an essential phosphocarrier and regulatory protein of the E. coli phosphotransferase system, PTS, are currently under study.

Although nearly all backbone and many sidechain NMR signals of S. nuclease had been assigned using 2D NMR techniques, severe signal overlap precluded assigning most of the signals of the large functionally important sidechains such as Lys, Arg and Glu. We have recently assigned the sidechain signals of these residues by applying newly developed 3D NMR experiments to a uniformly ^{13}C enriched protein sample. This development opens the way for detailed studies of structure and dynamics at the enzyme surface particularly at the active site. These studies are of particular importance because present evidence suggests that the structure of the active site observed in the crystal differs from the structure in solution.

Comparisons of the structures of wild type and mutant forms of S. nuclease are also underway. The mutation E 43 D causes a 10^3 fold reduction in catalytic activity. Although E 43 is thought to function as a general basis in catalysis, it is difficult to see why D 43 cannot do the same unless the mutation also causes structural perturbations. We are currently analyzing 3D NOESY spectra of the wild type and mutant enzymes to see if a structural basis for the change in activity can be identified.

A second mutant of interest is Δ (44-49) in which six residues of the disordered omega loop in the native enzyme have been diluted. This mutant is considerably more stable than the wild type enzyme but its activity is 50 fold less. This observation supports the hypothesis

that the flexibility of the omega loop enhances activity by allowing the enzyme to adjust its conformation to accommodate the shape of the substrate. We are using 3D NOESY spectra to check the hypothesis that the enhanced stability of the mutant is a consequence of the replacement of the omega-loop by a beta-turn.

Encouraged by the progress that has been made with S. nuclease, work has begun to determine the three-dimensional solution structure of III^{Glc}, an essential PTS phosphotransferase and regulatory protein whose structure is unknown. Collection of the various #-D NMR spectra of ^{15}N and $^{15}N/^{13}C$ uniformly labeled proteins is underway. Analysis of these spectra will provide signal assignments and these assignments together with NOESY data will be used to derive information about structure. The ultimate goal of this project is to relate the three dimensional structure of III^{Glc} to the various functions of the protein.

PROTEOGLYCAN CHEMISTRY SECTION

The Proteoglycan Chemistry Section continues to conduct research on the structure, metabolism and function of proteoglycans in a variety of biological models. These range from projects on intracellular proteoglycans (those found in the storage granules of hematopoietic cells such as the human HL-60 and murine monocytic leukemic M1 cell lines) to projects on cell surface proteoglycans (those on rat ovarian granulosa cells and a rat parathyroid cell line) to projects on extracellular matrix proteoglycans (those in the corneal stroma, cartilage matrix and the intercellular matrix of the cumulus cell-oocyte complex). The following highlight results in several of these projects.

A proteoglycan, referred to as serglycin, is synthesized selectively by cells of hematopoietic origin and concentrated in storage granules. This proteoglycan has a small core protein, ~ 20 kDa, with an embedded serine-glycine repeat sequence that serves as the attachment site for the multiple glycosaminoglycan chains on the mature proteoglycan. In a murine leukemic cell line, this proteoglycan contains 3-5 chondroitin sulfate chains and an overall molecular weight of ~135 kDa. Its synthesis is stimulated 4-5 fold without significant changes in structure when the cells are induced to differentiate into macrophages. Pulse-chase kinetics clearly distinguish two pools; one that is secreted rapidly from the cell after an ~1 hour lag, and a second that is stored and catabolized within the cell with a halflife of ~3.5 hour, (McQuillan et al., J. Biol. Chem. 264:13245-13251, 1989). The structure of the related proteoglycan in human HL-60 promyelocytic cells was similar as was its metabolic fate (Lohmander et al., J. Biol. Chem. 265:5802-5808, 1990).

The heparan sulfate proteoglycans synthesized by ovarian granulosa cells were shown to be associated with the plasma membrane by two different mechanisms. Phospholipase C, which specifically cleaves phosphatidylinositol membrane anchors, released ~25% of the heparan sulfate proteoglycans from the cell surface indicating that this proportion is bound to the plasma membrane via the fatty acyl groups on this membrane anchor. A photoactivatable, lipophilic reagent was used to tag plasma membrane bound molecules. The reagent

covalently bound to the cell surface heparan sulfate proteoglycans, but not to the dermatan sulfate proteoglycans synthesized by these cells. Approximately 30% of the marker on the purified heparan sulfate proteoglycans was released by phospholipase C. The results indicate that 65-70% of the heparan sulfate proteoglycans are intercalated into the plasma membrane, presumably via a membrane spanning hydrophobic polypeptide. Some evidence was found suggesting that the core proteins for the two membrane associated forms of proteoglycan are different (Yanagishita and McQuillan, J. Biol. Chem. 264:17551-17558, 1989).

The cell surface heparan sulfate proteoglycan synthesized by a rat parathyroid cell line has a structure similar to that for the intercalated form found on the ovarian granulosa cells. However, the proportion of the proteoglycan on the cell surface depends directly on the concentration of Ca^{2+} ion in the medium. At physiological Ca^{2+} levels almost all of these proteoglycans are sequestered in an intracellular (endosome) compartment, while at ~5% of physiological Ca^{2+} they redistribute between the endosome and cell surface and cycle between these two compartments with a t(1/2) of ~10 min per cycle. Pulse-chase experiments indicate that newly synthesized cell surface heparan sulfate proteoglycans enter into the endosome (physiological Ca^{2+}) or endosome/cell surface (low Ca^{2+}) compartments ~20 min after completion in the Golgi complex, and after ~4 hour enter into a lysosomal compartment where they are totally degraded. These proteoglycans, then, are probably localized in membrane compartments involved in the selective recognition of Ca^{2+} concentration, the main effector molecule for these cells (Takeuchi et al., J. Biol. Chem. in press).

Cumulus cells in cumulus cell-oocyte complexes initiate synthesis and deposition of an extracellular matrix in the preovulatory follicle during the 5-10 hours after the oocyte resumes meiosis in response to a gonadotropin surge. The process results in expansion of the complex, and it occurs *in vitro* when the isolated complexes are incubated in medium with follicle stimulating hormone and serum. Double labeling methods with ^{35}S sulfate and ^{3}H glucosamine were developed to monitor hyaluronic acid and proteoglycan synthesis during complex expansion *in vitro*. The major structural component of the extracellular matrix is hyaluronic acid. Experiments showed that the oocyte produces a soluble factor(s) which is necessary to induce hyaluronic acid synthesis, that the follicle stimulating hormone acts synergistically with the oocyte factor(s) during this stimulation, and that a serum factor(s) is necessary for the retention of the newly synthesized hyaluronic acid in the extracellular matrix and hence for successful expansion of the complex (Salustri et al. J., Biol. Chem. 264:13840-13847, 1989; Salustri et al., Dev. Biol. 138:26-32, 1990).

Experiments were initiated to study proteoglycans synthesized by a rat osteosarcoma cell line. However, a sulfated glycoprotein co-purified with the proteoglycans. This glycoprotein was purified and identified as bone sialoprotein. ^{35}S Sulfate and ^{3}H glucosamine or ^{3}H tyrosine were used to label the sialoprotein metabolically. Structural analyses revealed that almost half of the tyrosine residues are sulfated, with most of these localized to the carboxyl terminal half of the molecule in a region surrounding a cell attachment RGD amino acid sequence. Additionally each of the 3 N-linked oligosaccharides contains ~1 sulfate ester as do ~2 of the ~25 O-linked oligosaccharides on each molecule (Midura et al., J. Biol. Chem. 265:5285-5291, 1990).

Corneal buttons obtained after keratoplasty of patients diagnosed as having type 1 or 2 macular corneal dystrophy were cultured with ^{14}C leucine, ^{3}H glucosamine or ^{3}H mannose. Proteoglycans and glycoproteins were isolated and characterized. Type 1 corneas synthesize a normal dermatan sulfate proteoglycan, but an abnormal keratan sulfate proteoglycan in which the lactosaminoglycan chains are not sulfated to form normal keratan sulfate. This is consistent with the current hypothesis that such patients have a defect in a sulfotransferase required to sulfate lactosaminoglycans. The type 2 cornea studied synthesized a normal keratan sulfate proteoglycan, but an abnormal dermatan sulfate proteoglycan in which the dermatan sulfate chain was significantly shorter than normal. Thus, distinctly different defects in proteoglycan metabolism can yield similar patterns of the matrix disorganization characteristic of this corneal dystrophy (Midura et al., J. Biol. Chem. in press).

The core protein precursor (~210,000 molecular weight) of the larger cartilage proteoglycan was isolated from rat chondrosarcoma chondrocyte cultures metabolically labeled with ^{3}H serine. Serine residues substituted with carbohydrate structures were converted to alanine residues by a β-elimination mechanism in alkaline-borohydride. Unsubstituted serines are not reactive. The conversion of ^{3}H serine to ^{3}H alanine for the core protein precursor was less than 15% of the value expected if all the serine residues were substituted with xylose, the initiating sugar for the addition of the chondroitin sulfate chains. The result substantiates our previous kinetic studies which indicate that the addition of xylose occurs late in the intracellular lifetime of the core protein precursor, probably in an early Golgi compartment, and not as others have suggested cotranslationally in the rough endoplasmic reticulum (Lohmander et al., J. Biol. Chem. 264:18775-18780, 1989).

SKELETAL BIOLOGY SECTION

The members of the Bone Cell Biochemistry group, under the direction of Dr. Pamela Gehron Robey, are continuing to define parameters of osteoblastic metabolism, and examine the effect that variables such as animal species, developmental and cultural age, cell cycle and transformation exert on the phenotypic expression, with a particular emphasis on the synthesis of extracellular matrix. In order to compare the matrix secretion of cells *in vitro* to osteoblasts *in vivo*, the expression of several of the bone matrix proteins was examined by *in situ* hybridization and immunohistochemistry in developing human tissue. It was found that while osteonectin is transiently expressed in a number of basement membrane-producing tissues, it is found constitutively only in bone, and in the salivary and distal renal tubule epithelium. Synthesis of osteonectin (rather than adsorption from the circulation) was demonstrated in a renal tubule epithelial cell line ($LLCPK_1$) *in vitro*. In comparing the two small proteoglycans, decorin was found in most soft connective tissues, with a somewhat uniform distribution in developing bone, whereas biglycan was found primarily at cell surfaces in areas active in morphogenesis and in bone, in the osteoblastic layer and osteocytic lacunae. The sialoproteins, osteopontin and bone sialoprotein, had a more limited distribution. Osteopontin was localized in a number of tissues and in certain osteoblasts and mononuclear cells near the mineralized surface, as well as in other tissues. Bone sialoprotein was found

only in osteoblasts, trophoblasts of the pregnant uterus, at low levels in cartilage, and in osteoclasts.

The expression of hyaluronan and the four proteoglycans synthesized by this cell type was defined, and changes in their levels studied as a function of time (and matrix deposition) in culture. While the heparan sulfate proteoglycan and the small proteoglycan, decorin, remained relatively constant, the other small proteoglycan, biglycan, dramatically increased with extended time (and to a lesser extent, the large "space capturing" proteoglycan also increased), pointing to a role for biglycan at this stage of matrix formation. While studying the cell cycle in osteoblastic cells, it was found that the maximal proteoglycan synthesis peaks earlier in G_1 than most other proteins, and that the expression of alkaline phosphatase activity on the surface of the cells is cell cycle dependent, reaching maximal levels during S phase, followed by shedding into the medium during G_2 and M phases.

Since the nature of mineralization in monolayer cultures is focal, and not uniform across the dish, alternative methods of cell culture were investigated. In cultures where cell attachment is inhibited, aggregates formed that produced an extracellular matrix with a different composition compared to monolayer matrix. In tissue culture insert experiments, it was found that osteoblasts exhibit vectorial secretion. However, in both cases, the extent of mineralization was not increased over monolayer cultures.

Bone metabolism is influenced by an ever increasing number of hormones and factors, and the effects of several of these were studied using the bone cell culture system. Several early studies have touted fluoride as a possible treatment of osteoporosis. However, in this model system, fluoride, either by itself or in combination with other factors and varying levels of serum, had no effect on bone cell proliferation or on protein synthesis. This data indicates that any beneficial effect on fluoride is mediated via another cell type, or in combination with factors not yet identified. Bone cells have also been shown to contain estrogen receptors, and estrogen has been shown to be effective when administered to postmenopausal women with osteoporosis. *In vitro*, estrogen did not stimulate proliferation, but did increase the level of mRNA for alpha 1 of type I collagen between 30-40 hours following addition.

Changes in the biosynthetic capacity of bone-forming cells can result in the formation of abnormal bone as exemplified by the genetically acquired brittle bone disease, Osteogenesis Imperfecta. In initial studies of native bone, it was determined that the levels of several non-collagenous proteins were either abnormally high or low, and the hydroxyapatite crystals in young patients are smaller. The biosynthetic pattern of bone cells derived from patients with Osteogenesis Imperfecta is also deranged, independent of a detectable collagen defect. In another genetic disease, Turner's syndrome, affected individuals are females that are deficient in one X chromosome (X), and are of extremely short stature. Recently it has been determined that the gene for biglycan is located on the X chromosome. Studies of fibroblasts from these patients, and from patients with higher X values indicate that the level of biglycan is directly proportional to the number of X's, and suggests that the regions controlling gene activity are located on the short arms of the X and Y chromosomes.

Finally, assays have been developed to address function of bone matrix proteins. Specifically, cell attachment assays have indicated that bone matrix contains four RGD (the cell attachment consensus sequence) proteins. However, while fibronectin and thrombospondin mediate adhesion, neither support long term spreading of adult human bone cells, whereas osteopontin and bone sialoprotein are active even for extended periods of time under serum-free conditions. Interestingly, a fragment of BSP that does not contain RGD can support cell attachment, and cells attached to this fragment can not be displaced by RGD in the medium. Another approach to determine function of specific proteins was developed using phosphorothioate-derivative oligo DNA with sequences complimentary (antisense) to the region surrounding the start codon in mRNAs for several proteins. It was found that TGF-beta antisense inhibited DNA synthesis in adult human bone cells, suggesting that it is an autocrine growth factor for these cells. Using an osteonectin specific antisense, it was found that only a small proportion of total osteonectin synthesis was inhibited, which indicates that there may be multiple forms of osteonectin synthesized by bone cells.

In the Molecular Biology Group, headed by Dr. Marian F. Young, the structure and expression of several bone and tooth matrix genes was investigated by isolating cDNA and genomic DNA encoding the proteins. In addition, a previously unknown gene was isolated that appears to be related to osteopontin that we have called osteopontin 2. The discovery of alternatively processed forms of OP1 along with a second gene, OP2, indicates that osteopontin may be part of a multigene family. The differential expression of bone sialoprotein, biglycan and osteopontin was observed in a novel, *in vitro* model of bone cell differentiation. The genes for biglycan and bone sialoprotein were isolated and the intron exon organization determined by a combination of polymerase chain reaction amplification and DNA sequencing. The genes are relatively small and simple in structure and contain 5′ flanking DNA that is currently being characterized for functional activity. Analysis of the *cis* acting elements that regulate the osteonectin gene indicate that multiple intragenic and extragenic elements control its cell specific expression. Potentially regulatory, *trans* acting elements were identified that interact specifically with the osteonectin promoter one of which appears to be related to the transcription factor AP2. cDNA was isolated from an ameloblast library that encodes a protein with the size and character of enamelin. Antibodies to the gene specifically immunoreact to ameloblasts in intact tissue and to "tuft" preparations indicating the clone is specific for this unique structure in developing teeth. Amelogenin cDNA clones have been used to determine that 1) the gene is potentially expressed from both the X and Y chromosomes and 2) that the heterogeneity of the amelogenin protein is likely to be generated from the differential processing of mRNA from one genomic locus.

The Protein Biochemistry Group, under the direction of Dr. Larry W. Fisher and in collaboration with the Molecular Biology Group, have completed the cloning and sequencing of several human bone protein cDNAs and one genomic clone. Osteopontin la cDNA sequence corresponded to our previously described bone protein but a related cDNA, osteopontin 2, discovered during the cloning of OP la, has not yet been described as a functional protein of bone cells. The cDNA sequence of the human bone sialoprotein has given us insight into the possible functional roles that this protein may play in bone cell-matrix interactions and has lead to a series of cell attachment experiments. The human gene

for the small proteoglycan, biglycan, has now been shown to contain 8 exons, including one in the 5′ flanking region. The promoter region of the gene was typically GC-rich and contained SP-1 sites but lacked any CAT or TATA boxes. This group was the first to isolate large quantities of nondenatured bone sialoprotein and bioactive fragments. We have also begun to purify nondenatured BSP containing different post-translational modifications (including expression in bacterial cells that result in no modifications) to determine the effects of these modifications on bio-activity and a variety of physical-chemical measurements. We have also continued to develop a variety of useful antisera to both the collagen and noncollagenous proteins of human bone. The Protein Biochemistry Group has been instrumental in a number of the experiments described above for the Molecular Biology and Cell Biology Groups.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00012-28 BRB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

B.O. Fowler Research Chemist BRB, NIDR

COOPERATING UNITS (if any)

ADAHF, NIST, Gaithersburg, MD
NIST, Gaithersburg, MD

LAB/BRANCH: Bone Research Branch

SECTION: Mineral Chemistry and Structure Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (e.g., hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependence and polarization) are then utilized to establish composition and structural details of the apatites in question which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00074-18 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L.W. Fisher	Research Chemist	BRB, NIDR
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G.R. Hawkins	Chemist	BRB, NIDR

COOPERATING UNITS *(if any)*

SIU, School of Dentistry, Edwardsville, IL
University of New Mexico, Albuquerque, NM

LAB/BRANCH
Bone Research Branch

SECTION
Skeletal Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.20	1.70	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The extracellular matrix proteins of the bones and teeth are key elements in the structure and metabolism of these tissues. The goal of this project is to study matrix proteins specific to each mineralizing skeletal tissue in order to understand their molecular structure and biological function.

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binging, RIA, ELISA, etc.) have been developed to quantitate the levels of noncollagenous proteins in bone including osteonectin, bone sialoprotein, osteopontin, bone proteoglycans BGN and DCN, dentin phosphophoryn and the N-propeptide and C-propeptide of type 1(I) collagen in (a) surgical specimens of bony tissue and (b) serum (osteonectin). Changes in the noncollagenous protein profile with age and variety of bone (and tooth) diseases have been observed in man and several animal models. We have been highly successful at producing antisera against synthetic peptides for all of the human bone noncollagenous proteins. These antisera have proven useful in immuno precipitation studies, immunolocalization, immunodetection and on Western blots. We have successfully cloned and sequenced human bone proteoglycans I (biglycan) and II (decorin), sialoprotein (BSP) and two members of the osteopontin family (OPN-1 and OPN-2). The human biglycan gene has been cloned and sequenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00088-17 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical, Structural and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

E.D. Eanes	Chief, MCSS	BRB, NIDR
D. Skrtic	Visiting Fellow	BRB, NIDR
A.W. Hailer	Chemist	BRB, NIDR
V.C. Hascall	Chief, PCS	BRB, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Bone Research Branch

SECTION
Mineral Chemistry and Structure

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	2.00	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium phosphate salts provide the hardness and rigidity which uniquely characterize normal, healthy bone and teeth. Developmental defects in the deposition of these salts or their destruction and loss by disease can severely impair the function of these skeletal tissues. The purpose of this project is to study the physical, chemical, and ultrastructural properties of these salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable these salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. The principal endeavor currently being pursued is the use of artificial lipid vesicles (i.e., liposomes) as in vitro models for investigating the physico-chemical aspects of calcium phosphate precipitate formation in matrix vesicles. The liposome experiments are being conducted with the goal of better understanding how matrix vesicles, the loci for early mineralization in many vertebrate hard tissues, can initiate precipitation in their membrane-bound interior spaces and control the expansion of this initial precipitate into the surrounding extracellular space. Present findings show (1) that the ability of high membrane cholesterol levels to interfere with transmembrane Ca fluxes necessary for initiating intraliposomal precipitation is independent of the phospholipid composition of the membrane, and (2) that the action of extraliposomal proteoglycans to control precipitate expansion involves both oligosaccharide as well as glycosaminoglycan components.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00134-16 BRB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Structure and Biosynthesis of Proteoglycans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

V.C. Hascall	Chief, PCS,	BRB, NIDR
M. Yanagishita	Visiting Scientist	BRB, NIDR
R. Midura	Staff Fellow	BRB, NIDR
A. Calabro	Guest Worker/Biologist	BRB, NIDR
S. Shibata	Guest Worker	BRB, NIDR
A.M. Nahir	Guest Worker	BRB, NIDR

COOPERATING UNITS (if any)

Rush-Presbyterian-St. Luke's Medical Center; Univ. of Lund, Sweden; University of North Carolina; University of Michigan

LAB/BRANCH: Bone Research Branch

SECTION: Proteoglycan Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.00	5.00	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the project is to study the chemical and physical properties and metabolism of proteoglycans in a number of tissue and cell systems. Topics of present interest include: 1) Protein chemistry and immunology of the core protein of proteoglycans from the Swarm rat chondrosarcoma; 2) Biosynthesis of core protein precursors and processing to mature proteoglycans; 3) Effects of growth factors (insulin-like growth factor 1) on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilages; 4) keratan sulfate-proteoglycan and dermatan sulfate-proteoglycan in chick, cat and human cornea; 5) analyses of sulfated oligosaccharides on proteoglycans and sialoproteins in bone and cartilage tumor cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00157-15 BRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Studies of the Structure, Dynamics and Function of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

D.A. Torchia	Chief, Protein Biophysics Section	BRB, NIDR
S.W. Sparks	Staff Fellow	BRB, NIDR
H.B.R. Cole	Staff Fellow	BRB, NIDR
D.M. Baldisseri	IRTA Fellow	BRB, NIDR
J.G. Pelton	Staff Fellow	BRB, NIDR

COOPERATING UNITS *(if any)*

LCP, NIDDK, NIH; University of Maryland; Johns Hopkins University

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	3.00	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Protein structure and dynamics were investigated in solution and in the crystalline state using nuclear magnetic resonance spectroscopy in order to understand function. Studies were carried out on (a) staphylococcal nuclease, a calcium dependent phosphodiesterase and (b) III^{Glc} the phosphate carrier protein component of the bacterial PTS system.

A. S. nuclease We have succeeded in assigning nearly all proton, carbon and nitrogen NMR signals of the S. nuclease/pdTp/Ca^{2+} ternary complex by combining isotopic enrichment with sophisticated two- and three-dimensional NMR techniques. These assignments together with NOE and relaxation measurements show that the enzyme backbone structure in solution is similar to that in the crystalline state except in the omega loop and at the active site, both of which are more flexible than in the crystalline state. The flexibility in these regions provide an attractive explanation of the diversity of substraits hydrolyzed by the enzyme.

Current work is focused upon studies of the interaction of Ca with the enzyme and upon relating changes in sequence with changes in structure and function.

B. III^{Glc} We are determining the three dimensional solution structure of III^{Glc} in order to provide a basis for understanding the phosphate transporting and regulatory functions of this protein. A variety of high-quality heteronuclear edited three-dimensional NMR spectra have been obtained and are currently being analyzed to obtain signal assignments. These assignments together with heteronuclear 3D NOESY spectra will then be used to derive the protein structure.

The significance of the project is the development of techniques that provide protein structures in solution that will permit one to obtain a rational basis for understanding function in terms of structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00379-07 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Bone Matrix Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M.F. Young	Research Biologist	BRB, NIDR
J.D. Termine	Chief	BRB, NIDR
J.M. Kerr	IRTA	BRB, NIDR
K. Ibaraki	Visiting Associate	BRB, NIDR
A.M. Heegaard	Visiting Fellow	BRB, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Bone Research Branch

SECTION
Skeletal Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.85	3.60	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective in this investigation is to study the structure and function of these macromolecules and to understand the regulation of their expression.

The structure of the bone and tooth matrix proteins has been studied by constructing recombinant cDNA libraries from bone or ameloblast cell mRNA. cDNA encoding several bone and tooth matrix proteins was isolated using expression vectors and monospecific antisera directed against individual bone and ameloblast proteins. The clones were used to determine the primary structure and mode of expression of the genes in cultured cells and in intact tissue. The corresponding genomic DNA has also been isolated and used to determine the intron-exon organization of these genes and the elements that potentially regulate their expression during development.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00380-07 BRB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Metabolism of Bone Cells in Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. Gehron Robey	Biologist	BRB, NIDR
J.D. Termine	Chief	BRB, NIDR
N.S. Fedarko	IRTA	BRB, NIDR
J.B. Kopp	Staff Fellow	BRB, NIDR
T.E. Hefferan	Biol. Lab Technician	BRB, NIDR
R.J. Martin	Summer Research Fellow	BRB, NIDR
U.K. Vetter	Visiting Associate	BRB, NIDR
W.J. Grzesik	Visiting Associate	BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Bone Research Branch

SECTION
Skeletal Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.45	3.95	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bone cells derived from a variety of animal species including human, bovine, ovine and rodent, and of varying developmental ages, have been utilized to: 1) study the biosynthesis and deposition of extracellular matrix proteins such as collagen, osteonectin, bone proteoglycans and other bone proteins, and alterations of matrix production in the disease Osteogenesis Imperfecta; 2) study the responsiveness of the cells to a variety of hormonal and pharacoloical factors (such as estrogen and fluoride); 3) elucidate the production and interaction of growth factors (such as TGF-ß, insulin-like growth factors and platelet-derived growth factors); 4) study the potential function of bone matrix proteins through the use of functional assays and the use of anti-sense DNA to inhibit specific protein synthesis; and 5) serve as a source of mRNA and DNA for studies of the proteins at the genomic level.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00431-04 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Proteoglycans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. Yanagishita	Visiting Scientist	BRB, NIDR
V.C. Hascall	Chief, PCS	BRB, NIDR
Y. Takeuchi	Visiting Fellow	BRB, NIDR
D. McQuillan	Visiting Associate	BRB, NIDR
L. Masi	Visiting Fellow	BRB, NIDR
D. Hiscock	Visiting Fellow	BRB, NIDR

COOPERATING UNITS *(if any)*

Second University of Rome, Italy; University of Lund, Sweden;
K. Sakaguchi, NIDDK, NIH

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.33	2.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to study biochemical and physical properties, biological function and metabolism of proteoglycans under physiological and various pathological conditions using a number of tissues and cell systems. Topics of present interest include: (1) analysis of proteoglycans in a parathyroid cell line; (2) analysis of plasma membrane-associated heparan sulfate proteoglycans in rat ovarian granulosa cells; (3) analysis of proteoglycans and hyaluronic acid in the mouse cumulus cell-oocyte complex; and (4) analysis of proteoglycans in a human leukemic cell line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00507-01 BRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NMR Studies of the Structure and Interactions of an HIV-Inhibitor Complex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

D.A. Torchia	Chief, Protein Biophysics Section	BRB, NIDR
D.M. Baldisseri	IRTA Fellow	BRB, NIDR

COOPERATING UNITS *(if any)*
LCP, NIDDK, NIH

LAB/BRANCH
Bone Research Branch

SECTION
Protein Biophysics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	.50	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to develop NMR methods to study the dynamics and structure of HIV and related proteins in the crystalline state and in solution. The purpose of this work is to obtain a better understanding of the structure-function relationship. In a specific application, we will determine the structure of an HIV protease-inhibitor complex. Our ultimate goal is to use the knowledge of structure to develop a rational design of inhibitors of the HIV protease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00510-01 BRB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Regulation of Cartilage Matrix Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T.I. Morales	Senior Staff Fellow	BRB, NIDR
K.D. Smith	Biologist	BRB, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Chemoprevention, NCI
Laboratory of Pathology, NCI
The Orthopedic Laboratories, NIAMS

LAB/BRANCH: Bone Research Branch

SECTION: Proteoglycan Chemistry Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	1.00	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The long term objective of this program is to elucidate the intrinsic regulatory mechanisms that control the structure and function of the articular cartilage that normally protects and cushions underlying bones. We recently showed that TGF-ß has the ability to prevent the spontaneous proteoglycan loss that occurs in basal cartilage organ cultures. Our present efforts are directed at 1) gaining a precise understanding of the effects of exogenous TGF-ß administration on the cartilage matrix structure and 2) understanding the endogenous physiology of the TGF-ß isoforms in articular cartilage in order to determine their role in the control of metabolic homeostasis of the tissue.

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

The Clinical Investigations and Patient Care Branch (CIPCB) functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinical and basic research programs; (2) to offer consultation on oral and dental problems to other institutes and render clinical care to specified patients; (3) to encourage and facilitate clinical research activities of other branches and laboratories within the Institute; and (4) to sponsor an oral medicine training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research-oriented dental clinicians.

This past year has seen continued significant progress in both our basic laboratory and clinical research programs. The basic laboratory research effort remains focused on understanding the regulation of glandular epithelial cell secretory processes (i.e. ion fluxes and fluid movement, gene expression), while the clinical research efforts remain directed at four areas: salivary gland hypofunction, management problems posed by patients with congenital and acquired dental/skeletal disorders, aging and oral physiology, and oral manifestations of AIDS.

PATIENT CARE AND CLINICAL STUDIES SECTION

The primary responsibility of the Patient Care and Clinical Studies Section (PCCSS) is to conduct the daily operation of the NIDR Dental Clinic and, as such, it is the focus of clinical oral and dental health concerns at NIH. This year A.D. Guckes took over as Chief, PCCSS from M.W. Roberts, who retired from the USPHS after 8 years with the CIPCB. The Section provides a wide range of diagnostic and consultative services to NIH clinical care and research programs, integrating oral health care concerns to total patient management.

PCCSS staff members continue their deep commitment to our oral medicine training program, the Dental Staff Fellowship. They are primarily responsible for the clinical training, and introduction to clinical research, of the Dental Staff Fellows. Scheduled rounds, a Fellowship lecture series, oral medicine seminar series and a journal review are conducted weekly throughout the academic year. The lecture and seminar series brings in speakers from outside the NIH as well as from other NIH programs.

The PCCSS has markedly increased its research efforts. This situation, which represents a marked change from that present when the CIPCB was reorganized in 1982, is a result of having new senior attending staff with committed research interests in addition to clinical care goals. As noted above, a major area of investigation is the management of patients with acquired and congenital dental and skeletal disorders. The PCCSS over the past few years

has conducted clinical studies examining (i) oral physiological performance following orthognathic surgery and (ii) the utilization of titanium dental implants in patients with unusual hereditary disorders of mineralized tissues. These long-term studies have now begun to yield results. For example, it is well-known that smooth and rapid anterior and superior hyoid motion is essential for swallowing. Muscles from the hyoid attach to the mandible and support the tongue base. Any surgically (or otherwise)-induced change in the mandible may, therefore, alter such musculoskeletal relations and affect tongue-hyoid and mandibular activity needed to achieve a swallow. The CIPCB has previously pioneered efforts to study the oral-pharyngeal phases of swallowing using ultrasound imaging. We have employed this approach, in conjunction with conventional oral-motor, speech, dental and cephalometric X-ray examinations, to examine the effects of orthognathic surgery on mandibular-hyoid relationships during swallow. Fourteen subjects having either mandibular advancement or set-back procedures have been evaluated before surgery, and at 3, 6 and 12 month intervals thereafter. The major adjustment to new mandibular-hyoid positioning occurred within the first 3 months. However, functional changes, particularly in hyoid elevation times and oral swallow with only endogenous secretions (dry swallow), continued throughout the 1 year post-surgical period. Other PCCSS studies originally focused on the rigorous longitudinal evaluation of oral function and patient acceptance of dentures supported by titanium dental implants versus conventional dentures. These have evolved into an effort to understand the biology of the bone-implant interface. This directional change in part came about from an off-shoot of the original study in which the use of titanium implants was studied in patients with ectodermal dysplasia. The apparent (because data are still being collected and analyzed) success of implants in these patients led to their use in a patient with Erdheim-Chester disease, a rare histiocytosis also known as lipoid granulomatosis. Endosseous titanium implants were placed in the patient's anterior mandible with a guarded prognosis for integration of the fixtures due to fatty degeneration of the bone marrow. Of five implants placed all were successful prompting inquiry as to the nature of the biologic events occurring in tissues adjacent to newly placed implants. We have just initiated what we hope will be a successful experimental collaboration between members of the CIPCB, the Bone Biology Section, and the Mineralized Tissue Branch, NIDR, to examine these events.

Another major, long-term area of emphasis within the Section involves studies of oral physiological status during normal aging. We now have seen more than 650 normal volunteers from the National Institute on Aging's (NIA) Baltimore Longitudinal Study of Aging (BLSA) since we initiated the oral physiological component. Each volunteer is subjected to a detailed evaluation of salivary gland, oral motor and oral sensory performance, and oral mucosal status. We have extended these aging studies by using a cohort of healthy, non-medicated men and women seen in a cross-sectional aging program operated by the NIA at the NIH in Bethesda. These individuals represent a control group for a NIA study on dementia of the Alzheimer type (DAT). As reported in the past year, we have also begun to study oral function in patients with DAT. More than 125 subjects were seen this year in the BLSA, including 42 who were seen ~10 years ago by CIPCB staff. Thus we have begun the first-stage of our longitudinal evaluations of oral health and function. We have, also, begun to study subjects with certain well-maintained medical conditions. In the past, our primary focus has been on healthy, non-medicated persons of different ages so that we could measure

oral physiology uncomplicated by frank disease or therapy. This approach was exceedingly important for it allowed us to understand what normal oral function is across the lifespan and, thus, to recognize age-altered and disease-altered, performance. Our initial effort studying subjects with a single well-maintained medical condition is focused on diabetes.

Because little is understood about the relationship between menopause, hormonal replacement and oral function, we have, also, examined the effects of menopausal status and estrogen therapy on subjective reports of oral dryness and objective major salivary gland output in 43 healthy pre- and post-menopausal women. With the exception of post-menopausal females on hormone replacement therapy, no subjects were utilizing prescription medications. In this population no complaints of xerostomia or burning mouth were present, nor were differences observed in saliva production between pre- and post-menopausal subjects. The results suggest that menopause or hormone replacement therapy per se does not affect salivary gland function among healthy women.

It is well-accepted that saliva is important for the preservation and maintenance of oral health. Indeed, much of the CIPCB research effort is directed at salivary gland function for this reason. As part of our aging studies, we have attempted to ask how much saliva is required to maintain normal oral function. Major salivary gland flow rates, objective measures of oral health and subjective complaints of oral problems were assessed in 102 different-aged, healthy, non-medicated persons. Despite a great variation in major salivary gland flow rates observed, the oral health of all individuals studied was similar and generally good. It appears that in healthy persons, normal oral health was supported by a wide range of salivary fluid output. Analysis of the results suggests that the comparison of major salivary gland flow rates of an individual to population standards, to identify patients susceptible to the effects of salivary dysfunction, will be unreliable. Due to the wide range of normal performance, it is likely that changes in salivary function in an individual over time are a more meaningful gauge of the impact of saliva on oral health. It is, therefore, preferable that clinicians, as part of routine oral examinations, assess salivary function in order to monitor an individual's saliva production and identify patients with declining salivary gland output. This would allow appropriate intervention to limit or prevent the deleterious consequences of salivary gland dysfunction.

The last major area of PCCSS research involves studies of oral considerations in, and oral manifestations of, HIV-1 infection. These studies involve considerable collaboration with the Clinical Investigations Section (CIS) and have primarily focused on saliva and salivary glands. For example, we have previously reported that saliva contains a factor which inhibits the ability of lymphotropic HIV-1 to infect normal peripheral blood lymphocytes. CIPCB staff, in collaboration with the Laboratory of Immunology, NIDR, have further examined this phenomenon. By rigorous testing, using multiple different experimental approaches, these workers have confirmed and extended our earlier observations, including demonstrating that saliva was capable of preventing the infection of human monocytes by monocyte-tropic virus. In other earlier studies with AIDS patients, we demonstrated that soon after HIV-1 infection, asymptomatic generally-healthy individuals exhibit elevated levels of anti-microbial components in saliva. These observations have been extended through a longitudinal study

with 12 HIV-1 positive males seen over a median time interval of 14.5 months. Over this period these patients did not change their health or medication-use status. In general, the salivary proteins lactoferrin, IgA and lysozyme remained elevated, and parotid secretions, which at earlier visits were relatively unaffected, began to show changes seen previously in submandibular saliva.

We also have examined a subset of AIDS patients who develop enlargement of the major salivary glands and have complaints of xerostomia (dry mouth). Histopathological examination of their labial minor salivary glands reveal changes similar to those seen in Sjögren's syndrome. However, the distribution of lymphocyte subsets infiltrating the gland and the peripheral blood profile are different from those seen in autoimmune-mediated Sjögren's syndrome. We have compared parotid gland sialochemical parameters of patients with HIV-associated salivary gland disease (HIV-SGD) with those of controls (HIV seropositive and seronegative males) and patients with primary Sjögren's syndrome. The HIV-SGD patients had a number of sialochemical alterations which were similar to the Sjögren's syndrome patients but were less pronounced. IgA, lactoferrin and lysozyme were elevated compared to HIV seropositive controls. IgA rheumatoid factor was elevated as well, but was less than found in Sjögren's syndrome patients. Overall, the changes in the HIV-SGD group were similar to, but not as dramatic as, those seen in Sjögren's syndrome. The increases in IgA and IgA rheumatoid factor suggest that the parotid glands of patients with HIV-SGD are a site of active local immunoglobulin production.

CLINICAL INVESTIGATIONS SECTION

This principal focus of the CIS has been and remains understanding the etiology of, and developing treatments for, specific salivary gland secretory dysfunctional states and associated oral disorders. The central CIS activity remains the dry mouth (xerostomia) clinic, in which we have now enrolled more than 700 patients. Considerable progress continues in our efforts to develop more specific diagnostic and treatment approaches for patients with dysfunctional salivary glands. About 40 new individuals per year are now admitted as inpatients for intensive study under the protocol "Evaluation and treatment of salivary gland dysfunction". Approximately 60 persons per year are studied as outpatients. We, also, have continued treatment protocols for patients with salivary dysfunction which were begun previously. These include the oral use of the parasympathomimetic drug pilocarpine in patients post-head and neck irradiation and in patients with Sjögren's syndrome, as well as the use of anti-inflammatory drugs (steroid, non-steroid) for the latter patient group. Pilocarpine remains an extremely effective therapy for patients with some remaining functional gland parenchyma. Results from studies with anti-inflammatory drugs continue to be collected and the full double-blind code has yet to be broken. Analysis on their effectiveness hopefully will be completed during this year.

A major diagnostic tool which we have employed is salivary scintigraphy with 99m technetium petechnetate. A major general limitation to the routine use of this technique has been (i) a lack of well-defined normal values for various elements of the scan and (ii) a clear

demonstration of the relationship between gland saliva output and technetium handling. Accordingly, 5 CIS staff members independently examined scintiscans of 33 healthy, non-medicated individuals with no evidence of salivary gland dysfunction. Normal values (±2SD) were determined for each of the following elements of the scans: time of initial uptake and maximum concentration of radionuclide, time of unstimulated appearance of tracer in the oral cavity, and response to gustatory stimulus. These values were then formulated into a rating scale which was used to evaluate scans from 22 patients with dry mouth complaints. A very good correlation (~0.7) was achieved between scan ratings of the 5 examiners and salivary function. This rating scale should increase the utility of scintigraphy in the evaluation of the xerostomic patient.

We have also begun to study lymphocyte subsets which infiltrate the labial minor salivary glands of patients with Sjögren's syndrome. Using immunohistochemical and in situ hybridization procedures, we observed that the majority of T-cells in focal lymphocytic infiltrates bear the helper (CD4) phenotype and are activated since they express the class II (HLA-DR) MHC products. The majority (77%) of the infiltrating T-cells exhibit the memory helper/inducer phenotype (UCHL-1) and express LFA-1 molecules. Additionally, most T-cells express the $\alpha\beta$ receptor while only ~3% express the $\Gamma\delta$ receptor. These findings strongly suggest that T-cell infiltrates in Sjögren's syndrome patients may be responsible for the B-lymphocyte hyper-reactivity observed in their exocrine glands. In collaboration with CIPCB laboratory staff, the CIS also observed that the proto-oncogene c-myc was detected primarily in ductal elements of minor glands from primary Sjögren's syndrome patients. Lymphocytes infiltrating the glands did not show such reactivity. This finding was strongly correlated with the disease duration and age of the patient. Importantly, similar findings were not seen with gland samples from patients with either rheumatoid arthritis or sarcoidosis, or with normal controls.

We have previously documented age-related declines in taste threshold sensitivity and the perception of taste intensity. During this year CIS investigators have begun to apply their expertise to sensory problems of patients with salivary gland dysfunctions. We have shown that both Sjögren's syndrome patients and individuals who have undergone irradiation of the head and neck exhibit taste performance differences, relative to unaffected controls, that are in the same direction as those between older and younger healthy adults. Average taste detection threshold sensitivity for each of the four basic taste qualities declined significantly for all patients compared to healthy, age-matched controls. Although these average differences were in the same direction as those between younger and older individuals, they affected all qualities whereas the age-related sensitivity changes were unique, i.e. quality specific. We have also previously shown that the stability of taste intensity judgements across repeated presentations of the same stimulus is reduced with age. Repeatability deficits, like threshold deficits, are more frequent among patients than age-matched controls and occur in a distinctive pattern. As with threshold sensitivity deficits, salivary dysfunction patients show multi-quality alterations rather than single quality changes. These findings suggest that the mechanisms responsible for the taste deficits associated with Sjögren's syndrome or irradiation are different from those observed in a healthy, aging population.

A major strength of our Branch is the linkage of our laboratory and clinical components. Because of (i) our location, within the NIH Clinical Center, (ii) our mixture of basic scientists and clinicians, and (iii) the close physical approximation between our various groups, we are able to enhance each other's research efforts and help to fulfill our mission. This is especially obvious in the many interactions which occur between Branch basic scientists and CIS investigators (noted above). Another example is the considerable progress which has been achieved between CIS staff and members of the Membrane Biology Section to utilize microfluorometry to assess neurotransmitter signal transduction pathways in labial minor salivary glands of Sjögren's syndrome patients. We are now able routinely to disperse glands obtained from biopsy, affix them to coverslips, and monitor Ca^{2+} mobilizing responses to various neurotransmitter agonists in minor gland acinar and ductal elements which have been loaded with fura-2. Similar approaches with model glands (rat submandibular and parotid, see below) suggest that studying the regulation of pH and membrane potential in these cells is feasible. This "functional diagnostic" approach should allow the detection of specific alterations in signaling pathways which result in secretory hypofunction and, by extension, this should lead to the development of more rational forms of corrective therapy.

MEMBRANE BIOLOGY SECTION

The Membrane Biology Section (MBS) has as its major focus understanding the ion transport events involved in the formation of saliva. It is well accepted that saliva has a critical role in the defense, and functional maintenance, of all oral tissues. Saliva contains water and electrolytes, derived from serum, and specific exocrine proteins synthesized by glandular epithelial cells. Salivary glands are useful models of secretory processes and studies with these glands have proved important to our understanding of basic concepts of secretion and to appreciating pathogenesis in conditions such as cystic fibrosis.

Recent studies have indicated that fluid secretion in many exocrine glands is related to transepithelial anion movements. It has been suggested that a model first proposed by Silva et al. for the shark rectal gland applies to a number of these tissues. In this model, four plasma membrane ion transport systems act in concert to move Cl^-, and ultimately fluid from the interstitial space to the luminal space in response to secretory stimuli. These transport systems are (i) an electroneutral, loop diuretic-sensitive Na/K/Cl cotransporter located in the basolateral membrane of the secretory cell, (ii) a basolateral K^+ channel, (iii) an apical Cl^- channel, and (iv) the Na/K ATPase. According to this model the electrochemical gradient for Na^+ generated by the Na/K ATPase causes Cl^- to be driven into the secretory cell against its electrochemical gradient via the basolateral cotransporter. Stimulation of the gland results in the opening of the basolateral K^+ channel and the apical Cl^- channel. These increases in basolateral K^+ conductance and apical Cl^- conductance allow K^+ and Cl^- to flow out of the cell down their electrochemical gradients resulting in an accumulation of Cl^- ions in the lumen. Na^+ is thought to follow Cl^- by leaking through the tight junctions between the cells in order to preserve electroneutrality. The resulting osmotic gradient for NaCl causes a net transepithelial movement of water from interstitium to lumen.

Previous studies by the MBS have contributed much evidence supporting this model in salivary glands. MBS staff members have demonstrated the presence of a Na/K/Cl cotransporter in rat and rabbit parotid basolateral membrane vesicles and, in parallel studies, showed that this transporter was a major Cl^- entry pathway in both resting rat parotid acini and in acini stimulated with the muscarinic agonist carbachol. Other experiments indicated that Cl^- loss in response to carbachol stimulation was inhibited by the anion channel blocker diphenylamine-2-carboxylate, consistent with the proposed existence of an apical Cl^- channel. In additional studies, evidence was uncovered for the existence of Cl/HCO_3 and Na/H exchangers in parotid basolateral membrane vesicles, suggesting these transporters may play a role in the fluid secretion process. Subsequent studies showed ~25% of transepithelial cell Cl^-flux involved in fluid secretion was due to the Cl/HCO_3 exchanger. Further experiments showed that parotid acinar cells also secrete HCO_3^- in response to muscarinic stimulation and that this HCO_3^- secretion is driven by Na/H exchange. The Na/H exchanger also is responsible for intracellular pH buffering during secretion. During this past year considerable effort has focused on this exchanger, using the pH-sensitive fluorescent dye BCECF. Muscarinic stimulation results in two distinct types of upregulation of the Na/H exchanger. One is a relatively long-term effect (requiring ~10 min) and we previously have reported the characterization of this effect. This year our studies focused on the second type of upregulation, a short-term effect (within 30 sec of stimulation) which is independent of extracellular Ca^{2+} and requires an order of magnitude lower carbachol than the long-term effect (0.3 versus 3.0 µM). This second effect is not blocked by protein kinase or calmodulin inhibitors, but is mimicked by the Ca^{2+} ionophore ionomycin. Stimulation of Na/H exchange is also observed when acini are exposed to a hypertonic shock (100mM sucrose). Since acinar shrinkage follows muscarinic stimulation (due to net Ca^{2+}-dependent KCl loss), comparative studies of the upregulatory events and shrinkage phenomenon were conducted. All activities were blunted by the K^+ channel blocker charybdotoxin. Also, the shrinkage phenomena showed a comparable agonist dose response curve for short-term Na/H exchanger stimulation. It, thus, appears that both events are related.

In parallel with these studies, MBS staff members have continued the detailed characterization of the parotid Na/K/Cl cotransporter. The primary aim here was to understand the nature of the biochemical, biophysical and molecular processes involved in the functioning of this important membrane transport system. We have previously demonstrated that this transporter is inactivated by the sulfhydryl reagent N-ethylmaleimide (NEM) and can be protected against NEM by the specific Na/K/Cl cotransport inhibitor bumetanide. Using this information MBS investigators attempted to label the cotransporter with ^{14}C-NEM. The resulting ^{14}C-labeled membranes were then solubilized and analyzed by SDS-PAGE and autoradiography. Autoradiographs showed several ^{14}C-NEM labeled bands of equal density in both bumetanide-pretreated and control lanes. However, a single band at 160 kDa was markedly denser (more heavily labeled) in the bumetanide-pretreated material. The labeling of this bumetanide protectable NEM reactive site was also consistent with the known specificity of bumetanide binding to the cotransporter. Thus, when bumetanide pretreatment was carried out under conditions where bumetanide does not bind to the cotransporter (i.e. chloride-free medium), no enhanced labeling of the 160 kDa band was observed. When ^{14}C-NEM labeled membranes were treated with endoglycosidase F (to remove sugar residues) before SDS-

PAGE, the position of the bumetanide protected band shifted downward by ~30 kDa. These results provide strong evidence that the rabbit parotid bumetanide binding site, which represents all or a major portion of the Na/K/Cl cotransporter, is a 160 kDa glycoprotein.

According to the two-stage hypothesis of salivary formation proposed by Thaysen et al., saliva is initially formed as a nearly isotonic plasma-like "primary" secretion in the acinar lumen. This primary saliva is then modified (the "second stage" of Thaysen's hypothesis) by the removal or addition of various ions in the salivary ducts with no further secretion or absorption of water. There are now considerable data supporting Thaysen's two-stage hypothesis and the fluid secretory mechanisms operating in the acinar cells are at least qualitatively understood. Little is known, however, about the ion transport systems in salivary ducts. A major reason for this lack of information is that the ducts make up only a small fraction (10-30%) of the total glandular tissue. Thus, a tissue digest of the type commonly employed is essentially an acinar preparation. Over the past year several projects have been initiated to attempt to clarify the role of salivary ducts in the secretory process.

Using a recently acquired SPEX microfluorometer we have been able to characterize the responses of individual salivary ducts and acini to various secretagogues. In these experiments a collagenase digest of the rat submandibular gland is used. These tissue fragments (ducts and acini) are centrifuged (2000 rpm) onto glass coverslips to which they spontaneously adhere. The coverslips are than examined under an inverted microscope and a single duct or acinus can be selected for study. This specimen is then irradiated directly on the microscope stage with the appropriate excitation light for the fluorescent indicator employed. Emitted fluorescence is monitored by a photomultiplier tube coupled to the microscope optics. To date we have concentrated on the ability of secretagogues to mobilize intracellular Ca^{2+}. Resting $[Ca^{2+}]_{in}$ levels were ~100 nM in both ducts and acini. Consistent with earlier observations on whole gland digests we find that acinar $[Ca^{2+}]_{in}$ rises in response to muscarinic (carbachol), α-adrenergic (epinephrine) and substance P stimulation. The latter response is transient owing to the well-known desensitization of the substance P receptor. The rise in $[Ca^{2+}]_{in}$ seen in response to carbachol and epinephrine is sustained in the presence of extracellular Ca^{2+} but only transient in its absence. These results indicate that the increase in $[Ca^{2+}]_{in}$ is due to both Ca^{2+} release from intracellular stores and Ca^{2+} entry from the extracellular solution. The response of ducts to carbachol and epinephrine was similar to those of acini. However, no increase of ductal cell $[Ca^{2+}]_{in}$ was observed in response to substance P suggesting that ducts are without substance P receptors. As noted above, in collaboration with the CIS, similar studies have recently begun on acini and ducts obtained from human minor salivary gland biopsies.

We, also, have recently developed a method by which we can physically isolate acini and ducts after collagenase digestion. This method employs a 65% Percoll gradient centrifugation step and results in two distinct bands; one acinar, the other ductal. Ca^{2+} mobilization responses can be studied with acini and ducts obtained by this procedure and results are consistent with the microfluorometric studies described above. The ductal elements were also shown to secrete kallikrein in response to α-adrenergic stimulation. Epinephrine (used as an α-adrenergic agonist) yielded a potent response (10× basal at maximal doses), while

muscarinic stimulation (carbachol, 2×) and β-adrenergic stimulation (isoproterenol, 50% increase) were considerably weaker at eliciting kallikrein secretion. Isoproterenol, however, markedly elevated cAMP levels in ductal cells (360 versus basal levels of 5 pmol/mg protein).

Our experimental results on salivary ducts demonstrate clearly that these structures can be isolated and studied under conditions where their functional properties are preserved in a relatively intact state. To our knowledge, the above data collected using microfluorometry and purified ductal preparations represent the first direct characterization of intralobular salivary ducts to be obtained by any method.

In addition to these results on native salivary ducts, we have also continued to study human salivary ductal epithelial cell lines. HSY cells are thought to be derived from the intercalated duct/acinar region of human parotid glands. These cells respond to both muscarinic stimuli (by increasing $[Ca^{2+}]_{in}$) and β-adrenergic stimuli (by increasing intracellular cAMP levels) but not to α-adrenergic agents. Over the past year we have studied the muscarinic response of these cells in detail. Carbachol stimulation increased $^{86}Rb^{+}$ influx and efflux resulting in no change in the net cellular content of $^{86}Rb^{+}$. The Ca^{2+} ionophore A23187 gave similar results. $^{86}Rb^{+}$ fluxes were inhibited by two K channel blockers (quinine and charybdotoxin) suggesting these responses occur via maxi-K channels. In order to probe the Ca^{2+} handling machinery of single HSY cells at a more fundamental level, MBS staff members investigated the response to muscarinic stimulation using microfluorometric techniques. When single HSY cells, loaded with the intracellular Ca^{2+} indicator fura-2, were stimulated with carbachol, regular oscillations in $[Ca^{2+}]_{in}$ with a frequency of 1-2 oscillations/min were observed. These oscillations could continue for long time periods (>1 hr) and could typically be stopped and restarted repeatedly by removal and reapplication of the muscarinic stimulus. When extracellular Ca^{2+} was removed, the oscillations persisted for several minutes indicating that they originate from intracellular calcium stores which are apparently reloaded from the extracellular space. Oscillations were inhibited by calmodulin inhibitors and by the active phorbol ester phorbol 12-myristate 13-acetate. Calcium oscillations are presumably the result of the complex feedback mechanisms involved in the control of intracellular calcium levels. These results indicate that both protein kinase C and calmodulin-dependent events modulate the frequency of carbachol-induced calcium oscillations in HSY cells and, thus, may be involved in the regulation of $[Ca^{2+}]_{in}$.

SECRETORY PHYSIOLOGY SECTION

The Secretory Physiology Section (SPS) is primarily directed at understanding the mechanisms by which a neurotransmitter stimulus results in functional secretory responses of exocrine epithelial cells. Specifically these efforts are directed (i) at the regulation of second messenger formation (cAMP, inositol trisphosphate, IP_3, and Ca^{2+}) and (ii) understanding the post-receptor "switches" which account for the growth control and differentiation (i.e. tissue specific gene expression) of salivary cells. These mechanistic questions are not only central

to understanding neurotransmitter regulation of salivary secretion but, indeed, are pivotal questions central to all hormone regulated physiological processes.

Neurotransmitter signals are transduced from specific cell surface receptor proteins to intracellular effector enzymes via guanine nucleotide binding regulatory proteins (G proteins). Considerable effort has been devoted to elucidating mechanistic characteristics of these G proteins. For example, hormone-induced Ca^{2+} mobilization in parotid acini is mediated via an as yet uncharacterized G protein which activates phospholipase C. In some cell types the G protein responsible for phospholipase C activation is sensitive to treatment with pertussis toxin while in other cells this treatment is without effect. Classically, pertussis toxin catalyzes the ADP-ribosylation of the alpha subunit of an inhibitory G protein which blunts agonist-stimulated cAMP formation, so called Giα. Thus, signal transduction resulting in phospholipase C activation is clearly heterogenous and cell type specific. Accordingly, SPS staff members have utilized pertussis toxin in an effort to understand the Ca^{2+} mobilization process in rat parotid acinar cells. When parotid acinar cell membranes are incubated with [^{32}P] NAD and pertussis toxin, then subjected to electrophoresis, nitrocellulose blotting and autoradiography, only one protein is ADP-ribosylated. This protein has an M_r ~41 kDa, a molecular size comparable to Giα. The level of ADP-ribosylation in this protein is reduced by ~90% in membranes prepared from glands of rats treated in vivo with ~1.5 μg pertussis toxin. Additionally, the in vivo pertussis toxin treatment resulted in a >80% block in the ability of the muscarinic agonist carbachol to inhibit β-adrenergic agonist-induced stimulation of cAMP formation in parotid cells. Toxin treatment, however, was without effect on electrophoretic or [^{32}P] GTP binding characteristics of three, low molecular weight guanine nucleotide binding proteins (ras-like, M_r ~18,22,25 kDa) in parotid membranes. Thus, the in vivo regimen of pertussis toxin used is highly effective in markedly altering Giα and its associated functional response. Yet when muscarinic agonist-induced Ca^{2+} mobilization responses were studied in parotid cells from pertussis toxin-treated rats, no differences from controls were seen in (i) agonist sensitivity, i.e. concentration response effects; (ii) peak intracellular Ca^{2+} release from intracellular pools; or (iii) sustained extracellular Ca^{2+} entry and refill of the agonist-sensitive intracellular Ca^{2+} pools. Also, activation of Ca^{2+} mobilization by AlF_4^- (a general activator of heterotrimeric G proteins) was comparable in cells from control and toxin-treated rats. These studies demonstrate that the G protein involved in transducing a Ca^{2+} mobilizing signal from the muscarinic receptor in parotid acinar cells is of a class which is insensitive to pertussis toxin.

β-Adrenergic stimulation of adenylyl cyclase involves activation of a stimulatory G protein, Gs. We, also, have studied this signaling system in great detail. Alpha subunits of characterized signal transducing G proteins possess endogenous high affinity GTPase activity. G proteins are activated following binding of GTP to the Gα subunit. Inactivation results from the endogenous GTPase hydrolyzing GTP to GDP, thus allowing reassociation of α, β and Γ subunits forming an inactive heterotrimer. Studies by the SPS showed that parotid membranes contain two proteins which could be ADP-ribosylated by [^{32}P] NAD and cholera toxin. These proteins displayed an M_r ~45 and 51 kDa and are similar to Gs observed in other tissues. These membranes also contain high affinity GTPase activity which hydrolyzes

[Γ-^{32}P] GTP. This represents ~15% of all GTPase activity and is saturable, displaying a Km ~450nM and Vmax ~94 pmol/mg protein. Isoproterenol, a β-adrenergic agonist, stimulates this GTPase ~50% (to ~143 pmol/mg protein) without changing the Km. This increase is, also, blocked by the β-adrenergic antagonist propranolol. Isoproterenol and GTP each increase cAMP formation by these membranes ~2-fold, while in combination a ~6-fold change is observed. When the non-hydrolyzable GTP analogue GTPΓS is used, basal cAMP formation is increased ~18-fold and that elicited by isoproterenol ~70-fold. The latter activity is comparable to that resulting from AlF_4^- (i.e. the presumed maximal response). When membranes are treated with cholera toxin, basal GTPase activity was unaffected. However, the isoproterenol-induced increase in these membranes was ~80% reduced. Consistent with this finding, GTP significantly increased both basal and isoproterenol-stimulated cAMP formation in cholera toxin-treated membranes. Together, the data demonstrate that the high affinity GTPase studied by us is the "turn-off" step for adenylyl cyclase activation following β-adrenergic stimulation of rat parotid glands.

Last year we reported evidence for regulatory interactions, involving phospholipase C, between different receptor signaling systems in parotid acinar cells. For example, we reported that submaximal concentrations of α_1-adrenergic and muscarinic-cholinergic stimuli did not result in additive inositol trisphosphate IP_3 responses despite the presence of ample IP_3 precursor, phosphatidylinositol 4,5-bisphosphate (PIP_2). In order to examine such examples of "receptor crosstalk," we have developed an in vitro assay for measuring agonist stimulated PIP_2-phospholipase C activity in parotid membranes. Exogenous [^{3}H]PIP_2 is added to plasma membranes as mixed micelles (with deoxycholate) and IP_3 release is monitored. Optimal assay conditions include 10nM free Ca^{2+}, and 0.8 mM deoxycholate. The muscarinic agonist carbachol alone has no effect on IP_3 formation but, together with GTPΓs, can increase levels of IP_3 30-50%. Carbachol's effects are concentration dependent and blocked by atropine. Essentially all IP_3 generated is 1,4,5 IP_3, the expected hydrolysis product of PIP_2. This preparation undoubtedly will prove a valuable tool in direct, mechanistic crosstalk studies.

The SPS has had a long interest in understanding Ca^{2+} entry pathways into parotid acinar cells. While intracellular Ca^{2+} stores can initiate the fluid secretory process, the sustained entry of extracellular Ca^{2+} is required for the prolonged secretion of saliva which is observed physiologically (e.g. with meals). In order to study directly Ca^{2+} influx into parotid acini we have used the surrogate cation Mn^{2+}. This cation can enter cells via pathways that allow Ca^{2+} but Mn^{2+} remains in the cytosol since it cannot be transported by the Ca^{2+} ATPases. Thus, assessing the quenching of fura-2 fluorescence by Mn^{2+} is, in theory, an excellent means to study the magnitude of cation entry in parotid cells. SPS staff members have established that Mn^{2+} can enter parotid acini and displays characteristics similar to Ca^{2+} entry. Mn^{2+} entry is activated following agonist stimulation of cells in a dose dependent manner and is sensitive to antagonists. Following stimulation of acini with carbachol, Mn^{2+} entry is detected after a lag of about 12-15 sec and does not coincide with the initial phase of intracellular Ca^{2+} release, which is seen within 6 seconds. This delayed entry, similar to that of Ca^{2+}, is seen even in cells stimulated with AlF_4^-. Manganese entry is sustained and can be observed after 10 min of carbachol stimulation. Additionally, Mn^{2+} entry (with [Mn^{2+}]

between 12.5-200 μM) can be observed even in the presence of 1.28mM Ca^{2+} in the extracellular medium, suggesting a high affinity of the entry mechanism for this cation.

We, therefore, have studied the regulation and route of extracellular divalent cation entry into rat parotid acinar cells by following Mn^{2+} influx into fura-2 loaded cells. Previous studies from our laboratory and others have shown that on prolonged stimulation of acini in Ca^{2+}-free medium the internal Ca^{2+} pools are depleted. Refill of these pools requires the addition of an antagonist and can be assessed by following intracellular Ca^{2+} release induced by a subsequent stimulation with a second agonist. Our studies have shown that in agonist-stimulated, pool-depleted acini there is a 2-fold increase in the magnitude of Mn^{2+} entry. When the pools are allowed to refill with Ca^{2+}, Mn^{2+} entry is decreased as a function of the extent of refill. This demonstrates that Mn^{2+} entry into parotid acini occurs via the pathway involved in Ca^{2+} pool refill. Based on these findings, SPS investigators have tested the hypothesis which suggests that extracellular Ca^{2+} enters non-excitable cells via the intracellular storage pool. Data from our studies with parotid acinar cells, however, demonstrate that this hypothesis is generally unsupported. For example, following intracellular pool depletion due to stimulation of cells by carbachol in a nominally Ca^{2+} free medium, the addition of the muscarinic antagonist atropine does not alter the rate of Mn^{2+} entry. However, under these conditions, the IP_3 sensitive Ca^{2+} release channels on the agonist-sensitive pool would be closed, yet refill is promoted. Mn^{2+}, furthermore, does not accumulate in any intracellular pools, i.e. none is released by ionophore treatment. Thus, in rat parotid acinar cells, Mn^{2+} (Ca^{2+}) entry is dependent on the refill status of the intracellular agonist-sensitive pool and occurs directly into the cytoplasm.

The exact nature of Ca^{2+} entry pathways in non-excitable cells has remained elusive. Previous work by the SPS has suggested that cultured salivary ductal epithelial cells appear to have two modes of Ca^{2+} entry, one sensitive to changes in membrane potential and one which is insensitive. Recent studies by us have made similar observations (of membrane potential sensitivity) in parotid acinar cells. Mn^{2+} (Ca^{2+}) entry is enhanced in a medium containing the impermeant cation N-methyl-D-glucamine$^+$ instead of Na^+ (a hyperpolarizing medium) and is inhibited in a medium containing high K^+ (a depolarizing medium). Refill of the agonist-sensitive Ca^{2+} pool is, also, inhibited by membrane depolarizing conditions and this can be overcome by increasing extracellular [Ca^{2+}].

As mentioned earlier, SPS investigators, also, study neurotransmitter control steps which are believed to be involved in regulating the growth and differentiation of salivary cells. Previous studies by us have shown that β-adrenergic receptor stimulation leads to increased steady state levels of the c-fos and c-jun proto-oncogene mRNAs in rat parotid acinar cells. Following 9 days of administration of the β-adrenergic agonist isoproterenol, rat parotid glands are dramatically enlarged. While there is no correlation of c-fos or c-jun expression with these growth responses, chronic isoproterenol treatment resulted in high levels of expression of the c-abl proto-oncogene. The c-abl transcripts were highly unusual, 1.5 and 1.3 kb versus the typical 6.5 and 5.3 kb transcripts found in other tissues. These unusual transcripts resulted from sequences at the C terminal end of the c-abl gene. The 5' end of the isoproterenol-inducible c-abl transcripts was mapped using primer extension analysis. This

established the position of the cap site (transcription initiation site) for the c-abl mRNAs to be 356 nucleotides upstream of the XhoI site in the C-terminal segment of the abl reading frame. A cDNA clone corresponding to the 1.5kb c-abl transcript was identified using polymerase chain reaction methodology. This cDNA strongly hybridizes to the 1.5kb c-abl transcript and to a lesser degree to the 1.3kb mRNA. The 1.5kb c-abl cDNA has now been cloned to the Sma I site of M13mp18 and M13mp19 vectors for sequencing.

When parotid acinar cells are acutely stimulated in vitro with isoproterenol for 60 min, the secretion of the main parotid exocrine proteins (amylase, PSP and PRPs) is markedly increased and their synthesis, as measured by [^{14}C] leucine incorporation, is also increased. The enhanced level of protein synthesis is not, however, accompanied by elevations in their respective mRNA levels. Similar results were observed with in vitro incubations of submandibular cells by following kallikrein secretion and synthesis. These data indicate that the increased protein synthesis seen after β-adrenergic stimulation of salivary cells likely does not represent transcriptional level changes but rather suggests changes involving translation. Interestingly, chronic in vivo treatment of rats with isoproterenol results in a markedly different effect. The levels of PRP mRNA were highly increased but those for the other exocrine proteins (amylase, PSP and kallikrein) were dramatically decreased.

In order to facilitate mechanistic studies of gene expression in rat parotid acinar cells, SPS staff have utilized a variety of approaches (including plating on reconstituted basement membrane and growth in the continuous presence of isoproterenol) to maintain primary cell cultures in vitro. Thus far, cells with some important acinar characteristics have been maintained for >8 months. Morphologically, cells did not appear grossly acinar-like; they were flattened, elongated and formed multilayers. However, most cells still contained peroxidase-positive secretory granules and displayed abundant rough endoplasmic reticulum and a well-developed golgi apparatus. Most cells, also, were positive for PRP mRNA by in situ hybridization and positive for PRP protein by immunoperoxidase staining. Amylase mRNA and protein levels, though detectable, were low. The latter circumstance is somewhat analogous to the above-mentioned observation in parotid glands following chronic isoproterenol treatment of rats. We expect that these, and related, studies will enhance our understanding of the factors regulating acinar cell differentiation.

Summation

The CIPCB has a unique mission in the NIDR. Our success in achieving this mission is made possible by the careful blending of a staff of clinical problem-oriented (yet basic science appreciating) individuals with basic science-oriented (yet clinical problem appreciating) individuals. We try to apply basic science knowledge and methodologies to address significant clinical concerns. We continue to make substantial progress in these goals. Our considerable success, thus far, is due to the high level of idealism, the strong sense of mission and the capacity for hard work generally manifested by Branch staff. We believe that we have an important responsibility to NIDR, and to dentistry, as the institute's major research group functioning within the NIH Clinical Center. We have the opportunity to make many contributions to clinical dentistry, to oral science, as well as to fundamental

biology. Our Branch recognizes this and works creatively and enthusiastically toward meeting this goal. We anticipate continued forward movement in our efforts to address questions of importance to the understanding and management of oral disease.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00212-14 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Taste and Its Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Weiffenbach, James	Research Psychologist	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR

COOPERATING UNITS *(if any)*
LSB, NIA; BPB, NIMH

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.97	.87	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to elucidate the mechanisms by which oral sensory and perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. These methods, applied to the study of age-associate changes, have provided insights into basic mechanisms of normal chemosensory perception. Functional variation under pathologic circumstances is assessed through objective evaluations of oral sensory disturbances occurring in association with systemic disease, salivary gland dysfunction, therapeutic X-irradiation, eating disorders or as an isolated complaint. Assessments of olfactory identification as well as sensitivity to local pressure on the tongue and to variation in the temperature or the viscosity, of an oral bolus are obtained when they can contribute to an understanding of oral sensory function in relation to the complex stimuli encountered in everyday life.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00332-09 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Guckes, Albert D.	Dep Clin Dir/NIDR	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
McCarthy, George M.	Senior Staff Dentist	CIPC	NIDR
Ruttimann, Urs E.	Biomedical Engineer	DSB	NIDR
Shern, Roald J.	Senior Staff Dentist	CIPC	NIDR

(see attached continuation page)

COOPERATING UNITS *(if any)*
Laboratory of Clinical Science, NIMH; Inter-Institute Genetics Program, CC; Diagnostic Systems Branch, NIDR

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.64	1.89	1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical research, including case studies of unusual conditions, are conducted on a variety of subjects related to the oral cavity and oral health. Methods utilized include chart and literature reviews and clinical evaluation of various therapeutic regimens. A recent case study documented the oral complications in a patient with Erdheim-Chester disease. The patient lost all teeth due to rapidly progressing periodontal disease related to fatty infiltration of bone marrow tissue associated with E-C. Endosseous dental implants were utilized to replace lost mandibular teeth. Despite the abnormal bone marrow tissue present in the E-C patient, the implants appear to be functioning successfully to support a fixed bridge. Another case study documented the transformation of a long standing oral lichen planus of the dorsal surface of the tongue to squamous cell carcinoma. Evaluation of various therapeutic regimens have included (1) use of a dentin bonding agent and a composite resin to restore posterior teeth, (2) a collaborative study with the Upjohn Company evaluating the use of a non-steroidal antiinflammatory agent in the treatment of periodontitis, and (3) the surgical treatment of jaw immobilization associated with fibrodysplasia ossificans progressiva. Chart reviews and clinical examinations were used to document oral conditions associated with anorexia and bulimia nervosa.

PHS 6040 (Rev. 1/84) GPO 914-918

Ship, Jon	Senior Staff Dentist	CIPC	NIDR
Kobel, Mary L.	Dental Hygienist	CIPC	NIDR
Valdez, Ingrid	Clinical Associate	CIPC	NIDR
Vermillion, Cheryl	Dental Hygienist	CIPC	NIDR
Wright, William	Periodontal Consultant	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00336-09 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Salivary Gland Secretion Mechanisms During Normal and Altered Functional States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Ambudkar, Indu S.	Senior Staff Fellow	CIPC	NIDR
Dai, Y.	Visiting Fellow	CIPC	NIDR
Hiramatsu, Yukiharu	Visiting Fellow	CIPC	NIDR
Horn, Valerie J.	NRC Fellow	CIPC	NIDR

COOPERATING UNITS *(if any)*

LCMB, NIA

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Secretory Physiology Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.0	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize in vitro dispersed cells, and cultured epithelial cells of salivary glands, to understand mechanisms controlling saliva formation. We have focused these studies on autonomic neurotransmitter regulation of secretory events and associated signalling mechanisms. During this reporting period the primary focus of study has been directed at examining the role of GTP-binding regulatory (G) proteins in secretory stimuli in the rat parotid gland. In particular, we have shown that the Ca^{2+} mobilization response elicited by the muscarinic receptor agonist carbachol is unaffected by treatment with pertussis toxin (i.e. involves a pertussis toxin-insensitive G protein). We observed comparable carbachol sensitivity, and the ability of the agonist-sensitive intracellular Ca^{2+} pool to be refilled by extracellular Ca^{2+}, in control and toxin-treated cells. Similarly, Ca^{2+} responses to AlF_4^- were unaffected in cells from pertussis toxin-treated rats. We also have characterized the high affinity GTPase activity in Gs-enriched parotid membranes. The β-adrenergic receptor agonist isoproterenol can increase this GTPase activity (Vmax, ~50%; no effect on Km) and stimulate cAMP formation in these membranes. Adenylyl cyclase activity is increased markedly in the presence of isoproterenol and GTPYS. Cholera toxin treatment of membranes decreases isoproterenol-induced high affinity GTPase activity and increases cAMP formation induced by GTP (± isoproterenol). These data suggest the agonist-induced increase in high affinity GTPase activity is involved in the deactivation of β-adrenergic stimulated responses of rat parotid acinar cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00337-09-CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Physiological Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fox, Philip C.	Dental Officer	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Kohn, William	Dental Officer	CIPC	NIDR
Kousvelari Eleni E.	Senior Staff Fellow	CIPC	NIDR
Kurrasch, Regina	Medical Staff Fellow	CIPC	NIDR
Lane, H. Clifford	Medical Officer	LIR	NIAID

(see the attached continuing sheet)

COOPERATING UNITS *(if any)*
RM, CC; DR, CC; HGB, NICHD; LIR, NIAID; MD, NIDDK; LNS, NIA; LSB, NIA; Columbia University; Boston University; SUNY, Stony Brook; University of California, San Francisco.

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	3.6	1.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the function of various oral tissues in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness) utilizing the inpatient and outpatient services of the Dry Mouth Evaluation Clinic. Specific diagnostic approaches have been developed to aid in establishing the causes of salivary gland dysfunction and defining criteria necessary for management decisions. Normal parameters have been established for salivary scintigraphy with technetium pertechnetate in a healthy control population and a simple rating scale for scintiscans constructed. A treatment protocol for patients with secretory hypofunction related to active or past radiation therapy continues, employing a regimen of oral administration of the parasympathomimetic drug, pilocarpine. Clinical and laboratory studies focusing on the causes and character of the salivary gland component of Sjogren's syndrome, an autoimmune exocrinopathy, have advanced. Treatment protocols for primary Sjogren's syndrome are continuing. In addition, detailed studies of salivary-associated oral complaints (eg. taste and oro-pharyngeal swallowing disorders) have progressed. The cells infiltrating the labial minor salivary glands in patients with Sjogren's syndrome have been studied. These are predominantly T-lymphocytes of the memory (helper/inducer) subtype, although only a small percentage were found to express the $\gamma\delta$ form of the T cell receptor. There was also increased expression of specific proto-oncogenes in these cells.

Macynski, Alice A.	Research Nurse	CIPC	NIDR
Pillemer, Stanley	Senior Staff Fellow	OD	NIAMS
Skopouli, Fotini	Visiting Fellow	CIPC	NIDR
Shern, Roald J.	Dental Officer	CIPC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC
Valdez, Ingrid H.	Dental Staff Fellow	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00412-05 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Clinical Study of Oral Endosseous Titanium Implants in Edentulous Subjects and Subjects with Ectodermal Dysplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Guckes, Albert D.	Dep Clin Dir/NIDR	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
McCarthy, George M.	Senior Staff Dentist	CIPC	NIDR
Rudy, Sue	Clinical Nurse	CIPC	NIDR
Stables, Gloria	Chief, Clin.Nutr.Serv.	NUTR	CC
Siebring, Nancy	Nutr. Res. Specialist	NUTR	CC
Sonies, Barbara C.	Speech Pathologist	RM	CC
Gracely, Richard H.	Research Psychologist	NAB	NIDR

COOPERATING UNITS *(if any)*
Rehabilitation Medicine Department CC; Nutrition Department, CC; Surgical Services Department, CC; Diagnostic Systems Branch, NIDR; Commissioned Officers Dental Clinic, CC

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.55	.95	1.60

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the use of endosseous dental implants in completely edentulous patients, or patients with ectodermal dysplasia and associated congenitally missing permanent teeth. Removable dentures are considered a significant handicap related to mastication, speech, esthetics, reduction of the residual ridges of the mandible and maxillae, and body self image. Individuals with ectodermal dysplasia often have several congenitally missing teeth resulting in a lack of development of the alveolar bone which normally is present to support the permanent teeth. Lack of alveolar bone not only makes it difficult for a patient to wear a removable denture but also makes the placement of dental implants more difficult and possibly less predictable. This study is attempting to determine if dental implants can be used to successfully replace missing teeth in conventional patients and patients with ectodermal dysplasia, and if such treatment with an implant supported fixed denture significantly effects loss of vertical dimension of occlusion, satisfaction with treatment, food choice and nutrition, perception of difficulty of chewing selected food, and body self image; when compared to treatment with a conventional removable denture. In addition the project is investigating if patients identified as being difficult to satisfy with conventional dentures are more satisfied when the prosthesis is fixed and supported by implants. Many investigators have assumed that a patient's body self image and the ability to adapt to conventional removable dentures is related to their personality. Data from this project will provide information concerning the relationship of personality to body image and the ability to adapt to oral prostheses of various types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00415-05 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*
Ion Transport and Fluid Secretion in Salivary Glands

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Neme, title, leboretory, end institute effilietion)*

Turner, Roy James	Visiting Scientist	CIPC	NIDR
George, Janet N.	Chemist	CIPC	NIDR
Lee, Syng Ill	Visiting Associate	CIPC	NIDR
Paulais, Marc	Visiting Fellow	CIPC	NIDR
Dehaye, Jean Paul	Visiting Associate	CIPC	NIDR
Moran, Arie	Visiting Associate	CIPC	NIDR
Wellner Robert B.	Guest Workers	CIPC	NIDR
Patton, Lauren L.	Dental Staff Fellow	CIPC	NIDR
Valdez, Ingrid H.	Dental Staff Fellow	CIPC	NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Membrane Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.16	4.06	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Saliva is the principle protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations in the salivary secretory mechanism can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of salivary fluid secretion and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following.

(1) The regulation of the rat parotid acinar Na/H exchanger and Na/K/Cl cotransporter by secretagogues was studied in order to clarify the role of these transporters in the fluid secretory process.

(2) Studies of the functional properties of salivary ducts, in particular their response to various secretagogues, were begun using both microfluorometric methods and a recently devised ductal preparation procedure.

(3) The parotid Na/K/Cl-dependent bumetanide binding protein was identified using bumetanide sensitive [^{14}C]-N-ethylmalemide labeling techniques.

(4) Muscarinic agonist-induced oscillations in intracellular calcium concentration were studied using microfluorometry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00438-04 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms Regulating Calcium Flux in Salivary Glands

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ambudkar, Indu S.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Horn, Valerie J.	NRC Fellow	CIPC	NIDR
Mertz, Lawrence M.	IRTA Fellow	CIPC	NIDR
Hiramatsu, Yukiharu	Fogarty Visiting Fellow	CIPC	NIDR
Lockwich, Timothy	Staff Fellow	CIPC	NIDR

COOPERATING UNITS *(if any)*
Departments of Medicine (Nephrology Division) and Physiology, Johns Hopkins University, School of Medicine; Department of Biological Chemistry, University of Maryland School of Medicine; and LB, NHLBI.

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Secretory Physiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.78	3.18	.60

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fluid secretion in salivary glands is modulated by changes in the cytosolic $[Ca^{2+}]$, which involves Ca^{2+} release from intracellular pools and Ca^{2+} entry from the external medium. We have directed our efforts towards understanding the processes which regulate cytosolic $[Ca^{2+}]$. Three main areas are being investigated: (i) regulation of signal generation, (ii) regulation of intracellular Ca^{2+} mobilization, and (iii) regulation of Ca^{2+} flux in cell membranes. In the present reporting period a major effort was directed towards characterizing the mechanism of Ca^{2+} entry in rat parotid acini. We report that agonists stimulate Mn^{2+} entry into parotid acini in a manner similar to Ca^{2+}. Using Mn^{2+} as a tool we show that divalent cation entry likely occurs via a cytosolic route and is regulated by the $[Ca^{2+}]$ in the intracellular pool. We also show that hyperpolarizing conditions stimulate while depolarizing conditions inhibit cation entry, independent of intracellular $[Ca^{2+}]$. We have observed that in isolated basolateral membrane vesicles the permeability to Ca^{2+} is increased by the presence of permeant ions and inhibited by La^{3+} and Mg^{2+}. Additionally continuing our studies on the regulation of IP_3 generation, using an in vitro assay for the PIP_2-specific phospholipase C, we show that GTPγS and carbachol, but not carbachol alone, can stimulate this activity in an isolated, plasma membrane-enriched, membrane preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00458-03-CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
β-Adrenoreceptors and Gene Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kousvelari, Eleni	Senior Staff Fellow	CIPC NIDR
Fox, Philip C.	Dental Officer	CIPC NIDR
Lazowski, Krzysztof	Visiting Fellow	CIPC NIDR
Mertz, Prema	Staff Fellow	CIPC NIDR
Pluta, Agnieszka	Visiting Associate	CIPC NIDR
Yeh, Chih-Ko	Staff Fellow	CIPC NIDR

COOPERATING UNITS *(if any)*
Department of Pharmacology, University of Minnesota; MGH Cancer Center; Squibb Institute of Medical Research.

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.05	2.75	.30

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The composition and secretion of saliva as well as the morphology of parotid and submandibular glands in rats are influenced by β-adrenoreceptors (β-AR). Stimulation of these receptors by isoproterenol in vitro or in vivo increases levels of cAMP, protein phosphorylation, secretion, induces hyperplasia in these glands and alters salivary gland-specific gene expression. Our studies are designed to; (i) identify the mechanisms by which information from β-AR is transmitted to the nucleus and thus regulates these processes and (ii) examine the alterations in gene expression, protein synthesis and gland morphology in salivary gland disease. We primarily have focused on the role(s) of the proto-oncogenes c-fos, c-jun and c-abl in eliciting the β-AR responses in rat parotid acinar cells (RPAC) and in a salivary cell line (A5). In particular we studied the possible coordinate expression of these proto-oncogenes with salivary gland-specific genes during agonist stimulation, gland development and salivary gland disease. During this reporting period we have demonstrated that; 1) the isoproterenol-inducible c-abl mRNAs represent part of the C-terminal segment of the gene; 2) stimulation of β-AR in vitro regulates proline-rich protein (PRP), amylase, parotid secretory protein (PSP) and kalikrein synthesis and secretion post-transcriptionally, while in vivo chronic isoproterenol administration alters the transcription rates of these molecules; 3)only short times (5 min) of β-AR stimulation or exposure to cAMP are needed for the induction of c-fos and c-jun genes in A5 cells; 4) cultured RPAC, for 8 months, maintain some characteristics of their phenotype and; 5) c-myc, c-fos and c-jun expression in the salivary glands of patients with Sjogren's syndrome show a distinctly different pattern.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00499-01 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Health and Salivary Function in HIV-1 Infected Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Ship, Jonathan A.	Dental Officer	CIPC	NIDR
Yeh, Chih-Ko	Staff Fellow	CIPC	NIDR

COOPERATING UNITS *(if any)*
LME, NIDR; LIR, NIAID; Boston University, University of California, San Francisco; Virginia Polytechnical Institute, Medical College of Virginia, Centers for Disease Control

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.91	1.51	1.40

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on oral changes associated with HIV-1 infection. We have examined saliva, a critical part of the oral defense system, for changes following infection in cross-sectional and longitudinal studies. In all studies, the antimicrobial proteins lactoferrin, lysozyme, histatins, and salivary IgA were elevated in males positive for HIV-1 antibody. Of these proteins, only lysozyme continued to increase with time. Changes were most dramatic in the stimulated submandibular saliva. Salivary secretions from HIV-1 positive patients with a salivary gland enlargement which mimics that seen in Sjogren's syndrome were analyzed. Higher elevations of salivary IgA, lactoferrin, and IgA rheumatoid factor (RF) were present in HIV-1 positive patients with salivary gland enlargement than in HIV-1 positive patients with no enlargement. However, the saliva from patients with primary Sjogren's syndrome had much higher levels of salivary IgA, lactoferrin, and IgA RF. Recently, a study has started to examine periodontal status associated with HIV-1 infection (also see Z01 DE 00498-01). Preliminary data suggest that HIV-associated periodontitis is not frequently seen, and HIV-associated gingivitis is more common. These studies hope to establish a logical rationale for treatment of the oral problems seen in AIDS. We have also examined the incidence of AIDS among older persons, and observed that greater than 10% of cases in the U.S. occur in patients aged 50 years or older.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00500-01 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Physiology of Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ship, Jonathan A.	Dental Officer	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Cherry-Peppers, Gail G.	NRSA Staff Fellow	CIPC	NIDR
Ebbs, William L.	Dental Staff Fellow	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Patton, Lauren L.	Dental Staff Fellow	CIPC	NIDR
Shern, Roald J.	Dental Officer	CIPC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR

COOPERATING UNITS *(if any)*
LNS, NIA; LSB, NIA; RM, CC; Howard University

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.85	.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the status of various oral tissues during physiologic aging. The current emphasis is to study cross-sectional and longitudinal effects of aging on oral physiology in a variety of populations: healthy caucasians and blacks of different ages, and patients with dementia of the Alzheimer type. Healthy caucasians are being followed in two studies, at the Clinical Center in Bethesda, MD and at the Baltimore Longitudinal Study of Aging in Baltimore, MD. A newly initiated protocol proposes to examine healthy community dwelling blacks in a collaboration with Howard University Dental School. Additional studies on oral physiology have been initiated concerning the influence of systemic disease on aging and its impact on oral health. Clinical evaluation of volunteer participants involves an oral health questionnaire, collection of unstimulated and stimulated parotid and submandibular gland salivas, a comprehensive examination of dental, periodontal, and mucosal tissues, an oral motor exam, and the determination of pressure, gustatory, and olfactory sensitivities. During this reporting period, results from cross-sectional studies utilizing healthy subjects suggest: (1) the condition of the oral mucosa is unaffected by increasing age; (2) menopause and hormone replacement therapy have no observable effects on salivary gland function; (3) in individuals of different ages, normal oral health is supported by a wide range of salivary fluid output. Results from a group of unmedicated patients with early-stage dementia of the Alzheimer type suggest the presence of a selective impairment in submandibular gland function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00502-01 CI

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Maxillofacial Surgery - Rigid Versus Nonrigid Fixation Following Orthognathic Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
Folio, John	Consultant	CIPC	NIDR
Rudy, Sue	Clinical Nurse	CC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC
Gracely, Richard H.	Research Psychologist	NAB	NIDR

COOPERATING UNITS *(if any)*
Rehabilitation Medicine Department CC: Nutrition Department, CC; Surgical Services Department, CC: Diagnostic Systems Branch, NIDR; Commissioned Officers Dental Clinic, CC

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.52	1.17	1.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to determine the preferred method of fixation to avoid relapse following maxillary and mandibular osteotomy to correct facial developmental deformities. Correlations are being established between rigid and nonrigid fixation techniques, and the degree of relapse as measured by cephalometric techniques utilizing metallic markers implanted in the maxillae and mandible 6 months prior to orthognathic surgery. Changes in the height or the width of the attached gingiva are being recorded. Pre and post-operative changes in facial contours and occlusion are being measured. In addition, movements of the tongue, mandible and associated soft tissues during speech and swallowing are being assessed with ultrasound imaging procedures. Initial results observed at 3, 6 and 12 months did not demonstrate a significant difference in relapse between the two groups. However, patients treated with semirigid fixation experienced better diet, speech, and oral hygiene compared to patients with rigid internal fixation. Initial results of the swallowing tests demonstrate that maximal physical adjustment in the mandibular-hyoid relationship occurs in the first three months following the surgery. Functional changes continue to occur throughout the year. There appears to be a positive relationship between the surgical procedure and swallowing function.

PHS 6040 (Rev. 1/84) GPO 914-918

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

The Neurobiology and Anesthesiology Branch (NAB) is concerned with the study of oral-facial sensation with particular emphasis on mechanisms of pain and the development of new methods of pain control. Correlative multidisciplinary approaches are used to answer questions about the functional organization of nociceptive systems in normal and pathological states. The clinical component of the Branch focuses on the assessment and treatment of acute and chronic pain and often follows up advances made in the laboratory to determine their applicability to the clinical situation.

The Branch was established in 1974 and has grown from a small group of physiologists and anatomists studying nociceptive pathways to a major program spanning basic and clinical research on pain and its control. The research conducted has direct relevance to our understanding of mechanisms of neuronal function, in general. In the last few years, there have been major technical advances in the neurosciences that have given us the opportunity to move our research in new and exciting directions. Our previous studies of the organization and neurochemistry of nociceptive systems have provided a foundation for recent studies on the function of the system following tissue inflammation and nerve injury in animal models. Our clinical models have also focused on chemical mediators associated with surgical trauma and neurohumoral mechanisms of pain and analgesia following the stress and discomfort of surgical procedures. The NAB continues to provide the leadership and coordination of the activities of the NIDR/NIH Pain Research Clinic in the Clinical Center ACRF.

In the last few years we have developed animal models of peripheral tissue inflammation and nerve injury in the rat in order to study changes in the nervous system associated with acute and persistent pain. We have become very interested in the molecular events that accompany the neuronal plasticity seen in these models, and have performed combined studies using neurochemical, biochemical and electrophysiological methods.

Investigators in the Branch have received considerable recognition for their research accomplishments. A number of our senior staff organized topical seminars and presented invited papers at the Sixth World Congress on Pain held in Adelaide in April. Dr. Dubner was a plenary speaker at this Congress. Drs. Bennett and Iadarola were invited speakers at a Dahlem Conference held in Berlin in December. An international meeting mainly of U.S. and Japanese scientists included Drs. Dubner, Kenshalo and Ruda as invited speakers. Finally, Dr. Dubner was elected Treasurer of the International Association for the Study of Pain and was the recipient of the Public Health Service Distinguished Service Award.

Research accomplishments of the Branch are presented in detail below.

Chemical Mediators of Inflammatin, Pain and Stress

This year we have continued to study the effects of peripheral tissue inflammation and resulting pain. Inflammation is a common component of many acute and chronic pain conditions. Using models of inflammation in rat and in human subjects, we have continued to examine changes in the inflammatory exudate and surrounding tissues. We have used microdialysis probes to measure the release of bradykinin into the inflammatory exudate. In the oral surgery studies, tissue levels of immunoreactive bradykinin increased nearly ten-fold over a four hour observation period following the extraction of impacted third molars. These levels are more than 200-fold greater than blood levels of immunoreactive bradykinin. These observations indicate that the bradykinin system is significantly activated in inflamed tissue during the development of acute postsurgical pain. In our rat model, where we have previously demonstrated increases in bradykinin levels following the development of inflammation produced by the injection of carrageenan, a bradykinin antagonist significantly blocked hyperalgesia, hypothermia and edema. Taken together, the clinical and animal findings suggest that drugs which alter the bradykinin system will have analgesic and possibly anti-inflammatory activity in patients experiencing acute postsurgical pain.

Related studies in rat have revealed that there is an increase in immunoreactive calcitonin gene-related peptide (CGRP) in axons in inflamed tissue. Associated with this change is a 40 percent elevation in the number of CGRP-containing neurons in the dorsal root ganglion on the inflamed side. This increase occurred among all the different classes of dorsal root ganglion cells. These findings are of interest because of the suspected role of CGRP in mechanisms of plasma extravasation and edema.

This year we have extended our research on factors regulating the pituitary release of beta-endorphin under conditions of stress. Human subjects undergoing the removal of impacted third molars received epinephrine in conjunction with local anesthesia which resulted in a 30-fold increase in circulating epinephrine levels. Elevated epinephrine levels significantly reduced beta-endorphin levels in response to surgical stress in comparison to a control group which did not receive epinephrine. These results do not support the hypothesis of a stimulatory effect of epinephrine on beta-endorphin release and, instead, suggest that an inhibitory relationship between the two chemical mediators may exist in humans experiencing stress.

Neuronal Plasticity Following Peripheral Tissue Inflammation

Inflammation also results in central nervous system changes that involve the activation of specific opioid peptides. We have previously shown that inflammation induced by various inflammatory agents results in significant increases in the dorsal horn content of the opioid peptide, dynorphin, and a rapid increase in preprodynorphin mRNA. This year we have continued studies of the mechanisms of dynorphin gene transcriptional regulation by examining the expression of the immediate-early gene, c-fos, a proto-oncogene involved in transcription control. There is a marked and rapid increase in c-fos mRNA in spinal cord during inflammation. This change is transient, with a time course of 1/2 to 4 hours. In

contrast, the short-lived increase in mRNA gives rise to a prolonged increase in Fos protein. Using immunocytochemistry, we have found that Fos positive cells in the dorsal horn are present during the entire period of peripheral inflammation. We are very interested in the relationship between c-fos activation and the increase in dynorphin gene transcription. Therefore, we have begun to map protein binding sites on the dynorphin promotor. Using our antibody to the Fos protein we have established a gel mobility shift assay to determine potential Fos binding sites. We have shown that Fos proteins synthesized during inflammation can form AP-1 complexes with an oligonucleotide containing the AP-1 consensus sequence. Current studies are using this technique to assess AP-1 like sites in the dynorphin and enkephalin promotors.

Other studies in the laboratory have shown that c-fos and dynorphin gene transcription are localized to cells in the superficial and deep laminae of the spinal cord. To further study the possible transcriptional control of the dynorphin gene by Fos proteins, we examined the colocalization of increases in Fos protein with increases in dynorphin mRNA following peripheral inflammation in the rat. These studies utilized in-situ hybridization histochemistry to identify preprodynorphin mRNA followed by immunocytochemistry to identify Fos protein nuclear labelling. Increased levels of dynorphin message and Fos protein were observed ipsilateral to the inflamed hindpaw. In laminae I and II of the spinal dorsal horn, greater than 85% of the neurons exhibiting increased dynorphin message co-localized Fos protein in their nucleus. These data suggest that Fos proteins may be a component of the transcriptional control mechanisms underlying the increase in dynorphin gene transcription following inflammation. However, it appears that Fos protein may exert control over other transcriptional events following peripheral inflammation and hyperalgesia since the number of neurons exhibiting Fos protein immunoreactivity was far greater than those co-localizing Fos protein and dynorphin message.

We are also interested in the first messenger systems (neurotransmitters, neuromodulators) that lead to c-fos gene activation. In order to examine the role of different primary afferent fibers in this activation, we destroyed a subpopulation of peptide-containing small-diameter primary afferents by the administration of capsaicin, the active ingredient in red peppers, to neonatal rats. In this model, capsaicin administration leads to an almost complete loss of the neuropeptide, substance P, contained mostly in unmyelinated afferents. In this study, capsaicin-treated rats at 5-8 weeks of age exhibited hyperalgesia that was of a similar magnitude as that found in control rats. These findings indicate that capsaicin-sensitive primary afferent fibers are not necessary to produce hyperalgesia following peripheral tissue inflammation. In contrast, Fos protein activation was significantly reduced in the dorsal horn on the inflamed side by the destruction of the capsaicin-sensitive afferents. Thus, Fos protein activation appears to be dependent on small-diameter, peptide-containing afferents, suggesting a possible role of neuropeptides such as substance P in the initiation of c-fos gene activation.

We have continued to study the physiology of the neurons in the superficial spinal dorsal horn in the same inflammation models in order to examine changes in the physiological properties of the neurons associated with hyperalgesia and changes in dynorphin gene expression. Our initial observations of lamina I neurons studied ipsilateral to an inflamed

hindpaw revealed an increased incidence of neurons with large receptive fields and increased neuronal excitability. These changes occurred as early as 5-6 hours and persisted for days, and were closely correlated with the development of behavioral hyperalgesia in this rat model of inflammation. We were interested in whether dynorphin or kappa agonist drugs might play a role in the increase in excitability and receptive field expansion of these cells. Approximately 30% of the superficial dorsal horn neurons studied demonstrated an expansion of their receptive fields within 15-20 min after spinal application of dynorphin or the kappa agonist, U-50,488H. In addition to receptive field changes, neurons studied after the application of U-50,488H were likely to exhibit changes in responsiveness to mechanical stimuli. The majority of cells that demonstrated enhanced mechanical sensitivity also had expanded receptive fields. A small number of cells exhibited decreased responsiveness to mechanical stimuli that was not accompanied by receptive field changes. Responses to thermal stimuli were dose-dependent; lower doses of the kappa agonist tended to produce facilitation and higher doses, inhibition. Since there is a large increase in dorsal horn levels of dynorphin following peripheral inflammation, these findings have led us to postulate that dynorphin levels are related to enhanced excitability and development of expanded receptive fields. Expanded receptive fields will result in more neurons excited by a noxious stimulus, and may therefore result in an increase in the magnitude of the pain perceived.

The significance of the above series of studies is that they reveal which opioid neurons in the spinal cord are activated in response to inflammation and pain. Further elucidation of the pivotal role of the spinal dynorphin system in pain mechanisms may provide new approaches to the pharmacology of pain and new insights into chronic opioid use.

We have begun to study the possible changes in content of other neurochemical mediators in the dorsal horn following peripheral tissue inflammation. We have found that there is a large increase in the number of neurons that contain glutamic-acid decarboxylase (GAD), the synthesizing enzyme for gamma aminobutyric acid (GABA). This increase is highest in the deeper laminae of the dorsal horn and in the region around the central canal. There also appears to be an increase in GABA content measured by spectrofluorometric analysis which begins between 12 and 24 hours after induction of inflammation and is highest by the sixth day. These findings indicate that there is a marked increase in the spinal cord levels of a major inhibitory transmitter that presumably is involved in nociception. Further studies are needed to determine whether these changes are related to inhibitory or disinhibitory mechanisms.

Neuronal Plasticity Following Nerve Damage

Injury to peripheral nerves in humans often leads to persistent pain that is resistant to standard analgesic medications. These painful neuropathies remain a major problem in diagnosis and treatment whose incidence is increasing because of our aging population and the use of pharmacological agents in cancer and AIDS that damage nerves. In our laboratory, we have developed a rat model of neuropathic pain that is very similar to the neuropathic pain state in humans. Nerve constriction injury produced by loose ligation of the sciatic nerve results in hyperalgesia, allodynia and spontaneous pain in the rat. In the peripheral nervous system, the

injury does not produce the death of primary afferent neurons at a time when the abnormal pain sensations are fully expressed. Distal to the site of injury, however, there is an almost complete degeneration of the myelinated axons. The unmyelinated axons, in contrast, are nearly all intact and appear normal in nerves studied up to 8 days after the injury.

The experimental peripheral neuropathy is accompanied by an abnormality of the sympathetic vasomotor innervation. On the side of the nerve damage, there is almost a complete loss of sympathetic vasomotor efferents demonstrated using the histofluorescence method for visualizing norepinephrine, or the immunocytochemical methods for demonstrating the norepinephrine synthesizing enzyme, dopamine beta-hydroxylase, or neuropeptide Y, a peptide that is co-localized in norepinephrine-containing sympathetic efferents. The depletion is first detected at five days after the nerve injury and by 30 days postinjury is nearly complete. Of further interest is the finding that these animals demonstrate temperature abnormalities that have a temporal evolution that resembles that seen in humans with causalgia or reflex sympathetic dystrophy: the affected limb is initially hot and then progresses to a chronic cold status. The temperature abnormality, however, is not due to abnormal sympathetic discharge. Many animals with an affected extremity that is abnormally cold have no norepinephrine-containing axons so that the effect cannot be due to excessive vasoconstrictor discharge; cutting the nerve in some cases results in warming of the limb, indicating that receptor supersensitivity is not the mechanism and that the vasoconstriction is mediated by nerve activity. Our present hypothesis is that the rat neuropathy may evoke an abnormality in the central control of sympathetic function. These findings suggest that the temperature abnormalities in humans in later stages of the disease may not be due to abnormal sympathetic discharge.

The rat neuropathic model also results in central nervous system changes. Previous work in our laboratory has shown a large increase in the level of the opioid peptide, dynorphin, but not in the level of enkephalin, in dorsal horn neurons. We have now shown that there is a 200% increase in the amount of preprodynorphin mRNA in the spinal cord within 2 days of the nerve injury. This increase in gene expression peaks by 5 days and returns to control levels by 20 days post-injury. Preproenkephalin mRNA is not increased. As mentioned above, we previously showed that unilateral hindpaw inflammation also results in an increase in dynorphin gene expression in the dorsal horn. Thus, dynorphin synthesis may represent a common central nervous system response to injury with resultant hyperalgesia and pain.

Nociceptive Circuitry

In previous years we have developed a thermal discrimination task in which monkeys make fine thermal discriminations in the noxious range. With this behavioral model, we have studied the role of different neuronal populations in the encoding process by which monkeys perceive noxious stimuli. In addition, we employ this model to examine the effect of different chemical mediators on the monkeys' discriminative capacities.

This year we have continued our studies of the role of wide-dynamic-range (WDR) neurons in sensory discrimination. We previously showed that these neurons participate in the

encoding process by which monkeys perceive the intensity of noxious heat stimuli. We now have evidence that they also play an important role in the localization of a noxious stimulus. There was a strong correlation between neuronal discharge and the monkey's ability to detect noxious heat stimuli only when the stimulus was located in the central, most sensitive portion of the receptive field. These data suggest that information originating in the central portions of the receptive fields of WDR neurons encode intensity and location of a noxious stimulus.

Our pharmacological studies have examined the role of descending noradrenergic systems in nociceptive transmission in the dorsal horn. ST-91, a selective alpha-2 agonist, microinjected into the medullary dorsal horn, produced a dose- and stimulus intensity-dependent reduction in the monkeys' ability to detect small increases in noxious heat intensity. Systemic injections of idazoxan, an alpha-2 antagonist, and not prazocin, an alpha-1 antagonist, naloxone, an opioid antagonist, or saline, produced a significant attenuation of the effect of ST-91. There was no effect on the monkeys' ability to detect visual or cooling stimuli, indicating that the drug impaired heat detection without altering motor function or motivational and attentional components of the monkeys' performance. These results indicate that an alpha-2 adrenergic receptor agonist has a pharmacologically-specific effect on the sensory discriminative component of pain. Previously, we showed a similar effect of morphine microinjected into the medullary dorsal horn. We recently found that idozoxan produced a significant attenuation of the effect of morphine. This finding indicates that there is some interaction between opioid and noradrenergic inputs in the spinal cord. Since the opioid antagonist, naloxone, was not able to antagonize the effects of ST-91, the adrenergic effects in the dorsal horn appear to be downstream from the opioid effects.

We have found a similar interaction in our studies of opioids and alpha-2 agonists in the rat model of inflammation. Intrathecal opioids have an enhanced efficacy following inflammation with the dose-response curve shifted to the left as compared to stimulation of uninflamed limbs. In this model, naloxone and idazoxan blocked the analgesic action of morphine in rats with hindpaw inflammation. The effects of clonidine, an alpha-2 agonist, also revealed a leftward shift of the dose-response curve. The analgesic action of clonidine was blocked by idazoxan, but not by naloxone. These data support our previous findings of an interaction between opioid and adrenergic systems and suggest that during conditions of inflammation the enhanced analgesic action of spinal opioids may also depend on an adrenergic link.

Assessment and Treatment of Chronic Pain

In previous years we have completed a series of studies that examined new methods of treating chronic pain, with a particular focus on pain conditions often refractory to standard treatments, such as painful neuropathies. This year we have initiated clinical studies on the diagnosis and mechanisms of painful neuropathies in parallel with our basic studies on the mechanisms of neuropathic pain in the rat model. Detailed psychophysical analyses were performed in patients with reflex sympathetic dystrophy or causalgic-like pains. Touch-evoked pain was elicited by mechanical stimuli that were near the threshold for detection; on normal skin these same stimuli evoked innocuous tactile sensations. Similarly, transcutaneous

electrical stimuli also evoked pain at intensities near-threshold for evoking an innocuous tactile sensation. Reaction times to these sensations were too fast to include a contribution from unmyelinated or C afferent fibers. During ischemic block of the affected limb, the pain evoked by near-threshold tactile and electrical stimuli disappeared at the same time as impulse blockade occurred in the largest myelinated mechanoreceptive afferents. In these same patients, responses to noxious heat stimuli were essentially normal, indicating that unmyelinated nociceptors were not sensitized and did not contribute to the touch-evoked pain. These observations show that the touch-evoked pain of many of these patients is due to altered neuronal processing in the central nervous system so that input that normally evokes innocuous tactile sensations now results in pain.

This year we have also continued our clinical trial studies on new treatments for neuropathic pain. A crossover comparison of amitriptyline and desipramine was completed. The frequency of moderate or greater pain relief was 74% with amitriptyline and 61% with desipramine. The differences were not statistically significant. A crossover comparison of fluoxetine and placebo also revealed no statistically significant difference. Since over one-half of the patients participated in both crossover trials, and amitriptyline was significantly more effective than placebo, we were able to validate the sensitivity of the assay. These results suggest that desipramine has pain-relieving effects similar to amitriptyline in painful diabetic neuropathies, and is a useful alternative to amitriptyline in patients unable to tolerate the side effects of amitriptyline. Since the serotonin reuptake blocker, fluoxetine, was apparently without effect, it would appear that the potentiation of norepinephrine action, shared by desipramine and amitriptyline, may be an important mediator of the analgesic action of tricyclic antidepressants.

Pain Assessment

The purpose of these human pain studies is to develop psychophysical and behavioral models of pain perception in humans and to utilize these models in the understanding of pain mechanisms in humans and the development of new methods of pain control. We have developed an interactive staircase method which is a major refinement of the multiple random staircase method of psychophysical assessment. This method provides information about perceived intensity in units of stimulus intensity rather than in units of perceptual magnitude, provides information about an individual's scaling abilities, and provides information about the time course of an analgesic effect.

This year both the interactive staircase method and conventional psychophysical scaling procedures were used to assess the influence of meditation and progressive relaxation on the perception of thermal stimuli. The results show that meditation or a sitting control significantly reduced pain ratings in comparison to either no treatment or progressive muscle relaxation. In contrast, a questionnaire revealed that the subjects practicing progressive relaxation thought their method was the most effective. The results of both the staircase method and the scaling method were similar, with the staircase method showing higher sensitivity. This study is the first demonstration that meditation reduces experimental pain ratings. The surprising efficacy of the sitting control suggests that just taking time out from

daily activities and the freedom to pursue an individual relaxation strategy may be as efficacious as any routinized method.

Another study utilizing the interactive staircase method showed the effects of 50% nitrous oxide on thermal pain sensitivity. In comparison to oxygen, nitrous oxide significantly increased the stimulus temperatures required to maintain the same pain responses. The staircase method demonstrated both the time course and magnitude of an analgesic effect and the results show that it can be used to assess the kinetics of analgesia.

Another experiment used a method developed in our laboratory last year in which a track-ball is used to continuously measure pain levels on a graphic analog display presented by a computer. In this experiment the perception of thermal stimuli and mechanical thresholds were assessed on the hand during ischemic block of myelinated fibers. No differences in the intensity, onset latency or duration of ratings were noted for either innocuous or noxious thermal stimuli. These findings indicate that the component of thermal pain mediated by C fibers is a significant factor in the perceived pain sensation and that A-beta and A-delta fiber activity exert minimal if any influence on the perception of C-fiber mediated heat pain sensation.

Relief of Acute Pain

These studies consist of a series of clinical trials evaluating the efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as the model. This year we have continued our evaluation of spiradoline, a kappa receptor agonist. Preliminary data demonstrated little analgesic activity when the drug was administered by IM gluteal injection. In contrast, we have found that IM injection into the deltoid region of the highest dose results in analgesic activity comparable to 10 mg of morphine. Further study is needed to confirm this analgesic effect and to assess the side effect liability of the drug.

A parallel series of studies evaluated the safety and efficacy of drugs used for anxiety relief using the same oral surgery model. A study of oral triazolam in combination with nitrous oxide indicated that the 0.125 dose resulted in marginal therapeutic effect. The two higher doses, 0.25 and 0.5 were indistinguishable except that the 0.5 dose resulted in prolonged recovery. Based on these results, the combination of 0.25 triazolam and nitrous oxide was selected for further study in a factorial design with a placebo control and intravenous diazepam as a positive control. Results suggest that the effects of triazolam can be distinguished from placebo and triazolam appears to be comparable to intravenous diazepam. Further clinical trials will be needed to evaluate the therapeutic effectiveness of this oral drug as a substitute for parenteral sedation with diazepam or other oral benzodiazepines in combination with nitrous oxide.

Publications

Baum, B.J., Burstone, C.J., Dubner, R., et al.: Advances in Diagnosis and Detection of Oral Diseases. Advances in Dental Research, 3:7-13, 1989.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-22 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design and Computer Interfacing of Neurophysiologic Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Frederick J.	Electronic Engineer (Instru)	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects involve the design and construction of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory and central computer installations. Electronic circuit design, microcomputers, and assembly or machine language programming may be used in these instruments or interfaces.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-16 NA

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacologic Modulation of Neuroendocrine Responses to Stress and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hargreaves, Kenneth	Staff Fellow	NA NIDR
Dionne, Raymond	Research Dentist	NA NIDR
Costello, Ann	Post Doctoral Fellow	NA NIDR
Dubner, Ronald	Chief	NA NIDR

COOPERATING UNITS *(if any)*

Goldstein, David	Staff Scientist	ETB NHLBI

LAB/BRANCH
Neurobiology and Anestheisology Branch

SECTION
Clinical Pharmacology Unit, Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesa, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.15	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are 1) to evaluate the neuroendocrine responses to surgical stress and inflammation, 2) to determine the analgesic and anti-inflammatory effects of prototype and novel drugs which alter either the synthesis or the receptor activation of neuroendocrine mediators in an established animal model of inflammation, and 3) to evaluate the clinical utility of these novel drugs in controlled clinical trials.

This year, we have extended our research on factors factors regulating the pituitary release of beta-endorphin under conditions of stress. Subjects undergoing the removal of impacted third molars received epinephrine in conjunction with local anesthesia which resulted in a 30-fold increase in circulating epinephrine levels. Elevated epinephrine levels significantly reduced beta-endorphin levels in response to surgical stress in comparison to a control group which did not receive epinephrine. These results do not support the hypthesis of a stimulatory effect of epinephrine on endorphin release and, instead, suggest that an inhibitory relationship may exist in humans experiencing stress.

We have also extended our research on bradykinin with the use of microdialysis probes which permit accurate measurement of the levels of inflammatory mediators in inflamed tissue. In oral surgery patients, tissue levels of iBK increased nearly 10-fold over a 4 hour observation period. The peak in tissue concentrations of iBK precedes the peak in pain report and the two factors are significantly correlated. Blockade of bradykinin receptors with a bradykinin antagonist in a parallel study in rats significantly blocked hyperalgesia, hyperthermia, and edema. These observations suggest that bradykinin release is involved in clinical pain and that blockade of its release or its receptor mediated actions may result in analgesia and other anti-inflammatory effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-16 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Gaughan, Alexandra	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
2.15	1.35	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are (1) to assess psychophysical methods of experimental pain measurement: (2) to assess clinical pain measures in a dental setting: (3) to use these methods to evaluate underlying mechanisms of clinical pain syndromes; and (4) to evaluate the mechanisms and efficacy of pharmacological and non-pharmacological pain-control agents. The interactive computer-based staircase scaling method was used in five experiments and the continuous track-ball method in two experiments.

The first experiment found that 5-weeks of practicing a meditation procedure or spending the same time sitting quietly reduced pain ratings of thermal stimuli in comparison to progressive muscle relaxation or a no treatment control.

The second experiment manipulated baroreceptor activity by sitting or reclining postures. No difference was found in pain sensitivity, in contrast to a previous demonstration of analgesia when reclining in comparison to standing.

The third experiment assessed the magnitude and fast time course of nitrous oxide analgesia. The results demonstrated the sensitivity of the model, its ability to assess fast kinetics, and the weak potency of nitrous oxide.

The fourth experiment provides evidence that cardiac chest pain in patients with normal coronary arteries does not represent general hyperalgesia and is actually accompanied by reduced somatic pain sensitivity.

The fifth experiment found that subjects classified as "defensive" by a paper and pencil test rated thermal stimuli as equally intense but less unpleasant as non-defensive subjects.

The sixth experiment found little change in the latency, magnitude or duration of pain responses to thermal stimuli to the hand after an ischemic block that affects both A-beta and A-delta primary afferent fibers.

The seventh experiment showed that first and second pain sensation, and their suppression or summation by peripheral or central factors could be adequately evaluated by the track ball method in naive subjects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00276-12 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Brain Stimulation Analgesia in the Control of Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Max, Mitchell B.	Neurologist	NA NIDR

COOPERATING UNITS *(if any)*

Young, Ronald, UCLA, Los Angeles, California
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of the study are (1) Assess the effectiveness of chronic electrical stimulation of midbrain sites for the relief of chronic pain in humans; (2) Evaluate the efficacy and mechanisms of traditional narcotic analgesia and compare these to chronic electrical stimulation of midbrain sites; (3) Validate experimental models of pain and their potential diagnostic use in chronic pain patients; and (4) Determine and compare the impact of both traditional narcotic and chronic electrical stimulation therapies on the functional, intellectual and emotional well being of these patients. The effects of chronic brain stimulation in surgical patients will be compared to the effects of narcotics previously administered to patients and to effects of narcotic regimes in non-surgical chronic pain patients. In addition, the effects of narcotics on perceptual and neural mechanisms of experimental induced pain will be assessed in pain-free volunteers.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO DE 00286-11 NA

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Therapeutics for Acute Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond	Research Pharmacologist	NA NIDR
Costello, Ann	Postdoctoral Fellow	NA NIDR
Hargreaves, Kenneth	Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*

Samaha, Ramona	Nurse	CC Nursing

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.45	1.15	0.3

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as a model for minor surgical procedures with associated intraoperative and postoperative pain and perioperative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety.

Spiradoline, a novel analgesic which acts at the kappa opioid receptor, was evaluated in 75 oral surgery patients in comparison to 10 mg of IM morphine and placebo. Data from subjects who received the IM injection in the gluteus did not show any activity for spiradoline and only minimal activity of morphine. Preliminary results for IM injection in the deltoid suggest that spiradoline at 4.8 micrograms per kilogram is equipotent with 10 mg of morphine but that its duration of activity is only 30-60 minutes. The sample size is being increased to 100 subjects to allow collection of sufficient data following deltoid administration to support these preliminary findings. A second study is evaluating the analgesic efficacy of two antihistamine drugs in comparison to ibuprofen and placebo. Terfenadine, a H_1 histamine receptor blocker, and ranitidine, a H_2 histamine receptor blocker are administered one hour prior to oral surgery and the onset and severity of postoperative pain monitored for four hours postoperatively. A demonstration of analgesic activity for either antihistamine will provide a rationale for a factorial study comparing ibuprofen alone, an antihistamine alone, and the combination of an antihistamine and ibuprofen, to placebo. Drugs acting through separate mechanisms should result in additive analgesia and the lack of central effects for these two drug classes should provide a therapeutic advantage without increased side effects in ambulatory patients.

A parallel series of investigations are evaluating the safety and efficacy of drugs used for anxiety relief in patients undergoing minor surgical procedures with local anesthesia. A dose-range study of the combination of oral triazolam, a benzodiazepine, and nitrous oxide indicated that 0.25 mg of triazolam in combination with nitrous oxide resulted in therapeutic benefit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-11 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacological Characterization of Synpatic Circuitry in the Dorsal Horn

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ruda, Maryann	Research Biologist	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Humphrey, Emma L.	Bio. Lab. Tech (Elec. Mic.)	NA NIDR
Noguchi, Noichi	Visiting Fellow	NA NIDR
Hylden, Janice	Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neurocytology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesd, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.35	2.25	2.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The neural circuitry of the dorsal horn of the spinal cord forms the basis for the mechianisms of pain and analgesia. Our lab has made significant inroads in understanding the neuronal connectivity which subserves these sensory phenomena through experiments involving multiple markers to identify interactions between neural elements.

Fos protenin coded by the c-fos proto-oncogene appears to be useful as a marker for neuronal activity. In a rat model of peripheral inflammation and hyperalgesia, Fos protein has been shown to increase in the nuclei of a subpopulation of neurons concentrated in laminae I, II and V, areas of the dorsal horn known to receive noxious inputs. Dynorphin, an opioid peptide which also up-regulates in the same animal model can be colocalized to a subpopulation of Fos-labeled neurons using either a double immunocytochemical method or immunocytochemistry in combination with in situ hybridization histochemistry. These data provide insights into the transcriptional events that occur in response to peripheral inflammation and hyperalgesia.

A rat model of reduced small diameter primary afferent input to the spinal cord has been used to examine the role of these afferents in neuronal activiation and behavioral responsivity in a rat model of peripheral inflammation and hyperalgesia. Capsaicin, injected neonatally into rat pups destroys a subpopulation of nociceptive primary afferent neurons. However, they retain their ability to behaviorally withdraw from noxious stimuli but exhibit less dorsal horn Fos-activation than control animals. Thus inflammation-induced hyperalgesia likely involves non-capsaicin sensitive primary afferent axons while Fos activation appears to be closely related to capsaicin sensitive primary afferent axons.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-11 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microinjection of analgesic agents into the Medullary Dorsal Horn of the Behaving Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas, David	Staff Fellow	NA NIDR
Kenshalo, Daniel	Research Biologist	NA NIDR
Dubner, Ronald	Chief	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.30	1.20	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We examined the effects of ST-91 microinjected into the medullary dorsal horn (MDH) on the ability of monkeys to detect small temperature increases in the noxious heat range. The monkeys were trained to detect temperature changes of 0.4, 0.6 and 1.0¡C (T2) superimposed on an initial temperature shift to 46¡C (T1). Behavioral detection latency and the percentage of correct detections were used as indices of the monkeys perceived intensity of noxious thermal stimulation. ST-91 (1, 3, 10 and 30 μg), an alpha-2 adrenergic agonist, and morphine (5 μg; a dose previously found to be effective in this paradigm), an opiate agonist, were microinjected into the MDH. ST-91 in a dose- and stimulus-dependent fashion, and also morphine produced a decrement in the monkey's ability to detect noxious heat increments. There was no effect on the detection of innocuous coolings or visual stimuli, indicating that effects on the detection of noxious heat are independent of motivational, motoric and attentional factors. Systemic idazoxan (an alpha-2 receptor specific antagonist), but not prazocin (an alpha-1 receptor specific antagonist), naloxone (an opiate antagonist) or saline, significantly attenuated the effects of ST-91 on the detection of all noxious T2s. Morphine's effect were attenuated by both naloxone and idazoxan. These data demonstrate a pharmacologically-specific effect of an alpha-2 agonist on the perceived intensity of noxious heat stimuli at the MDH, the earliest central relay for noxious information. Further, they demonstrate an asymmetrical interaction of opioid and adrenergic pain control systems.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-09

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 chorecters or less. Title must fit on one line between the borders.)*

Discrimination of Thermal Stimuli Applied to the Face in Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenshalo, Jr. Daniel	Research Biologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Thomas, David	Postdoctoral Fellow	NA NIDR
Iwata, Koichi	Visiting Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project correlates behavioral responses with responses of neurons in the medullary dorsal horn produced by noxious thermal stimuli on the face of behaving monkeys. Medullary dorsal horn neurons encode noxious thermal information used by the monkey to perform a thermal detection task. Two populations of nociceptive neurons are found in the medullary dorsal horn: nociceptive-specific (NS) and wide-dynamic-range (WDR) neurons. Nociceptive-specific neurons respond only to intense mechanical and thermal stimulation, whereas wide dynamic range neurons respond to low-threshold mechanical stimuli, but their largest response is to noxious mechanical and thermal stimulation. We found that a subpopulation of wide-dynamic-range neurons encoded the intensity of noxious thermal stimulation and could account for the monkey's ability to detect noxious thermal stimulation. The discharge of nociceptive-specific neurons could not account for the monkey's ability to detect noxious thermal stimulation. An argument that has been used against the role of wide-dynamic-range neurons in pain sensation is that the large size of the receptive fields would not allow for localization of a noxious stimulus. However, we found that there was a strong correlation between neuronal discharge and the monkey's ability to detect noxious thermal stimulation only when the stimulus was located in the central, most sensitive portion of the receptive field. When the stimulus was located in the peripheral, less sensitive portion of the receptive field, a correlation was not found between neuronal discharge and the monkey's ability to detect noxious thermal stimulation. These data suggest that information from only the central portion of the wide-dynamic-range receptive field is important for pain sensation and the localization of a painful stimulus. Information from the peripheral portion of the receptive field does not appear to be important for oral-facial pain sensation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00366-08 NA

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analgesic Mechanisms in Patients with Chronic and Acute Postoperative Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Max, Mitchell	Neurologist	NA NIDR
Zeigler, Daryl	Visiting Fellow	NA NIDR
Gracely, Richard	Research Psychologist	NA NIDR
Bennett, Gary J.	Neurophysiologist	NA NIDR
Lynch, Sue	Medical Staff Fellow	NA NIDR
Dubner, R.	Chief, NA	NA NIDR

COOPERATING UNITS *(if any)*

Craig, Bradene	Nurse	CC Nursing
Muir, Joanne	Nurse	CC Nursing
Benjamin, Janice	Nurse	CC Nursing

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to elucidate the principles of treatment of acute and chronic pain syndromes, with particular attention to the drug treatment of pain caused by nerve injury and surgery.

In randomized, double-blind, crossover studies in 84 patients with painful diabetic neuropathy, 74% of patients had moderate or better relief with amitriptyline (an antidepressant blocking both norepinephrine and serotonin reuptake), 61% with the selective norepinephrine reuptake blocker desipramine, 48% with the selective serotonin reuptake blocker fluoxetine, and 41% with placebo. These results suggest that desipramine is a useful alternative in patients unable to tolerate amitriptyline side effects, that fluoxetine has little effect at the dose tested, and that norepinephrine may be a mediator of the analgesia produced by tricyclic antidepressants.

In a study of the noradrenergic agonist clonidine, administered by transdermal patch, 54% of 24 patients obtained moderate relief or better, but pain scores were not significantly lower than recorded during placebo treatment. A subset of 8 patients had a marked analgesic response to clonidine and not placebo, and in many of these that differential response has been replicated. Clonidine may be an effective treatment for a subset of patients with neuropathic pain, but studies need to be designed to maximize the power to detect response in a subset.

In patients with pain following orthopedic or gynecological surgery, a single does of desipramine, 50 mg, did not potentiate the effects of intravenous morphine unlike results in animals and in a small trial at another center that used chronic desipramine treatment. Future studies of this possible interaction should use either single doses higher than 50 mg or chronic treatment.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00413-05 NA

PERIOD COVERED

October 1, 1989- September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Neuropathy of Peripheral Nerve in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bennett, Gary J.	Research Biologist	NA NIDR
Kajander, Keith C.	Postdoctoral Fellow	NA NIDR
Wakisaka, Satoshi	Visiting Fellow	NA NIDR
Laird, Jennifer M.A.	Adjunct Postdoctoral Fellow	NA NIDR
Iadarola, Michael	Research Pharmacologist	NA NIDR

COOPERATING UNITS *(if any)*

Munger, P.	Professor	Hershey Medical Center, Hershey, PA
Ochoa, J.	Professor	Good Samaritan Hospital, Portland, OR
Seybold, V		University of Minneapolis, Minneapolis, MN

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A chronic constriction injury to the sciatic nerve of the rat produces a painful peripheral neuropathy that is very similar to the neuropathic pain states seen in man. In the peripheral nervous system, light- and electron-microscopy showed that within 8 days the injury results in a partial and differential deafferentation that interrupts nearly all of the myelinated axons but spares a large majority of the small unmyelinated axons. The neuropathy has been found to produce a near total depletion of norepinephrine, in the axons that comprise the sympathetic perivascular plexus. The time course of this depletion has been shown to parallel the time course of the rats' temperature abnormality. The temperature abnormality has been confirmed with infrared thermography. A time course study shows that the temperature abnormality has a temporal evolution that resembles that seen in humans with causalgia/RSD. Specifically, the affected hindpaw is initially hot and then progresses to a chronic cold status. We have documented a substantial amount of short-term (hours to days) variability in the temperature abnormality. It is a common clinical assumption that the temperature abnormality seen in causalgia/RSD is due to abnormal sympathetic discharge. However, this assumption may often be incorrect. Many animals with an affected extremity that is abnormally cold have no NE-positive axons on their vasculature, and thus the cold skin cannot be due to excessive vasoconstrictor discharge. The hindpaw temperature abnormality has a paradoxical response to α_2-adrenoceptor blockers. Instead of the normal warming, the affected hindpaw becomes cold. Previous work has shown that the neuropathy evokes a large increase in the level of the opioid peptide, dynorphin A1-8, but not in the level of enkephalin, in lumbar dorsal horn neurons. Measurement of mRNA levels have now shown that there is a corresponding up-regulation of the dynorphin gene but not of the enkephalin gene. The experimental neuropathy evokes marked changes in the levels of receptor binding for opioid ligands. At 2 days post-injury, there is an increase in binding in the superficial laminae of the dorsal horn for the mu, delta, and kappa sites. At 10 days, there is a decrease of mu binding in lamina V, a decrease in delta binding in laminae I-II, and also a decrease in kappa binding in laminae V and X. These data suggest that the neuropathic pain state may activate endogenous pain modulating circuitry, and also suggest that neuropathic pain patients may have an altered response to narcotic analgesics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-05 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CNS Neurotransmitter Regulation During Peripheral Inflammatory States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Iadarola, Michael J.	Research Pharmacologist	NA NIDR
Yeung, Choh Lun	Biologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanism Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.1	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns the role of central nervous system (CNS) neurons in sensory processes especially as they relate to pain and the control of pain. The main broad questions are: what are the molecular mechanisms and what are the physiological mechanisms regulating spinal nociceptive neurochemistry. A model of peripheral inflammation has been developed to investigate the relationship between spinal cord neurons, in particular those containing the opioid peptides enkephalin and dynorphin (but also other neurotransmitters and neuropeptides) and abnormal primary afferent input. Alterations in gene expression are assessed by measurement of peptide, protein of mRNA levels and cellular localization techniques. Our present goal is to understand the molecular mechanisms controlling transcription of the dynorphin opioid gene.

Transcriptional control involves specific DNA binding proteins that recognize nucleotide sequences in the promoter region of the dynorphin gene. These proteins are often first identified as oncogenes and we have shown that inflammation induces an increase in mRNA levels coding for the c-fos proto-oncogene. This nuclear protein is involved in transcriptional regulation and the increase in c-fos mRNA precedes the increase in dynorphin. Analysis of the Fos protein revealed a sequential, long-lasting increase in multiple Fos-immunoreactive proteins in nuclei isolated from dorsal spinal cord. The increase in Fos proteins far outlasts the elevation in c-fos mRNA and, essentially, occurs throughout the entire period of peripheral symptomology. These basic observations are being extended to other nuclear transcription factors such as NGFI-A and to an analysis of their binding sites on the dynorphin gene.

Using our antibody to the c-fos protein we have also established a gel mobility shift assay to determine potential Fos binding sites on target sequences of DNA. We have shown that the Fos proteins synthesized during inflammation can form AP-1 complexes with a double stranded oligonucleotide containing the AP-1 consensus sequence. Current studies are using this technique to assess AP-1 like sites in the dynorphin and enkephalin promoters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00440-04 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dorsal Horn Circuitry Related to Pain: Inflammation-induced Plasticity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hylden, Janice L.K.	Staff Fellow	NA NIDR
Nahin, Richard L.	Staff Fellow	NA NIDR
Traub, Richard J.	Postdoctoral Fellow	NA NIDR
Thomas, David	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the present research project, we have employed a combination of physiological, pharmacological and behavioral approaches to the study of somatosensory systems related to pain and analgesia.

The activity of superficial lumbar dorsal horn neurons was studied in rats before and after application of κ-opioid agonists to the spinal cord. The resulting changes in receptive field and neuronal responsiveness were compared to those observed in the presence of peripheral inflammation. The non-peptidergic κ-opioid agonist U-50,488H had both facilitatory and inhibitory effects on superficial dorsal horn neurons. The facilitatory effects often coincided with expansion of receptive fields. The endogenous κ-ligand, dynorphin, also had facilitatory effects on the receptive fields of superficial dorsal horn neurons. Similar expansions of receptive fields had been observed previously during inflammation or tissue injury. We postulated that enhanced excitability and expansion of receptive fields are related to inflammation-induced increases in dynorphin peptide.

We characterized the spinal antinociceptive activity of both opioid and adrenergic agonists during hindpaw inflammation by injecting agents intrathecally and determining thermal paw withdrawal latencies. Agonists with μ-, δ- or α_2 selective activity exhibited a leftward shift in the dose-response curve in rats with inflamed hindpaws as compared to control. The mechanism of enhanced efficacy may depend on the known synergistic action of opioid and adrenergic agonists at the spinal level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00460-03 NA

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuroanatomical mechanisms of pain transmission and modulation

PRINCIPAL INVESTIGATOR.*(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nahin, Richard	Staff Fellow	NA NIDR
Hylden, Janice L.K.	Staff Fellow	NA NIDR
Iadarola, Michael	Research Pharmacologist	NA NIDR
Dubner, R.	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

NIDR, NIH, Bethesda, Maryland 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using a number of complementary anatomical and biochemical techniques, the effects of hyperalgesia on the rat nervous system were studied.

Lumbar spinal cord neurons were retrogradely labeled after injections of the inactive subunit of cholera toxin (subunit-B) into the caudal midbrain of normal rats. Spinal cord sections from these animals were processed for dynorphin (DYN) immunoreactivity. A larger percentage of projection neurons were contacted by DYN immunoreactive varicosities ipsilateral to a hyperalgesic hindpaw. This ipsilateral-contralateral difference was most pronounced in lamina I, where significantly more DYN immunoreactive varicosities were in apposition to retrogradely-labeled neurons.

Spinal cord sections of animals with a hyperalgesic hindpaw were processed for gluatamic acid decarboxylase (GAD) immunoreactivity. We observed that the number of neurons immunoreactive for GAD was substantially greater in spinal cord sections ipsilateral to the hyperalgesic hindpaw. In concurrence with this observation, an enzymatic-based spectrofluorometric assay of spinal cord revealed a 36% increase in gamma amino butyric acid (GABA) content ipsilateral to the hyperalgesic hindpaw.

We examined alterations in the content of calcitonin gene-related peptide (CGRP) in primary afferents innervating a hyperalgesic hindpaw. It was found that there is significantly more CGRP within L_4 dorsal root ganglia associated with the hyperalgesic vs. normal paw.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00509-01

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurosensory mechanisms in painful peripheral neuropathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bennett, Gary J.	Research Biologist	NA NIDR
Gracely, Richard H.	Research Psychologist	NA NIDR
Lynch, Sue A.	Clinical Associate	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanism Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

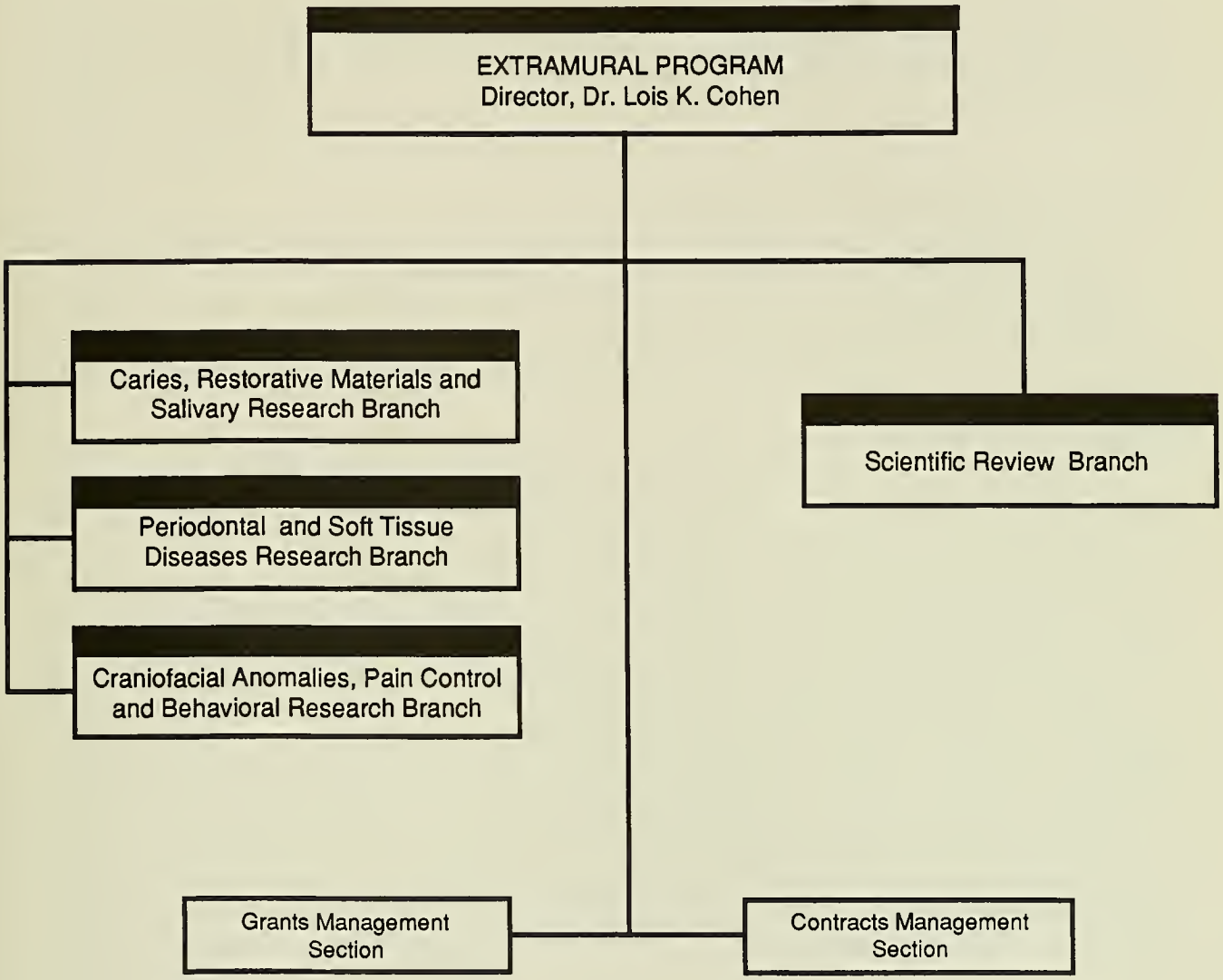
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Detailed psychophysical analyses were performed of the abnormal pain sensations found in patients with reflex sympathetic dystrophy/causalgia. Touch-evoked pain (mechanical allodynia) was elicited by v. Frey hair stimuli that were near threshold for detection; on contralateral normal skin these same stimuli evoked innocuous tactile sensations. Transcutaneous electrical stimuli also evoked pain at intensities near threshold for detection; on normal skin these same stimuli evoke innocuous tactile sensations and activate Aβ low-threshold mechanoreceptors (Aβ-LTMs). Reaction times for the painful sensation evoked by threshold-strength electrical stimuli were too fast to include a contribution from unmyelinated C-fiber nociceptive afferents. During an ischemic block, the pain evoked by threshold-strength v. Frey and electrical stimuli disappeared at the same time as impulse blockade in Aβ-LTMs; pain returned in concert with Aβ-LTM function. In these same patients, thresholds for heat-evoked pain and stimulus-response ratings for suprathreshold noxious heat stimuli were essentially normal; thus indicating that C-fiber nociceptors were not abnormally sensitized and did not contribute to the touch-evoked allodynia. These observations show that the touch-evoked pain of many RSD patients is due to an abnormality of CNS processing of input that normally evokes innocuous tactile sensations. In a subset of patients whose touch-evoked pain was otherwise conclusively shown to be Aβ-LTM dependent, ischemic block relieved the allodynia within 3-6 min., a time that is prior to any detectable impulse blockade in Aβ-LTMs. This observation indicates that a subset of patients have pain influenced by some aspect of circulatory occlusion other than its effect on impulse conduction. Lastly, we analyzed an advantageous case who had a focus of extremely painful sensibility that was separate from areas that supported mechanical allodynia of the Aβ-LTM dependent type. Infiltration of local anesthetic into the painful focus completely eliminated the patient's allodynia without any indication of anesthetic spreading to the previously symptomatic skin. The implication of this observation is that a focus of ongoing pain input dynamically maintains an abnormal CNS processor that is responsible for the "misread" of Aβ-LTM input.

Extramural Program

NATIONAL INSTITUTE OF DENTAL RESEARCH



OFFICE OF THE DIRECTOR, EXTRAMURAL PROGRAM (EP)

The Extramural Program (EP) of the National Institute of Dental Research (NIDR) is responsible for the development, review, funding, and management of research grants and contracts for projects, research training, and manpower development. Three program branches support a wide spectrum of research that extends from basic research to epidemiology and new methods of prevention, diagnosis, and treatment.

The new Director entered the program in mid-November with a vision to broaden the scope of the program in accordance with the Institute's new program plan for the decade of the nineties. One of the first accomplishments was the production of an extramural report of funding patterns throughout the decade of the 80's in order to provide baseline data for the new decade. While specific data are contained in the report to the January 1990 National Advisory Dental Research Council, certain highlights should be noted.

Research project grants continue to predominate, amounting to 63.9 percent of the portfolio in FY 1989. Since FY 1985, the percentage devoted to other research grants has doubled to 11.3 percent in FY 1989, largely owing to the Dentist Scientist Award (DSA) program, while the percentages for centers, research training and research contracts have decreased slightly (to 15.0, 5.5 and 4.3 percent respectively). Looking back toward the eighties, periodontal diseases and dental caries received the most funds ($168.5 and $133.4 million respectively); congenital craniofacial malformations, restorative materials, orofacial pain and sensory-motor dysfunctions, salivary glands and secretions, soft tissues diseases, mineralized tissues, and dentofacial malrelations received between $25.3 and $52.2 million; and behavioral studies, fluoride studies, implants, replants and transplants, acquired craniofacial defects, pulp biology and nutrition research received between $1.4 million and $16.6 million. Major increases were evidenced in the periodontal research area, salivary glands and secretions and soft tissue diseases (including AIDS). Some decreases were apparent in mineralized tissues, implants and nutrition. Looking forward toward the areas of focus for the nineties, diseases and disorders of dental and craniofacial structures received 64.7 percent of FY 1989 extramural funds; systemic diseases and oral manifestations received 23.1 percent; and technologies and health behavior received 12.2 percent.

A major new initiative in FY 1990 was the stimulation of enhanced collaboration between the private sector and extramural researchers. Presenting this information on the state of extramural research funding to the American Association of Dental Research Institutional Section at their meeting in Cincinnati, to the American Dental Association Council on Dental Materials, Instruments and Equipment in Chicago, to the Extramural Program Management Committee ad hoc committee on alternative funding strategies or with individual corporations, heightened visibility and interest was in evidence by both the private sector and extramurally-

located researchers. The Request for Applications (RFA) on clinical dental research core centers included this item, the announcement on a new funding ceiling for program project grants similarly encouraged collaborative funding and plans for a FY 1991 working conference, "Industry-Government-University Research Alliances in the Public Interest: Models for the Future" was generated by the extramural community to include NIDR and NIH involvement. It is the hope that NIDR can play a major leadership role in the NIH--to expand alternative funding strategies by developing working guidelines which can preserve scientific integrity and forge productive partnerships to advance dental and other biomedical/behavioral research...and to enhance the U.S. competitive position globally. Taking advantage of National Science Foundation initiatives such as the University of Texas--San Antonio model of an Industry-Academic Collaborative Research Center, and other such models, NIDR can learn and act towards these ends. Discussions with the American Fund for Dental Health (AFDH) staff have taken place to explore respective roles in facilitating such initiatives, including the potential of AFDH to host a meeting of potential research sponsors.

Additional effort to develop partnerships in funding was made by staff within the NIH family of Institutes, Centers and Divisions and with other Federal agencies. For example, the new Agency for Health Care Policy and Research and the National Institute for Drug Abuse were able to fund some of NIDR generated grants. Meetings with the Veterans Administration, the National Institute of Aging and the NIDR are continuing to focus on a new manpower development vehicle in the geriatric dental research area. Internationally, staff is working to facilitate essential linkages between U.S. researchers and institutes and foreign investigators and to develop collaborative multinational sources of funding.

Processes have begun to develop plans for the evaluation of Center grants (P50), the Dentist Scientist Award program, research training (including short-term training) and manpower needs. Assessments of Requests for Applications and Program Announcements are being conducted as well as Merit Awards, FIRST Awards and Small Grants.

A broad array of award mechanisms is available to extramural scientists ranging from small grants to regular research project grants to large center grants. During FY 1990, the NIDR Extramural Program (EP) made 601 research and training awards for an estimated total of almost $ 95 million including 382 investigator-initiated research project grants ($62.4 million). The 382 research project grants accounting for 66 percent of the EP budget comprised 291 regular grants for $43.5 million; 16 program project grants for $10.5 million; 34 FIRST awards for $3.0 million; 21 MERIT awards for $4.1 million; and 20 small business innovative research awards for $1.2 million. Of the 382 research project grants, 104 were new awards or competing renewals.

The NIDR also funded 21 center grants for an estimated total of $15.7 million which was about 16.5 percent of the EP budget. The center awards supported four non-categorical centers, one Oral Health in Aging Center, five Periodontal Diseases Research Centers, two Caries Research Centers, one Pain Research Center, three Craniofacial Anomalies Research Centers, three Materials Science Research Centers. Two Clinical Dental Research Core Centers were also awarded in FY 1990.

The "Other Research" budget, approximately 11.4 percent of the EP budget, funded 36 small grants for $770,154 and 15 small instrumentation grants for $311,556. It also provided $79,477 as full or partial support for 3 conferences and $161,000 for the National Institute of General Medical Sciences Minority Biomedical Research Program. Eighty-two percent of the "Other Research" budget or $9.0 million funded 17 career development awards, 21 individual physician scientist for dentist awards, 25 individual dentist scientist awards and 9 institutional dentist scientist awards (81 individuals), all together providing support for a total of 144 individuals.

The $5.8 million National Research Service Award (NRSA) budget was disbursed as follows: $4.3 million in support of 27 regular institutional training programs and $364,624 in support of 19 institutional awards to provide short-term (summer) training opportunities for dental students; and $1,024,750 for 33 individual fellows. The 27 regular institutional training grants provided 24 predoctoral and 31 postdoctoral training positions while the short-term awards supported a total of 161 dental students.

NIDR extramural support for AIDS-related research reached $2.7 million. Included in the portfolio were 2 program project grants, 10 regular research grants, 1 FIRST award, and 1 career development award. NIDR awarded 2 Academic Research Enhancement Awards (AREA) in FY 1990 for a total of $170,587.

Regarding minority programs, the NIDR provided $144,630 in direct grant support for 1 regular research grant to a minority institution. In addition, the NIDR funded 14 minority research supplement awards totalling $641,839, a 300 percent increase over FY 1989. The NIDR contribution to the Minority Biomedical Research Support Program, administered by the National Institute of General Medical Sciences was $161,000, a 10 percent increase over FY 1989. These funds supported dental research projects at 3 different institutions. In addition, $70,282 was provided to the Minority Access to Research Careers program (MARC), also administered by the National Institute of General Medical Sciences in support of 10 ancillary training activities. NIDR contributed $15,000 to support 10 students on the Minority High School Student Research Apprenticeship Program, administered by the National Center for Research Resources. Funding ($10,870) also was provided for a short-term training program intended only for minority dental students. NIDR supported a minority individual on the NIH Intramural NRSA Institutional Training Program for $28,250. The total support for minority programs in FY 1990 was $1,071,871 which was greater than an 80 percent increase over FY 1989.

There were thirteen contracts ($2,098,000), six supplements ($286,000) and ten interagency agreements ($2,028,000) awarded during FY 1990. Contract support for AIDS-related research totaled $1,041,000.

EP activities were varied and extensive. The most demanding and time-consuming efforts of the year involved review by the Scientific Review Branch of 19 applications for Clinical Dental Research Core Centers. These reviews utilized the "applicant interview" format. The applicant interview was preceded by a "triage" review which reduced the number of

competitive applications from 19 to 10. Three Clinical Dental Research Core Center applicants then were site visited and two were subsequently funded.

Another major activity was the development of a Request for Applications (RFA) for the National Research Service Award - Institutional grants. Program Announcements which were issued were: "Nutrition Research and Oral Health", "Research on Salivary Glands and Secretions", and "Pathogenesis of Joint, Muscle, and Inflammatory Pain as Related to Temporomandibular Disorders". A new publication was issued, "NIDR General Guidelines for Program Project Grants."

The Programs Advisory Committee met twice during the year and provided needed direction on the subjects of oral cancer and the biology of the pulp. Increasingly important during FY 1990 was the stimulation of research proposals which are relevant to the "Research and Action Program to Improve the Oral Health of Older Americans and Other Adults at High Risk." Suggested designations for program priority in this area were brought to the National Advisory Dental Research Council for their discussion.

Extramural staff visited a number of grantee institutions as part of a continuing effort to familiarize NIDR management with relevant issues in the extramural community and to communicate to them the availability of NIDR funding opportunities and effective utilization of those opportunities as well as directions explicit in the Long-Range Research Plan for the Nineties. Staff also participated in a number of site visits, organized and/or participated in a number of workshops and conferences to stimulate further development of research advances.

RESEARCH TRAINING AND MANPOWER DEVELOPMENT

A RFA for institutional training grants (T32) was issued in the NIH Guide for Grants and Contracts in July 1990. It contained a number of changes in policy and guidelines: 1) although priority for appointments to a training grant continues to be dentists pursuing a basic science research, doctoral degree and dentists pursuing postdoctoral research training, appointment of individuals holding a Ph.D. degree pursuing traditional postdoctoral research training is allowed; appointment of traditional predoctoral trainees is still allowed; 2) the following paragraph, which was in several previously issued RFAs, does ***not*** appear, "Subject to availability of funds, it is the intention of the NIDR to provide stipend support until the completion of training for dentists already enrolled and participating in a doctoral degree program, in the event that a competitive renewal application is not funded"; 3) the training grant application should be relevant to the goals of the NIDR, as described in the *NIDR Long-Range Research Plan for the Nineties;* 4) in addition to addressing the issue of minority recruitment, program directors must include a section describing their proposed program of instruction about the responsible conduct of research; 5) although this RFA is issued to solicit training grant proposals primarily in the program areas of craniofacial anomalies and dental biomaterials, policy has been modified so that NIDR will accept applications in any area of biomedical/behavioral oral health research.

The fifth meeting of the program directors of the institutional (K16) Dentist Scientist Awards (DSA) took place in March 1990 in conjunction with the AADS/IADR/AADR meeting in Cincinnati. Issues discussed included: the impact of the budget on funding of new and renewal applications, numbers of positions to be funded for the next grant year, and carryover of unobligated balances and continued support for the awardees beyond the end of their fifth year; the problem of the first research grant support for new graduates; the meeting of the senior awardees in Bethesda in conjunction with the May Council meeting; recruitment, and sharing of names of qualified applicants; clinical certification of the awardees; circulation of a list of graduates available for employment; program balance of the awardees' clinical interests; the evaluation of the Physician Scientist Award for Dentists and the DSA program.

The first group of graduates or Clinical Research Scientists (CRSs) from the DSA program will be available for employment in the summer/fall of 1990. With the collaboration of the Office of Planning, Evaluation, and Communications, a system is being initiated to monitor the progress of the CRSs after completion of their training.

The NIDR sponsored a DSA Day in May, in conjunction with the National Advisory Dental Research Council meeting. Twenty individual awardees (K15s) and representatives from each of the institutional programs (K16s) participated. Each gave a brief presentation of their research project. This was followed by an open discussion of their experiences on the DSA

program. The following day, several gave presentations to the Council about their DSA program experience. The next day, under the auspices of the AADR's National Affairs Committee, they visited key congressional offices to become familiar with the legislative aspects of research support.

NIDR staff participated in discussions with counterparts in the NIA and VA to explore a new collaborative funding mechanism to support dental geriatric research training.

At the conclusion of this fiscal year, the NIDR will be supporting approximately 276 trainees, fellows, and clinical research scientists, at some stage of their research/clinical development. These individuals are being trained in the following program areas:

Program Area	Number of Individuals	Dollars[1]
Periodontal Diseases	65	2,861
Caries	24	935
Craniofacial Development	41	1,989
Salivary Glands & Saliva	22	1,011
Dental Biomaterials	16	435
Oral Soft Tissues	23	1,164
Pain	16	840
Dentofacial Malrelations & Trauma	7	294
Mineralized Tissues	9	544
Epidemiology	20	902
AIDS	2	108
Behavior & Disease, Dental Utilization & Treatment Outcomes	16	367
Oral Motor Functions	4	119
Nutrition	4	131
Pulp Biology	3	162
Dental Implants, Replants & Transplants	4	216
Totals	276	$12,078

[1] The dollar amounts (in thousands) are meant only to indicate the approximate amounts of manpower development and training funds in the respective program areas. They were obtained by adding individual and institutional awards. The latter figures were obtained by multiplying the number of positions by the average dollar amount per position for institutional program mechanisms, i.e., training grants (T32s) and institutional DSA (K16s).

Of the funds being expended for manpower development and training, approximately $6.1 million (44 percent) are in the Periodontal and Soft Tissue Diseases Research Branch, $3.4 million (25 percent) in the Craniofacial Anomalies, Pain Control, and Behavioral Research Branch, and $4.1 million (30 percent) in the Caries, Restorative Materials and Salivary Research Branch.

CARIES, RESTORATIVE MATERIALS AND SALIVARY RESEARCH BRANCH

In Fiscal Year 1989, the Branch supported 241 research project and center grants, research development contracts, interagency agreements, and research training and manpower development awards in the caries (108 awards), restorative materials (70 awards), and salivary (63 awards) research areas. Projects in dental caries research included epidemiological and clinical studies on caries experience in older Americans and in special populations, the role of hypobaric hypoxia in fluorosis-like hypomineralization of teeth, studies on the microbial etiology of root surface caries, development of artificial peptide sweeteners, mechanisms of bacterial attachment to teeth, and oral vaccines for caries prevention.

Research on restorative materials included development of a diagnostic system for recurrent caries; synthesis and evaluation of polymers for soft denture liners and maxillofacial materials; and preparation of composites subject to minimal polymerization shrinkage.

Research on salivary glands and their secretions included studies on enzymatic sulfation of mucus glycoprotein and the effects of drugs on this process; characterization and antimicrobial effects of histatins and proline-rich salivary proteins, and the effects of streptozotocin-induced diabetes on salivary secretion.

The following discussion of activities is presented in reference to research objectives as defined and coded in the NIDR Long-Range Research Plan, "Challenges for the Eighties."

A01 Determine the incidence and prevalence of dental caries among all target populations.

The prevalence of caries and periodontal diseases was determined in a group of over 65 year-olds, some of whom wore removable partial dentures. University of North Carolina investigators found that for blacks, coronal and root caries levels were greatest in teeth abutting the dentures. In whites, this was true for root caries only. Differences in coronal caries levels between abutment and non-abutment teeth were greater in older subjects, in males and in subjects who had difficulty eating; for root caries, the differences were greatest in subjects who had infrequent preventive dental visits and who had trouble eating.

A03 Further elucidate the cause of all types of dental caries.

University of Michigan investigators have studied the relationships between caries and bacterial and salivary parameters in children living in a Rio de Janeiro slum. Forty eight of 56 children were caries active. The patient-based caries prevalence rates for

the primary and secondary dentition were 86 and 53 percent, respectively. The corresponding tooth surface-based prevalence rates were 7.3 and 6.1 percent, respectively. There was a linear relationship between salivary *Streptococcus mutans* levels and surface-based prevalence rates in the primary dentition; no relationship was found with lactobacillus levels. The saliva secretion rate was inversely associated with the surface-based prevalence rate in the permanent dentition.

Clinicians at Case Western Reserve University have analyzed the bacterial flora associated with carious lesions in a Middle Eastern student population. Ninety nine percent of sites sampled on normal teeth had *S. mutans* present. In contrast, *S. mutans* was found in only 65 percent of incipient lesion sites and 60 percent of carious lesions. *Streptococcus sobrinus*, on the other hand, was present in only 8 percent of normal sites but was isolated from 35 percent of incipient lesion sites and 40 percent of carious lesions. A greater percentage of incipient sites in caries-active students had *S. sobrinus* (54 percent) than did similar lesions in caries-free students (16 percent). These data suggest that *S. sobrinus* may be a significant odontopathogen, in this population.

A04 Further characterize the composition and chemistry of plaque, tooth enamel, and other physiological systems.

Researchers at the Medical College of Georgia and the Karolinska Institute, Sweden, have found that chronic hypobaric hypoxia causes fluorosis-like hypomineralization of rat incisor enamel and that this fluoride sensitivity is limited to the late secretory or early transitional stage of amelogenesis. Pregnant rats were provided with low-fluoride food and water and at birth, neonates were randomly reassigned to dams. At 6, 13 or 17 days of age the dams and pups were exposed to hypobaric oxygen by placing them in a simulated altitude (5,200 m) chamber for 24 hours. The pups were killed at 24 days of age and the enamel of the mandibular molars was examined. Enamel from pups placed in the chamber at 17 days of age, or from control animals, was normal. Disturbances, similar to those found after exposure to high levels of fluoride, were seen in enamel of rats placed in the chamber at 6 and 13 days of age. Disturbances initiated during the secretory stage of amelogenesis (day 6) were more severe than those seen in rats placed in the chamber at day 13, when amelogenesis is at the early transitional stage. This suggests that enamel disturbances caused by fluoride and hypobaric oxygen share a common mechanism.

Certain bacteria that colonize oral surfaces produce an IgA protease, which splits immunoglobulin IgA1 rendering the antibody inactive against the bacteria. IgA proteases may serve as virulence or colonization factors for these bacteria. Scientists at the University of Florida, Gainsville, found that the percentage of IgA protease-positive organisms in tooth plaque was greater four hours after cleaning the tooth surface than before cleaning. They concluded that the IgA protease-positive organisms have an advantage over other organisms when colonizing a cleaned surface. IgA protease-positive strains of streptococci were better able to adhere to saliva coated

hydroxyapatite than were IgA protease-negative strains, suggesting that production of this enzyme may confer an additional advantage in colonizing tooth surfaces.

Investigators at the New England Medical Center and Tufts University have cloned the gene for the IgA protease of *Streptococcus sanguis*, using *E. coli* as host. The nucleotide sequence has been determined; it has a 5654 base open reading frame, which encodes for a 186 kDa polypeptide chain with protease activity. There is no obvious homology with protease genes of *Hemophilus* or *Neisseria*. The cloned product from *E. coli* has little or no similarity in primary structure to proteases of identical specificity from Gram-negative organisms. The product contains a pentapeptide which is characteristic of zinc-binding site of metaloproteinases. A protease negative mutant of *S. sanguis* has been constructed for use in determining the role of the protease in the pathogenesis of this organism.

A06 Elucidate the systems allowing bacteria to recognize and attach to different oral surfaces.

A group of oral bacteria, which are able to cause caries in animals and some of which are associated with dental decay in humans, has been termed "mutans" streptococci. They have some common physiological characteristics, including their ability to colonize teeth, but they are genetically and antigenically heterogeneous, and have been divided into six species. The mechanisms by which cariogenic bacteria attach to teeth has been studied, extensively. Microbiologists at Forsyth Dental Center found selective binding of "mutans" streptococci to salivary components adsorbed to hydroxyapatite (HA). Binding of all strains of *S. mutans*, *Streptococcus rattus* and *Streptococcus cricetus* to HA was enhanced by saliva. In contrast, binding of six of seven strains of *S. sobrinus* was not promoted by saliva. The binding of different species to HA was enhanced to varying extents by different salivary components. Adhesion of *S. mutans* was mainly promoted by the mucins, while binding of *S. rattus* and *S. cricetus* was mainly promoted by the acidic proline-rich proteins or amylase. Proline-rich protein 1 was more effective in promoting binding than prolyine-rich protein 3. In a related study, *S. cricetus* and *S. rattus* bound strongly to collagen treated HA, whereas *S. mutans* and *S. sobrinus* bound poorly or not at all. The investigators suggest that the collagen-binding ability of the former organisms may facilitate their penetration into exposed dentin and cementum and contribute to their cariogenic potential.

A12 Purify and characterize all potentially important *S. mutans* antigens for vaccine development.

There has been a great deal of interest in use of the non-toxic B subunit of cholera toxin (CTB) as an adjuvant for vaccines targeted for delivery to mucosal-associated lymphoid tissue. When antigens, which are weakly immunogenic, are conjugated to CTB their immunogenicity is markedly increased and they elicit serum and secretory antibodies. In some cases, conjugates prove ineffective, possible due to conformational changes induced in the CTB during chemical conjugation. Molecular biologists at

Virginia Commonwealth University have used gene fusion to avoid this problem. A synthetic nucleotide, encoding for 15 amino acid residues of the glucosyltransferase of *S. mutans*, was fused to the N-terminal end of the CTB gene. The protein, expressed in *E. coli*, retained the conformational features of CTB, possessed the antigenicity of both glucosyltransferase and CTB and was immunogenic when fed to mice. The preparation of chimeric antigens by genetic fusion illustrates an important new method of producing oral vaccines against caries, as well as against life threatening diseases. Another promising method of increasing the antigenicity of bacterial antigens is being tested at the University of Alabama at Birmingham. Cell-wall serotype carbohydrate (CHO) from *S. mutans* was purified extensively and incorporated into liposomes by hydrating a monolayer of phosphatidylcholine, cholesterol and diacetylphosphate with CHO. Oral administration of enteric capsules containing 500 µg of the CHO-liposomes for seven days produced salivary antibody responses in three of four subjects. The responses peaked at 26-32 days after immunization. Serum antibody responses varied between subjects. Reimmunization of one subject after seven months induced a more rapid response in salivary IgA. Further studies are necessary to optimize the responses that are protective against caries.

A17 Identify ways to reduce the caries-producing potential of dietary items.

A grantee at the University of California at San Diego is studying the molecular basis of taste. He is characterizing sweet peptides by spectroscopy and X-ray crystallography and using computer simulation to design new peptides, which are intensely sweet, non-cariogenic and non-caloric and are safe. The instability of aspartame to heat and during storage makes these studies particularly timely. A computer model can "taste" by examining the three dimensional architecture of synthetic compounds. The model can distinguish tastes among a family of compounds that includes aspartame and it can predict whether a compound will be sweet, bitter or tasteless from its size and structure. An immediate application will be to reduce the need for taste panels in the food industry. Long term, this work will explain how unrelated compounds, from plant sugars and proteins to simple peptides all provoke a similar response in the receptors of taste buds on the tongue.

P99 Develop controlled release diagnostic and therapeutic agents.

Reliable criteria for diagnosis of recurrent caries and for deciding whether to replace a restoration are not available. Radiographs often do not reveal recurrent caries. Researchers at the University of Florida, Gainesville, are developing controlled-release agents for diagnostic, preventive, and/or therapeutic applications. These materials will signal the presence of recurrent caries and could initiate prevention and treatment without operative intervention. Polymeric microspheres are being fabricated, which respond to changes in pH and release their encapsulated agents. Two polymers have been synthesized by emulsion polymerization as initial candidates for microsphere fabrication. The solubility and gel formation of these polymers is being evaluated in aqueous, buffered solutions. At pH 6 and above, swelling and dissolution of the

polymers is negligible for at least a 30-day period. This is encouraging since a polymer which is pH sensitive between 4.5 and 6.0 and pH insensitive above a pH of 6 is desirable. Caries activity associated with a restoration would reduce the local pH below 6.0. This research will provide the information needed to develop diagnostic liners to be placed beneath restorations.

Develop new maxillofacial materials and soft denture liners.

The number of patients requiring maxillofacial prostheses in the United States each year is of the order of 125,000. There are more than 25 million denture wearers in the U.S. A satisfactory maxillofacial prosthesis offers the opportunity for users to function normally in society. Unfortunately, the materials used in prostheses soon lose their physical and mechanical properties, stain and become unsightly. A large percentage of denture wearers suffer from chronic soreness and inflammation of their gums, although soft denture liners can reduce these problems. The requirements for successful soft denture liner materials and maxillofacial elastomers are similar. Preliminary studies by investigators at the University of Michigan indicate that it is possible to synthesize silicone block copolymers having physical and mechanical properties that can avoid the problems associated with existing products. These polymers should adhere well to the acrylic denture base, be resistant to invasion by microorganisms and staining, and provide improved mechanical properties such as tear strength and resilience.

P08 Elucidate the role of filler particles in the mechanical and chemical behavior of composites.

Polymerization shrinkage is one of the major problems associated with composite materials. New materials for dental composite matrices and adhesives, in which the deleterious effects of polymerization shrinkage are minimized or eliminated, are needed. This can be achieved by employing ring opening polymerization that can partially or entirely compensate for the volume contraction that normally accompanies the conversion of a monomer to its polymer. Investigators at the National Institute of Standards and Technology are developing ring-opening monomer systems, which possess adequate reactivity to effectively copolymerize with conventional methacrylate monomers. A single ring-opening model monomer has been synthesized, in the form of a vinyl-substituted acetal. The comparative polymerization of this compound will provide information about the effectiveness of the radical-stabilizing substituent on ring-opening. A preliminary investigation of the polymerization has shown that essentially complete ring opening was achieved.

H04 Elucidate the cellular mechanisms involved in the synthesis, transport, storage and release of inorganic and organic salivary gland products including the transcriptional, translational and post-translational events.

The oral mucosa, soft gingival tissue and tooth enamel are protected from a variety of exogenous and endogenous insults by copious quantities of viscous secretions elaborated by the major and minor salivary glands. Among the constituents of saliva implicated in the preservation of the health of the oral cavity are mucus glycoproteins. These large, highly glycosylated proteins promote salivary bacterial clearance, maintain the viscoelastic properties of saliva, and are part of the pellicle coating tooth enamel and the mucosa. The functional performance of salivary mucus glycoproteins depends upon the features acquired during their synthesis and processing. The early stage of mucus glycoprotein assembly involves the ribosomal synthesis of the peptide core, its acylation with fatty acids, translocation into the lumen of endoplasmic reticulum and the initiation of glycosylation. Further processing involves post-translational elongation of carbohydrate chains, subunit assembly and sulfation, principally of N-acetylglucosamine moieties. The sulfate groups impart a strong anionic character, promoting interaction with oral mucosa and tooth enamel and affecting physicochemical and functional qualities of saliva such as viscosity, lubrication and bacterial clearance. Thus, the sulfotransferase for mucus glycoproteins plays an important role in the maintenance of the salivary non-immune defense system. Yet, with the exception of studies on the incorporation of sulfate into salivary mucins by glandular tissue, little is known about this sulfotransferase and about factors affecting its activity. Investigators at the University of Medicine and Dentistry of New Jersey-Newark have used extracts of the Golgi-rich membrane fraction of the rat submandibular gland to study the sulfotransferase enzyme, which catalyzes the transfer of the sulfate ester group from 3′-phospho-adenosine-5′-phosphosulfate to gland mucus glycoprotein. Optimum enzyme activity was obtained at pH 6.8 and the apparent K_m for salivary mucus glycoprotein was 11.1 μM. Sulfotransferase activity was stimulated by 16,16-dimethyl prostaglandin E_2 at concentrations well below therapeutic levels and comparable to prostaglandin levels present in human saliva, suggesting that prostaglandins may play a role in mucin sulfation. Aspirin, commonly used for the relief of dental pain, inhibits sulfotransferase activity at concentrations occurring in the blood following the common therapeutic dose of aspirin. Consequently, a similar untoward effect of the drug on salivary mucin sulfation might be occurring in humans taking this analgesic frequently, thereby diminishing the non-immune protective properties of their saliva.

H05 Develop improved procedures for characterizing the molecular structure and function of the salivary proteins and other macromolecules important in oral health maintenance.

Grantees at Boston University Medical Center have isolated and purified to apparent homogeneity three major histidine-rich proteins of human parotid saliva. These three histatins, 1, 3, and 5, have molecular weights of 4929, 4063, and 3037, respectively, and contain 38, 32 and 24 amino acid residues, 7 of which are histidine. The data suggest that histatins 1 and 3 are derived from different structural genes, whereas histatin 5 is a proteolytic product of histatin 3. Histatins have an unexpected biological role in the oral cavity; they are part of the host's non-immune defense system. All three proteins have antimicrobial effects, including inhibition of growth of the

pathogenic yeast, *Candida albicans*. In a bioassay using direct microscopic quantitation, histatins 1, 3, and 5 killed *C. albicans* blastospores in a dose-dependent manner and their effectiveness to kill blastospores correlates inversely with molecular size. The availability of these naturally occurring homologous proteins will facilitate determination of their structure/function relationships with respect to killing of *C. albicans*.

Streptococcus milleri, is the major streptococcal species at the gingival crevice, comprising 20-60 percent of the flora at the gingival margin, and it is involved in early supragingival colonization. It is rarely found on tooth surfaces other than at the gingival margin. The mechanism for its localization at the gingival margin is unknown. Investigators at the Medical University of South Carolina-Charleston are investigating the mechanism of *S. milleri* colonization on hydroxyapatite (HA). *S. milleri* selectively adsorbs salivary proline-rich protein I (PRPI) in preference to other acidic proline-rich proteins (PRPs). The PRPs and the first complement component (C1) interact to form a stable complex; C1 associates with HA in dental pellicle formation, and increasing concentrations of C1 on HA are directly correlated with increased adherence of *S. milleri* to the HA. It is concluded, that adherence of *S. milleri* to HA is mediated by an interaction between PRPI on the bacterial surface and C1 in the dental pellicle. If the mechanism of early bacterial adherence at the gingival margin is understood, another approach to intercept inflammatory periodontal disease may be possible.

Actinobacillus actinomycetemcomitans, a small capnophilic, aerotolerant, gram-negative coccobacillus, is considered a minor component of the normal oral microflora of humans. It can be recovered from dental plaque. Although apparently an innocuous parasite in most instances, it has been implicated as an etiologic agent in periodontitis, endocarditis, and severe abscess formation. A number of host defense mechanisms present in saliva may normally act to control oral colonization by *A. actinomycetemcomitans* and prevent local or disseminated pathology. Lactoferrin, an iron-binding glycoprotein found in saliva and other exocrine gland secretions, can inhibit the growth of numerous microorganisms through simple iron deprivation. In addition to this bacteriostatic action, which is stimulated by the presence of bicarbonate in saliva, lactoferrin is thought to have direct bactericidal activity. Grantees at Emory University have shown that human lactoferrin is bactericidal for *A. actinomycetemcomitans*. Lactoferrin, devoid of iron and anions, produced a 2-log decrease in viability within 120 minutes at 37°C at a concentration of 1.9 µM. Besides exhibiting concentration dependence, killing kinetics were affected by minor variations in temperature and pH. Magnesium enhanced lactoferrin killing of *A. actinomycetemcomitans*, while other cations, such as potassium and calcium, had no effect. These data suggest that lactoferrin plays a significant role in secretory defense against this potential periodontopathogen.

H09 Identify and characterize those diseases, environmental factors and treatment regimens that affect the salivary glands.

Streptozotocin-induced diabetes in rats was previously shown by investigators at the University of Washington-Seattle to decrease the sympathetic secretory response of parotid salivary glands, while the protein concentration of saliva remained normal. The investigators have recently assessed the effects of streptozotocin-induced diabetes on submandibular gland response to sympathetic nerve stimulation. Salivary secretion in anesthetized animals was induced by stimulating the cervical sympathetic trunk using bipolar electrodes. The glands were significantly smaller than normal at 3 weeks and 3 months after inducing diabetes. Salivary flow rate per gram tissue was similar to controls at 3 weeks, but significantly increased at 3 months with a reduced concentration of protein. It was concluded that streptozotocin-induced diabetes in rats produces different metabolic effects on submandibular glands from those on parotid glands.

PERIODONTAL AND SOFT TISSUE DISEASES RESEARCH BRANCH

During FY 1990 the Periodontal and Soft Tissue Diseases Branch made a total of 146 awards: 108 for research on periodontal diseases and 38 for research on soft tissue diseases. Training and development support was provided for 80 individuals in periodontal disease research and for 24 individuals in soft tissue disease research.

Selected periodontal research highlighted in this report includes a clinical study on the aggravating influence on periodontal disease of diabetes mellitus and cigarette smoking, molecular biology studies of a leukotoxin from *Actinobacillus actinomycetemcomitans* (*Aa*), and research on immunosuppressive factors of periodontal organisms. Also included are studies on the significance of specific antibody avidity to periodontal pathogens and a description of an enzyme test to indicate risk for disease.

Research highlights in the soft tissue diseases and AIDS areas include a study on the role of Vitamin A compounds in cellular differentiation, a project to develop a new mucosal graft, and a project to develop new chemotherapeutic agents for herpes simplex virus infections. Also included are studies on the roles of a specific growth factor and papilloma virus in epithelial cancer development, and an AIDS-related project on nonimmune HIV-inhibitory factors in saliva. Preliminary findings on the orphan disease epidermolysis bullosa are also presented.

The following discussion of activities is presented in reference to research objectives as defined and coded in the NIDR Long-Range Research Plan, "Challenges for the Eighties."

B03 Identify systemic and environmental factors that influence the development and/or progression of periodontal diseases, and determine their relative importance.

Diabetes and Cigarette Smoking: A study by investigators from Tufts University, Forsyth Dental Center and the Joslin Clinic has produced findings to indicate that cigarette smoking is a significant environmental risk factor for periodontal disease both in diabetics and nondiabetics. Data from 81 patients with insulin dependent diabetes mellitus (IDDM) and 70 nondiabetics, aged 19 to 40 years, showed that 47 percent of the diabetics had periodontal disease, whereas only 30 percent of the controls were affected. Periodontal disease was defined as having at least one periodontal site with pocket depth of 5.0 mm or more and attachment loss of 2.0 mm or greater.

The study population was made up of current cigarette smokers, nonsmokers and former smokers. Some participants were diabetics. Epidemiological studies of older populations have suggested that both cigarette smoking and diabetes mellitus are risk

factors for periodontal disease. When data on smoking history of participants in the current study were adjusted for age and diabetes, it was apparent that former smokers are 2.4 times and current smokers are 6.9 times more likely to have periodontal disease than nonsmokers. Diabetics were 1.6 times as likely to have periodontal disease than non-diabetics.

B04 Elucidate the mechanisms leading to the destruction of soft tissue and bone in the periodontal diseases.

Molecular Biology Studies of Aa Leukotoxin: An apparent reason for the virulence attributed to *Aa*, the principal causative organism in juvenile periodontitis, is that it produces a leukotoxin which destroys PMNs and monocytes, two key immune cells which defend the body against infection. Scientists at the University of Pennsylvania and the University of Texas in San Antonio, working independently, have cloned the gene for this toxic protein, determined the DNA sequence, and expressed the gene in *E. coli*, with the production of active toxin. They have also obtained evidence that the *Aa* leukotoxin gene is closely linked to 3 other genes that appear to be related to activation and transport of the leukotoxin protein. All known members of this gene cluster in *Aa* show some degree of resemblance to genes with equivalent functions in three non-oral organisms.

Although the majority of *Aa* isolates from patients with periodontal disease are serotype b strains which produce the leukotoxin, most of the *Aa* isolates from healthy individuals are serotype c strains which do not produce the leukotoxin. Both serotypes contain the gene for the leukotoxin, but apparently the active toxin is not produced by the serotype c strains. Failure to express leukotoxic activity appears to be in the transcription process.

These studies also demonstrated molecular similarities between the *Aa* leukotoxin and that produced by a taxonomically closely-related organism, *Pasteurella haemolytica*, which causes pneumonia in cattle, and also with the hemolysin from *E. coli*, suggesting a common mechanism responsible for diseases caused by these three species.

These advances set the stage for determination of the structure of the toxic protein, and determination of how the *Aa* leukotoxin kills white cells. Such studies may lead to the design of new therapeutic strategies. Because of its unique target cell specificity against white cells, the leukotoxin may have value in the treatment of some leukemias.

Leukotoxins in Other Oral Organisms: Investigators at SUNY Buffalo found that several other periodontal pathogens also have the potential for producing a leukotoxin. Using 4 oligonucleotide probes, corresponding to regions of the *P. haemolytica* leukotoxin gene, these investigators found that oral bacterial DNA from several periodontal pathogens reacted with at least one probe. In *in vitro* tests, using polymorphonuclear leukocytes, the leukotoxin from *Aa* killed 58 percent of the cells tested, *Wolinella recta*, 10 percent, *Bacteroides ureolyticus* 14 percent, *Campylobacter*

sputorum, 16 percent. When HL-60 cells were used, the results were *Aa* and *B. ureolyticus* 70 percent, *W. recta* and *C. sputorum* 40 percent of the cells killed. The data indicate that the synthetic gene probes recognize cytolysins of varying specificity in potentially pathogenic periodontal organisms.

Immunosuppression: A general mechanism that could set the stage for periodontal tissue destruction is locally induced immunosuppression by oral organisms. There is some indication that oral organisms can initiate systemic immunosuppression that involves complex cellular interactions. Investigators at the University of Pennsylvania have found that 16 periodontal species inhibit human lymphocytes. While all of five species studied in detail suppress both B-lymphocyte and T-lymphocyte responses, each seems to alter different aspects of immune function. Examples are outlined below:

1. *Aa* induces proliferation of B regulatory cells and T suppressor cells.
2. *Treponema denticola* and *T. socranskii* activate suppressor monocytes.
3. *B. intermedius* interferes with T helper cell activity (Interleukin-2 synthesis).
4. *Centipeda periodontii* selectively kills lymphoid cells.
5. *Fusobacterium nucleatum* directly influences effector B and T cell functions.

Local immunosuppression need not be continuous to have a negative impact. Temporary suppression could enhance the virulence of pathogenic organisms and/or that of secondary opportunistic organisms and lead to episodes of disease and tissue destruction.

Injection of the purified immunosuppressive factor from *Aa* into experimental animals increased the number of T suppressor cells in the spleen and inhibited delayed hypersensitivity reactions in the skin. These results support findings by Forsyth investigators that periodontal bone loss was increased when athymic animals were given *Aa*-sensitized T suppressor cells.

B05 Clarify the role of the host's immunological system as either a protector of the periodontal tissue or as a possible source of destruction.

The Role of Antibody Avidity in Immune Protection: Numerous reports document that humans respond to putative periodontal pathogens by producing specific antibodies and the response usually precedes tissue destruction. The titers seem to vary and the significance of varying titers has not been established. The role of antibody avidity has been given little if any attention, but recent evidence indicates that it may be critical. Avidity is the net binding strength which results when multivalent antigens interact with specific antibodies. Avidity affects a variety of host defense mechanisms including complement activation, immune elimination and virus neutralization. Studies on periodontal patients and controls at the Universities of Washington in Seattle and Michigan at Ann Arbor, involved measurements of serum IgG titers and the avidity of antibodies to *Bacteroides gingivalis* (*Bg*) antigens.

In approximately 100 Seattle patients with rapidly progressing periodontal disease (RPP), the mean IgG titers to *Bg* were lower in than in 40 healthy controls, but the avidity of the antibody was higher. The finding of low titers in RPP patients was confirmed in another study. The Michigan investigators found that compared to control subjects, the patients with moderate periodontal disease had higher titers and antibody of greater avidity. However, compared to rabbit antibodies against the same *Bg* antigens, the avidity of the human antibody was less than one third that of the rabbit antibodies. With other common antigens, including streptokinase and tetanus toxoid, the avidity of human antibody is comparable to that of rabbits. Thus, the Michigan scientists concluded that the human response to the major periodontal pathogen *Bg* is to produce an antibody of very low efficacy. These and related studies indicate that antibody avidity is inversely related to disease; low antibody avidity, regardless of titer, indicates that the body's defensive cells are unable to handle the specific antigen efficiently. According to published studies, when avidity is low experimental animals experience more severe and more chronic disease, because antigen-antibody complexes persist in localized tissues. Thus, low avidity antibodies would be expected to contribute to the pathologic destruction of periodontal disease. This work indicates that the affinity of the human antibodies to periodontal pathogens may be critical in determining susceptibility to periodontal disease.

B06 Develop safe, sensitive, objective tests of disease activity and rates of progression.

Bacterial Enzyme Test to Detect Risk Factor: Investigators at the University of Michigan have developed an enzyme test that detects local signs of anaerobic infection and may serve as a reliable indicator of impending periodontal destruction. The test is based on the fact that three anaerobic organisms (*Bacteroides gingivalis*, *Treponema denticola* and *Bacteroides forsythus*) known to be present in pathogenic periodontal plaque produce a trypsin-like enzyme, which breaks down a synthetic substrate, called BANA. The BANA test has been used as a measure of disease activity in recruiting patients for studies, in monitoring compliance, and monitoring patients on long term maintenance. Data indicate that a positive BANA test after the completion of treatment predicts future periodontal attachment loss. The investigators believe that this test will be useful in the overall management of periodontally diseased patients.

F01 Elucidate the mechanisms governing epithelial-mesenchymal interactions in the growth and development of the oral mucosa.

Epithelial Differentiation: In a highly significant study at the University of Washington in Seattle, the role of retinoids (Vitamin A) in altered oral mucosal and skin keratinization in the Er mutant mouse is being studied. The Er mutant mouse has a lethal recessive defect which blocks epithelial differentiation, and causes hair loss in heterozygotes. Because retinoids are critical in epithelial differentiation, the investigators tested whether retinoid distribution is altered in affected mice. In adult heterozygotes, retinoids were elevated in liver (3.5 fold), the primary organ regulating retinoid homeostasis, as well as in skin and kidney, but plasma content of retinoids was

only half of normal. In affected newborns, lethal retinoids were elevated in the placenta (the organ regulating homeostasis *in utero*), but reduced in liver and elevated in other tissues. This suggests that the defect is correlated with altered delivery of Vitamin A to tissues.

Subsequent experiments led the investigators to postulate that the retinoid binding proteins in these animals have structural defects, which alter the distribution and accumulation of retinoids in target tissues. Such alterations would lead to incomplete epithelial differentiation. The investigators are using the polymerase chain reaction to amplify the altered gene to test this hypothesis. If it is verified, the Er mutant will be the first known mutant in which vitamin A homeostasis is altered and it could be an important tool in furthering our understanding of the role of retinoids in epithelial differentiation.

Oral Mucosal Graft: Using dogs, investigators at U.C.L.A. are attempting to produce a full thickness oral mucosal equivalent (FTOME) *in vitro* which mimics oral mucosa *in vivo*. Optimum growth was achieved in five weeks. Characterization studies revealed that the oral mucosa and FTOME produced *in vitro* differed in only one keratin. This compositional similarity accounts for the histological similarity of FTOME canine mucosa *in vivo* and *in vitro*. When a FTOME was grafted onto the edentulous alveolar ridges of dogs, subsequent biopsy revealed that grafted sites healed within 7 days as opposed to 14 days for control sites, and the mucosal barrier function persisted. Future studies will characterize the transplantation antigens of the FTOME to determine whether the tissue can be stored for use in emergency procedures. The FTOME could then be widely applied to burn and surgical cases.

F08 Identify the role of immunological mechanisms, genetics, and microbial agents in the pathogenesis of recurrent aphthous stomatitis and vesicula-erosive diseases such as pemphigus, pemphigoid, lichen planus and erythema multiform.

Epidermolysis Bullosa: Epidermolysis bullosa (EB) refers to a group of as many as 24 subtypes of hereditary disorders with unique and frequently debilitating manifestations of soft and hard tissues. Investigators at the University of Alabama are attempting to determine the prevalence of oral disease in each EB subtype and establish which clinical and histological features of dental tissues are diagnostically useful in their delineation. Manifestations of EB range from very mild to lethal depending on the subtypes, and include developmental defects, frequent blistering of the oral mucosa and rampant caries. Previous investigators have hypothesized that some of the oral complications associated with severe types of EB result from altered salivary function. Preliminary results from this study failed to show a relationship between salivary flow rate and oral disease in EB, or provide any data in this direction. Thus, the oral factors predisposing to rampant dental caries remain to be identified.

F04 Identify and test antiviral compounds and biological inhibitors such as interferon that might control infection, prevent development of latency, or prevent reactivation of latent herpes simplex infection.

Chemotherapy for Herpes Simplex: An investigator at Ohio State University is studying the role of deoxyuridine triphosphate nucleotidohydrolase (dUTPase) in the replication of HSV to determine if dUTPase can be used to develop anti-HSV compounds. The investigator found that mercurithio-derived compounds of dUTP inhibited purified HSV-encoded dUTPase but not human dUTPase. Current studies are examining whether similar derivatives of deoxyuridine can inhibit replication of HSV-1 (responsible for cold sores and encephalitis) and HSV-2 (responsible for genital infections). The investigator has found two such compounds which inhibit replication of both HSV-1 and HSV-2, *in vitro*. The compounds are not cytotoxic to human cells. Since acyclovir, the drug of choice for HSV infections, has lost some of its effectiveness, these studies take on added significance.

F99 Site and Role of Growth Factors in Oral Cancer

Growth Factors from Eosinophils: Investigators at Harvard School of Dental Medicine have studied the role of transforming growth factor-alpha (TGF-α) in the development of oral cancer. Until recently, epithelial tumor cells were thought to be the major source of the TGF-α contributing to cancer growth, since most malignant epithelial cell cancers had been shown to express TGF-α. However, when the source of TGF-α in experimental oral carcinomas in Syrian hamsters was studied by *in situ* hybridization techniques using a purified TGF-α RNA probe, they found that the cancerous epithelial cells were not the only cells showing signs of TGF-α. Instead, the major source appeared to be eosinophilic leukocytes infiltrating into the cancer tissues. In collaboration with Harvard Medical School investigators, they confirmed these findings in human specimens from cancers of the oral mucosa and large intestine. The novel finding that eosinophils express TGF-α permits a new direction in cancer research.

F06 Examine the roles of microorganisms such as viruses, fungi and other environmental host factors in the development of neoplastic lesions of the oral soft tissues.

Papilloma Virus in Oral Cancer: Viruses in general and human papilloma viruses (HPV) in particular are associated with oral cancer. Previous studies suggest that oral cancers may be due solely to HPV or to multiple factors including HPV. Researchers at the University of North Carolina and the University of Texas in Houston demonstrated HPV in 50-60 percent of patients with squamous cell carcinomas of the head and neck using the Southern blot technique. In the Texas study, 71 percent of the patients with HPV-positive tumors were tobacco users, whereas only 20 percent of the HPV-negative cancer patients used tobacco. Similar findings were reported from North Carolina. In more recent studies, both investigators used the highly sensitive, polymerase chain reaction to detect HPV, and found evidence of HPV in 90 percent of the lesions from oral cancer patients, a much higher percentage than reported

previously. These results suggest that HPV is important in oral cancer and that it may be synergistic with tobacco use.

Complications of Leukemia: Preliminary results of a study at the University of Alabama, show that during the first 8 months of cancer therapy, approximately 20 percent of pediatric leukemia patients exhibited a range of oral complications, including candidiasis, mucositis and lesions of the periodontium. As treatment for cancer is continued over time, the incidence and severity of the oral complications is expected to increase. The ultimate objective will be to design more effective treatments and minimize the severity of these complications.

F99 HIV Inhibitory Factors in Saliva

Parotid Factor: Investigators at Emory University have begun studies to characterize HIV inhibitory substances in human saliva. Their focus is on secretory leukocyte protease inhibitor (SLPI), which is believed to be part of a system of natural mucosal immunity found in normal saliva, nasal and bronchial secretions, but not in serum, plasma and rectal fluids. The SLPI being studied was produced by recombinant DNA technology, following isolation from tissue cultures of human parotid gland. The SLPI preparation is inhibitory to both HIV and a highly virulent simian immunodeficiency virus. SLPI prevents infection *in vitro* by blocking a critical proteolysis step before the HIV enters the cell. If SLPI inhibits by the same mechanism *in vivo*, more T cells would be protected and permitted to function normally. Thus, this new agent could be beneficial to patients already HIV-seropositive. A human milk fraction also showed a high degree of viral inhibition *in vitro*.

CRANIOFACIAL ANOMALIES, PAIN CONTROL AND BEHAVIORAL RESEARCH BRANCH

One hundred thirty-six awards were made during 1990 to support research and training on craniofacial anomalies. Cell and molecular biology, and genetics of normal and abnormal craniofacial development received increasing support. Additional clinical trials were initiated to evaluate orthodontic and surgical treatments for dentofacial malrelations. Seventy one awards were made for support of orofacial pain and behavioral research and training. The epidemiology, identification of risk factors and treatment of orofacial pain and the impact of social and behavioral factors on consistency of self care and on the levels of oral diseases received special attention.

The following discussion of activities is presented in reference to research objectives as defined and coded in the NIDR Long-Range Research Plan, "Challenges for the Eighties."

C02 Uncover the mechanisms that govern interactions between regulatory genes and the extracellular environment.

C03 Characterize the chemical mediators that underlie cell-cell and cell-matrix interactions.

Over the past two decades, it has become apparent that interactions between cells of different origins and between cells and macromolecules in the extracellular matrix surrounding them influence the behavior of the cells radically. These interactions guide the multiplication, movement and differentiation of cells during development of tissues in the embryo. Aberrations in these interactions during development lead to birth defects, including craniofacial anomalies. They are also critical to cellular processes of wound healing and tissue regeneration. Grantees at Tufts University, working with developing chick limbs, have shown that interactions between extracellular hyaluronic acid and cells are important in many facets of development, including formation of the skeleton and musculature. These interactions are mediated by receptor molecules on the surface of the cells that recognize extracellular macromolecules. As a result of binding to the receptors, information is transmitted to the inside of the cell and processed in such a way as to alter intracellular structures or metabolism. These alterations ultimately lead to changes in cell movement, multiplication and differentiation. In effect, they are controlling the way that genes are expressed during development. Much of the current effort is being applied to isolating and purifying the receptors in order to characterize them and determine how they convey information into the cell.

Tooth development results from a series of reciprocal interactions between epithelial cells, derived from oral epithelium, and mesenchyme, derived from neural crest tissue. Investigators at the Universities of Connecticut and Southern California are examining tissue interactions that occur during tooth root development. Differentiation of odontoblasts, the dentin forming cells of the tooth, results from interactions between the epithelial root sheath and dental papilla cells in the tooth root. This parallels the previously reported interactions between the epithelial enamel organ and the dental papilla that leads to odontoblast differentiation in the tooth crown. However, the interactions in root and crown are quantitatively and qualitatively different. Root odontoblasts and the extracellular matrix secreted by them have been shown to be morphologically and biochemically different from those in the crown. Tissue separation and recombination experiments have revealed an important role of the epithelial root sheath in the formation of the cementum and periodontal ligament attachment of the tooth root. Mineral is secreted by the dental sac but the presence of basal lamina, secreted by the epithelial root sheath, is essential in order for the mineral to adhere to the developing root. Similarly, the presence of epithelium is necessary for the formation of the periodontal ligament attachment.

C05 Explore the nature of genetic susceptibility or resistance.

Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects, affecting 1/700-1/1000 Caucasians. Several years ago, grantees reanalyzed Danish pedigree data. They concluded that a model of action of a major gene with autosomal recessive inheritance modified by additional genetic and/or environmental factors best fitted the data. This major gene was predicted to have a frequency of 3.5 percent and to account for about one-third of the cases of CL/P in the Danish population. A collaborative study between scientists from the University of Southern California and the Zhabei Eye Institute, Shanghai, China, yielded data from 1,500 Chinese families that indicated that a single autosomal recessive major locus could account for clefting in this population. Gene mapping studies are proposed to locate the gene responsible and use "reverse genetics" to identify the molecular mechanisms causing the clefts. Similar approaches are being used with U.S. populations.

An association study at the University of Iowa looked for genetic markers across a population of unrelated individuals with CL/P. Finding the disorder in association with a particular marker implies a causal relationship between the marker and the disorder. A number of growth factors, hormones and their receptors have been implicated in palate development. Mutations in the genes for any of these factors would be expected to interfere with normal palate development and possibly cause clefting. An association was found between altered DNA sequences in the region of the transforming growth factor-α (TGFα) gene and the occurrence of clefting. TGFα is one of the factors implicated in palate development. This suggests that an abnormality in either the TGFα gene itself or adjacent DNA sequences contributed to development of clefts in a portion of the cases.

Linkage analysis is being used with three generations of affected families to identify the gene involved in Van der Woude syndrome (VWS). VWS is a single gene, autosomal dominant disorder in which affected individuals have one or more of the following manifestations: cleft lip, cleft palate, missing teeth or lower lip pits. VWS accounts for one to three percent of all cases of CL/P. Several likely candidate genes for growth factors and structural cell components were eliminated from consideration. However, the VWS locus was tightly linked to the renin locus, which has been studied intensively. Renin is a kidney enzyme indirectly involved in blood pressure regulation. This study is the first step toward identifying the VWS gene, developing methods for prenatal diagnosis and identifying the molecular abnormality underlying this birth defect.

C13 Explore sensory and respiratory dysfunctions in relation to craniofacial malformations.

Clefts of the lip and palate frequently produce nasal deformities, which reduce the size of the nasal airway and interfere with breathing and speaking. Some authorities believe that impaired breathing leads to abnormal growth of the face and jaws. Surgical procedures to correct the deformed nose and palate may further compromise breathing. Investigators at the University of North Carolina have developed methods for assessing airway impairment and assessing the effects of treatment on airflow. In adults an airway of cross-sectional area of less than 0.4 cm^2 is not adequate for nasal breathing and produces symptoms of breathing difficulty. Almost all individuals with an airway less than this are mouth breathers to some extent. Seventy percent of individuals with cleft palate are chronic mouth breathers from birth. They continue to breathe through the mouth to some extent even when the nose is no longer impaired, suggesting that once the habit of mouth breathing is established it is difficult to break. If mouth breathing becomes habitual, early surgical intervention should be considered although any advantage of early treatment needs to be balanced with possible adverse effects on growth. Repositioning the upper jaw upwards, relative to the skull, improves nasal function by changing the shape of the aperture at the back of the nose and flaring the nostrils.

D07 Develop and evaluate new methods of diagnosis and treatment of acquired craniofacial defects.

E04 Expand studies on bone formation and remodeling.

J04 Increase research to elucidate the role of local growth factors in bone such as bone morphogenetic proteins and human skeletal growth factors.

Numerous approaches are being explored to develop methods to promote localized bone formation. If successful they would have widespread use in treatment of congenital and traumatic skeletal bone deficiencies, in periodontal diseases and in osteoporosis. Combinations of methods may prove more effective than using only one treatment. Studies on bone morphogenetic protein have been supported for many

years. Individual bone inducing factors have been isolated and, as reported previously, the genes for some of them are being cloned. Use of electric fields to stimulate fracture healing is being tested and is mentioned in another section. Systemic administration of large doses of prostaglandin (PGE-1) is known to promote bone formation. Investigators at the University of Massachusetts Medical School are using a minipump implanted in the necks of dogs to infuse PGE-1, via a catheter, to the lower jaw. In young and adult dogs the local, continuous infusion of PGE-1 for five weeks is accompanied by a remarkable osteogenic response characterized by rapid formation and mineralization of bone, primarily localized to the teeth bearing surface of the jaw. Microscopically, the new bone has the lamellar, structural characteristics of developing normal bone. The investigators hypothesize that this bone will develop to form structurally competent and mechanically responsive mature bone.

Another group of investigators at Washington University in St. Louis, has shown that there is extensive bone formation in rats in response to injections of negatively charged beads. The effect was seen in long limb bones and when the beads were applied to repair skull and jaw defects or when used as an onlay on the nasal surface. Positively charged beads did not induce bone formation. When a mixture of negatively charged beads and demineralized bone powder was placed in periodontal defects created in the jaws of rats, normal bone was formed and there was regeneration of tooth root cementum and the periodontal ligament. The implanted materials prevented the usual migration of the surface epithelial layer into the graft, allowing regeneration to take place. Future studies will optimize the system for delivering the beads, stabilize their location and maximize their effects.

A different approach is being used at Case Western Reserve University. These investigators propose that bone marrow stem cells, which are the precursors of bone forming cells or osteoblasts, can be grown in culture and reintroduced back into an animal to repair a bone defect. The cultured stem cells were incorporated into porous calcium phosphate ceramic, which was then introduced into a defective bone. By using cultured stem cells from quail marrow and implanting them into rats it was possible to show that the donor cells formed the initial layers of osteoblasts on the wall of the ceramic and about 30 percent of the bone filling the pores of the ceramic. These cells then died and were replaced by bone forming cells from the host, which continued to lay down bone. These findings have been extended to show that the periosteum from chick and human long bones contains stem cells, which can be cultured and will similarly induce bone formation.

E05 Expand studies of interceptive treatment aimed at growth modification.

E06 Study factors affecting the post-treatment stability of bones and teeth.

Growth deficiencies of the mandible or lower jaw occur in about five percent of the population. Significant advances in correcting the resulting dentofacial malrelations have been made in the past 25 years. Orthodontic, surgical or combined orthodontic-

surgical treatment procedures, which may improve facial esthetics and psychological state, are being advocated. A study at the University of North Carolina illustrates the difficulties in conducting retrospective evaluations of treatments. Orthodontic retraction of the upper front teeth and tipping the lower front teeth forward improved the malocclusion and camouflaged the jaw discrepancies. Surgical treatment was more effective in bringing the jaws into normal relationships and improving the malocclusion. Because the surgically treated group had greater aesthetic problems to begin with, the aesthetic improvement was greater than in the orthodontically treated group of patients. Consequently, the greater aesthetic improvement following surgery cannot be used to advocate surgical rather than orthodontic treatment. Prospective studies have been initiated to evaluate the efficacy of different forms of treatment.

Animal models are proving useful in comparing therapies and developing the theoretical basis for recommending treatments in patients. In adult rats about 40 percent of the surgical advancement of one side of the lower jaw is lost due to relapse, caused by the torsional, tensile and compressive forces placed on it during healing of the fractured jaw. The investigators at St. Louis University Medical Center, who developed this model, are studying the effects of stretching the jaw muscles prior to surgery in order to reduce forces on the fractured jaw during healing. They are also using electrical stimulation to speed the repair of the fractured jaw and allow less time for relapse to occur. Electrical stimulation has proved effective with limb bone fractures but has not been used on jaw fractures.

Following jaw surgery, the cut sections of bone are held together rigidly by metal plates and screws or the jaws may be wired together, with the upper jaw stabilizing the sectioned lower jaw. Patients generally prefer rigid fixation because they can eat and speak soon after surgery rather than waiting until the wires holding their jaws together are removed. However, there is some evidence that rigid fixation causes problems in the temporomandibular or jaw joint and that nerves may be damaged in the process. Studies at the University of Texas Health Science Center, San Antonio, show increased stability with the use of rigid fixation. The magnitude of the advancement was a reliable indicator of the extent of relapse. Some relapse was believed to be related to malpositioning of the lower jaw leading to poor articulation with the upper jaw, rather than the method of fixation. These investigators are going on to compare the efficacy and cost effectiveness of rigid and wire fixation in a prospective clinical trial.

C14 Explore the short and long-term psychosocial impact of craniofacial anomalies and their treatment on the patient, family, and society

M04 Develop sensitive, reliable indicators of the impacts of oral diseases and conditions and their treatments

University of North Carolina scientists have evaluated a wide range of personal and family characteristics in over 800 children and adolescents with cleft lip and/or palate (CL/P). They found no gender differences in patients' satisfaction with their facial

appearance and speech, despite cultural norms suggesting that facial appearance might be more salient for females. However, the parents of female patients expressed significantly more concern regarding their daughters' appearance that did parents of male CL/P patients.

Rates of divorce and separation among parents with a CL/P child far exceeded those characteristic for families in North Carolina, and were substantially higher when the first-born child had CL/P than if the affected child fell later in the birth order. Over one-third of first-born CL/P children in the sample lived with a single parent. These findings suggest the need for preventive counseling to strengthen family stability and help resolve dysfunctional marital or parental reactions to the birth of a CL/P child.

As regards measures of cognitive abilities, approximately 25 percent of the CL/P patients studied showed mental retardation or learning disabilities, with differential rates observed among cleft types. Fifteen percent of the patients with only cleft palate scored in the mentally retarded range on standardized intelligence tests, while 7 percent and 8 percent respectively of the cleft lip and palate, and of the cleft lip only group fell in that range. On a standardized school achievement test 50 percent of the CL/P patients performed below the 25 percentile, as compared with only 18 percent of students in North Carolina state-wide. These findings indicate elevated risk for cognitive or academic disabilities and suggest a need to identify any cognitive deficits early, so that extra stimulation or educational enrichment can be provided.

Another investigator at the University of Iowa showed that CL/P adolescents with lower academic achievement tended to overestimate their speech clarity and underestimate their facial disfigurement, as compared with CL/P adolescents with higher academic achievement. This finding suggests that unrealistic self-perception may be one important factor related to lower academic achievement in adolescents with clefts.

G09 Conduct basic and clinical studies of pain associated with the temporomandibular joint and other myofascial pain

G10 Study the role of anxiety, stress, and other psychosocial factors in relation to the etiology and symptomatology of a variety of motor dysfunctions and pain disorders.

Understanding the specific determinants of muscle and joint pain has recently been given greater programmatic emphasis, as is indicated by a FY 1990 program announcement encouraging research in these areas (Pathogenesis of Joint, Muscle, and Inflammatory Pain as related to Temporomandibular Disorders). NIDR-supported scientists have begun making strides toward understanding how muscle pain differs from cutaneous pain, and how muscle activity may produce or intensify jaw and headache pain.

U.C.L.A. investigators are studying relationships between facial muscle tension and chronic headaches in the temporalis region, commonly called "tension headaches." In these studies patients wear compact electromyography (EMG) devices generating day-long records of activity in the anterior temporalis muscles; they also complete visual analogue ratings of their stress and pain every 30 minutes. The EMG records of muscle activity did not predict when pain started or was present in headache patients. However, headache and non-headache patients showed significantly different overall levels of muscle activity, with both daytime and nocturnal recording being higher for the headache patients than for pain-free controls. Ratings for pain and stress levels in the headache patients were highly, and positively, correlated.

Specific mechanisms through which muscle activity influences the development of jaw and head pain are being studied. Studies at the University of Michigan, and Emory University are characterizing patterns of muscle activity seen in response to pain-producing stimulation; responses to both clinical and experimentally-induced, muscle pain are being studied. The biological consequences of sustained muscle hyperactivity are widely thought to play an important role in the development of temporomandibular disorders, but relatively few well-controlled basic studies have assessed the validity of this concept.

M01 Determine how behavioral, social and cultural factors relate to the incidence, prevalence, and distribution of oral diseases and conditions.

G09 Conduct basic and clinical studies of pain associated with the temporomandibular joint and other myofascial pain.

Among individuals showing some signs of a temporomandibular disorder (TMD), few physical characteristics differentiate those who seek health care from those who do not. Epidemiologists and pain researchers at the University of Washington found that TMD patients in *clinic* samples report higher overall pain levels and react more to facial muscle palpation than do subjects identified in *community*-based epidemiological studies (i.e., persons who show some TMD symptoms, but have not sought health care). However, reliable, carefully standardized examinations reveal that these groups differ minimally in measures of jaw excursion (except for vertical opening), joint sounds, or occlusion.

The investigators confirmed that TMD pain occurs very rarely in persons over 50. They also discovered that subjective pain ratings for younger individuals show episodic variation, but remain relatively stable overall. These and other epidemiological findings strongly suggest that TMD is not generally a condition resulting in progressive disability or progressively impaired oral function over time. Though TMD can persist over many years, adversely affecting quality of life, epidemiological studies support the view that TMD is an episodic, and ultimately self-limiting, condition.

Other scientists and clinicians see this same self-limiting temporal pattern as potentially reflecting incompletely-understood pathophysiological changes, such as joint remodeling, which, once completed, no longer generate pain. Preliminary support for this view is provided by magnetic resonance imaging and histological studies (University of Rochester and the University of Oregon) indicating strong age-related pathological changes in the temporomandibular joint and surrounding tissues when jaw sections from young and older cadavers are compared. Additional work in the laboratory, pain clinics, and communities will be necessary to determine the causes underlying the puzzling, but well-confirmed, age distribution characteristics of TMD.

M07 Determine the critical factors influencing dental professionals to adopt and integrate appropriate preventive procedures into practice

When scientific advances occur or new knowledge develops, dentists must modify their patient care practices, adopting new approaches. Understanding processes influencing dissemination and adoption of new technologies is critical to the success of major Institute initiatives such as the Research and Action Program to Improve the Oral Health of Older Americans and Other Adults at High Risk. These initiatives will require dental professionals, as well as their patients, to change critical behaviors influencing health.

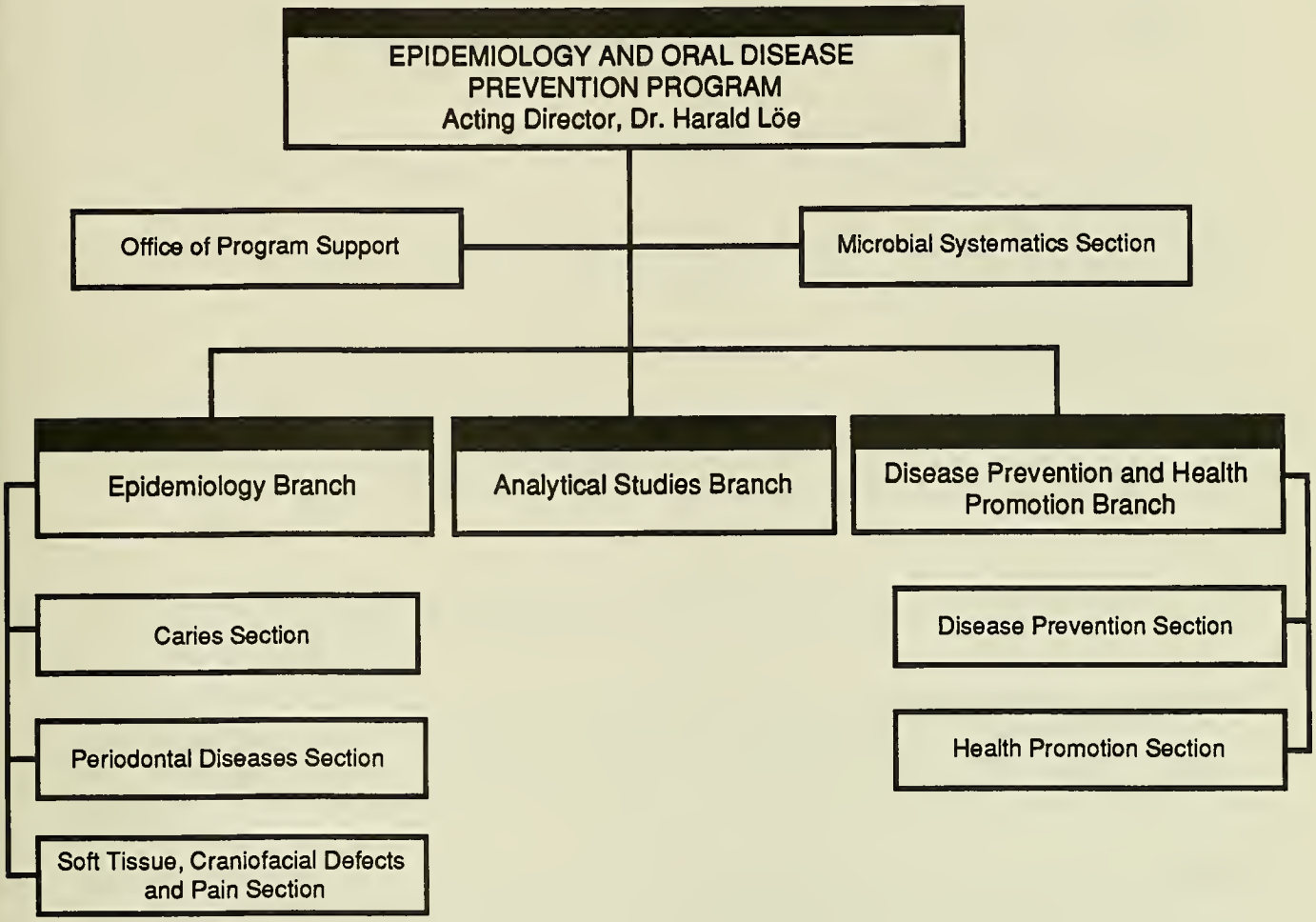
Investigators at the University of North Carolina and Albert Einstein Medical College-New York have developed and evaluated interventions to improve patient care in community dental practices. Such research is often challenging to implement, since it requires securing sustained cooperation from independent, busy professionals. The study in North Carolina focused on improving general practice dentists' identification, charting, and treatment of periodontal conditions in their regularly-attending patients. Participating dentists received a brief educational intervention including practice in certain diagnostic and clinical skills and specific practice-based feedback. Feedback utilized the researchers' independent observations of the periodontal status of selected patients the dentists had seen in their practice. Dentists who had participated in the educational intervention, and a control group of comparable dentists, allowed periodic patient record audits in their offices over the next year to assess changes in their charting and treatment of periodontal conditions. The group receiving the intervention showed major improvements in their chart notations of patients' periodontal status, some of which were maintained one year later. Positive, though less substantial, changes were also seen in the frequency of periodontal treatment services the dentists provided and in patients' observed periodontal status at the end of the study year.

A second intervention, designed to increase dentists' knowledge regarding effective pre-treatment antibiotic regimens for patients at risk of infectious endocarditis, was studied in a national sample of general practice dentists at least 40 years old and in solo practice. Prior studies had shown this group was least likely to know, and manage their "at risk" patients in compliance with, American Health Association guidelines for prevention of infectious endocarditis. The investigators discovered that a

simple, inexpensive intervention such as mailing a single-page laminated summary with specific guidelines for the dentist produced substantial, long-lasting changes in dentists' knowledge of how "at risk" patients should be identified and managed. Contrary to expectation, supplying brief additional materials summarizing theoretical underpinnings for the clinical guidelines resulted in no additional gains. Studies such as these are beginning to yield practical insights into how to develop and evaluate interventions to improve dental care.

Epidemiology and Oral Disease Prevention Program

NATIONAL INSTITUTE OF DENTAL RESEARCH



OFFICE OF THE DIRECTOR
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM

The overall mission of the Epidemiology and Oral Disease Prevention Program (EODPP) includes the following key interrelated objectives:

- To plan, develop, direct, and apply epidemiologic methodologies to investigation of oral diseases and disorders.
- To plan and conduct research analyzing the effect of changing oral disease patterns on dental research, education, and delivery systems.
- To plan and conduct clinical trials, field, demonstration, and related studies in the prevention of oral diseases.
- To plan and coordinate activities related to the prevention of oral diseases and disorders, targeted to the general public, health professionals, and the scientific community.
- To design, develop, and coordinate programs which facilitate or implement the transfer of research findings to application.

EODPP's activities in pursuit of these objectives are organized according to the organization chart. Further details on the functions, internal structure, and staffing patterns of these Program components are provided in the narrative which follows or in the separate Branch reports.

The Office of the Director is responsible for the overall scientific direction and administrative management of the Program. Dr. Harald Löe is Acting Director, Epidemiology and Oral Disease Prevention Program. Dr. Thomas F. Drury is Deputy Director. Ms.Phoebe Edwards, lead secretary for the Program, left in January to join the staff of the National Center For Health Statistics. The Office of the Director also includes Dr. Albert Kingman, Chief Statistician, NIDR, and Special Assistant to the Director of EODPP for Biostatistics, Clinical Trials, and International Epidemiologic Studies. Dr. Tullio F. Albertini, a Dental Public Health Administrator, joined the Program this September as Special Assistant for Program Management and Professional Affairs.

ORGANIZATION OF THE REPORT

The remainder of this report from the Office of the Director, EODPP, is organized into three main sections. The first section describes special initiatives of the Office of the Director. The second and third sections provide detailed reports for those sections of the Program that are located within the Office of the Director: namely, the Office of Program Support and the Microbial Systematics Section. More detailed, separate reports for each of the three Branches follow this report of the Office of the Director.

SPECIAL INITIATIVES OF THE OFFICE OF THE DIRECTOR

A major new initiative this past year was the creation of the position of Chief Statistician, NIDR. Initiatives begun last year in the areas of dental public health, minority health, and the epidemiology of pain have also continued, and there are plans to advance them further in the coming year.

Creation of a Chief Statistician Position

A notable new initiative this past year was the creation of the position of Chief Statistician for the Institute, which is located within the Office of the Director, EODPP. Dr. Albert Kingman, who was selected for this position in February, is the first incumbent of this newly created position.

The Chief Statistician is responsible for providing expert advice and consultation to the Director, NIDR; to the Scientific Director, Intramural Research Program; to the Director, Extramural Program; and to the Director, EODPP, regarding the application of biostatistical and epidemiologic principles in the development and implementation of their national programs of research, including laboratory, clinical, and field studies of oral diseases and conditions. In addition to a broad spectrum of activities related to these consultative duties, the incumbent is also responsible for a program of independent research in the development of multivariate risk assessment models, as well as models for evaluating stability and change in longitudinal measures of oral health and conditions.

During this past year, considerable time and effort has gone into consulting with Program and Institute staff on the analysis and interpretation of longitudinal measurements of periodontal disease, on the monitoring of examiner reliability in the oral health component of the Third National Health and Nutrition Examination Survey (NHANES III), on the analysis of experimental evaluations of oral hygiene approaches to the control of gingivitis, and on the analysis of a double-blind, placebo-controlled study of the effects of flurbiprofen on the rate of alveolar bone loss. Consultation was also provided to outside groups, including the University of Alabama at Birmingham on an NIH Grant for a 4-year clinical trial on root caries, which is being supported with supplementary funds from Johnson & Johnson Company. Two manuscripts for the *Journal of Dental Research* were reviewed, and five from *Community Dentistry and Oral Epidemiology.*

Independent research efforts during this same time period have focused on the role of measurement error in assessments of periodontal disease, on surface-specific attack rates in predicting dental caries, and on statistical issues that arise in gingivitis trials of superiority and equivalence.

Two of these foci—the analysis of surface-specific attack rates for dental caries and the analysis of salivary assays—are part of ongoing international collaborative studies with faculty at the University of Göteborg, Sweden. During July-August of this year, Dr. Kingman also planned and presented a "Short Course in Biostatistics" at Chulalongkorn University in Bangkok, Thailand. The course consisted of seven 3-hour lectures in biostatistics for dental researchers.

Liaison with Dental Public Health Organizations

Since the oral health of individuals in the context of community populations is a major concern or oral epidemiology and science transfer activities, liaison efforts have continued with dental public health organizations, including the Association of State and Territorial Dental Directors (ASTDD); the American Association of Public Health Dentistry (AAPHD); the American Board of Dental Public Health (ABDPH); the Association of Community Dental Programs (ACDP); the American Dental Association (ADA) Council on Community Health, Hospital, Institutional and Medical Affairs; the Dental Health Section of the American Public Health Association (APHA); the American Association of Dental Schools (ASDS); and the American Dental Hygienists' Association (ADHA).

The major foci of these liaison relationships are to keep in touch with the implications of what is happening in communities for programs of oral epidemiologic research, to examine the import of oral epidemiologic research for community-based programs, and to explore areas of possible collaboration.

A Research Agenda for Dental Health Professionals

Toward this end, the Program provided staffing and funding support this past year for an initiative of the Dental Health Section of the American Public Health Association to develop "A Dental Public Health Research Agenda." On February 12-13, a workshop was held in Rockville, Maryland, to draft a dental public health research agenda that, if enacted, could enhance practitioners' abilities to improve the nation's oral health. To achieve this overall goal of the workshop, an expert panel was charged (1) to identify the scope and components of "dental public health research," (2) to critique research recommendations that had been developed prior to the workshop through careful review of existing research agendas, as well as through special solicitations of comments and recommendations from dental public health organizations, (3) to develop the specifications and format for a dental public health research agenda, (4) to provide direction for the completion of the agenda, and (5) to develop strategies for implementing the agenda.

The expert panel for this workshop was chaired by Dr. Deborah Winn, an epidemiologist which the National Center for Health Statistics. The panel also included (in alphabetical order) Dr. James Bader, Dr. Brian Burt, Dr. Jean Frazier, Dr. Dennis Deverett, Dr. Irwin Mandel, and Dr. Edith Morrison. Other key staff participants included Drs. Stephen Corbin and Dushanka Kleinman (co-organizers of the project), Dr. Preston A. Littleton, Jr., Dr. Thomas F. Drury, and Dr. Linda Niessen, Director of the Geriatric Dental Program, Perry Point Veterans Medical Center. An additional 17 persons from government and nongovernmental organizations attended the workshop as observers and played a vital role in the process of the expert panel's deliberations, since many of them had been involved in the pre-workshop analyses of existing research agendas and policy documents.

Since the workshop was held, the results of the expert panel's deliberations have gone through several draft and review cycles, with a final working document anticipated later this winter.

Comparison of National, Regional, State, and Local Data

A second initiative is the development and evaluation of information obtained from existing state and local surveys of oral health in the context of what is currently known about oral diseases and conditions at the national and regional levels from recent NIDR surveys of adults, seniors, and U.S. schoolchildren, as well as from surveys conducted by the National Center for Health Statistics, the Health Resources and Services Administration, and other government and nongovernmental organizations. A professional services contract has been let for this purpose, with analyses and a report planned for the coming year.

The Oral Health of Minorities

As described elsewhere in this report, EODPP staff are currently involved in a number of research and science transfer initiatives directed at improving the oral health of minorities. Two of these initiatives have involved staff from across the Program's Branches and Sections: (1) a systematic planning effort to identify, clarify, and resolve issues that currently confound the epidemiologic study of Black oral health; and (2) an evaluation of existing NIDR and other databases as sources of information about the oral health of racial and ethnic minorities.

Issues in the Epidemiologic Study of Black Oral Health

In recent years, there have been numerous efforts to document the health status of Black Americans. Most notable among these efforts, of course, have been the Secretary's Task Force Reports on Black and Other Minority Health. However, even a cursory review of these latter, and other related, efforts highlights the fact that available data on the oral health of Black Americans are subject to a number of limitations, including insufficient sample sizes, limited coverage of the Black subpopulation, selected problems with the reliability and validity of measurements, limited scope of measurement of oral diseases and conditions, and lack of indepth contextual information. These limitations have had several effects on the current state of descriptive and analytical epidemiology of Black Americans' oral health.

(1) Current estimates of the oral health of Black Americans are conceptually limited and provide an incomplete, and possibly distorted, profile of their oral health; (2) generalizations about Black/White differences may be underestimated; (3) empirical checks on alternative interpretations of Black/White differences have been extremely limited; and (4) the manifold relationships between oral health status and general health status have been largely unexplored. Clearly, there is a major need for a comprehensive assessment of the oral health of Black Americans.

To address these issues, staff developed a project proposal this past year that would provide the resources for the planning of such a comprehensive assessment. As in other planning efforts of this kind, specific attention will be given to the following: (1) the formulation of major questions that most need to be answered about the oral health of Black Americans; (2) the evaluation of the adequacy of existing data bases to answer these questions; (3) identification of major data gaps for which new data collection efforts are required; (4) the outlining of a program of research (including baseline and followup surveys, clinical trials, demonstration projects, secondary analyses, and other studies) which together would provide the comprehensive and systematic epidemiologic information needed to fill these gaps; (5) the development of a detailed design for a comprehensive baseline survey of the oral health of Black Americans; and (6) the development of cost estimates both for this latter baseline study and for other projects included in the outline of a program of research in this area.

This project proposal was presented for concept clearance to the National Advisory Dental Research Council on January 22, 1990. A Request for Contract document was approved by Institute staff and Director on March 23rd. An RFP was issued on May 8th. This contract was awarded in late September 1990. Staff of the Health Promotion Section of the Disease Prevention and Health Promotion Branch are project officers for this planning study.

The Oral Health of Racial and Ethnic Minorities

A year ago an EODPP Working Group on Minority Health was formed to undertake a project entitled "The Oral Health of Racial and Ethnic Minorities in the United States." This project had three major objectives: (1) to critically evaluate what is currently known about the oral health of racial and ethnic minorities in the U.S. based on existing literature; (2) to derive from analyses of existing data comprehensive and systematic information on the oral health of these minority populations; and (3) to identify new research and action initiatives to improve the oral health of these minorities.

The planning and design activities of this Working Group provided the foundation for the development of the project on Black oral health described above and has produced a selected bibliography on the oral health of racial and ethnic minorities that has been shared with other researchers working in this area. The activities of this Working Group have also highlighted the enormity of the project that the Group initially set for itself. Accordingly, this recognition has led to the development of more modest, but realistic, proposals for analyses of existing NIDR data sets highlighting Black/White differences in oral health. These proposals are on the agenda of the Working Group for the coming year and are expected to lead to at least one

paper summarizing what is currently known about Black oral health based upon NIDR's recent surveys of adults, seniors, and U.S. children.

Pain Epidemiology Initiatives

The Office of the Director has also continued to provide scientific leadership for broad initiatives in the general area of chronic pain epidemiology and in the specific area of the epidemiology of orofacial pain syndromes.

Chronic Pain Epidemiology

The broad initiative extends work begun several years ago at the National Center for Health Statistics (NCHS) by the Deputy Director and is continuing in collaboration with NCHS staff. These ongoing collaborative projects involve the development of an inventory of currently available population-based survey data on chronic pain; a critical review of existing theoretical, methodological, and empirical perspectives; the clarification of concept-measurement problems in the survey measurement of chronic pain; and the codification of methodological principles involved in writing questions, designing questionnaires, and conducting interviews about chronic pain in general population surveys.

This past year "An Inventory of Pain Data Available from the National Center for Health Statistics" was completed that will appear in NCHS' *Vital and Health Statistics Series* devoted to descriptions of their major data systems. Final editing has also been completed on a series of 20 papers prepared by national and international experts in pain and epidemiology that together provide a comprehensive evaluation of the methodological quality, substantive adequacy, and epidemiologic usefulness of NCHS pain data. The introductory summary for this publication will be completed early this fall and submitted to NCHS' Publications Branch for publication in their *Vital and Health Statistics Series* devoted to health data policy and evaluation reports.

The Deputy Director is an invited participant on the Social Security Administration's Pain Assessment Instruments Development Advisory Panel. This expert panel of federal scientists is providing technical advice and review for the Social Security Administration's more than $3 million project to develop and validate pain assessment instruments for use in the disability determination process.

Epidemiology of Orofacial Pain

Efforts have also continued to clarify epidemiologic aspects of orofacial pain syndromes, including efforts to update current understandings of problems of case definition and case ascertainment in epidemiologic studies of chronic orofacial pain. The Deputy Director attended the "Second Annual Conference on Orthodontics, Occlusion, and the Temporomandibular Joint" on November 9-10 at the University of Michigan, and was a discussant of the presentation by Dr. Christian S. Stohler, "Epidemiology of Temporomandibular Disorders," at the *Head and Neck Pain Symposium: Review of TMJ*

Management Guidelines which took place this past March in Cincinnati at the time of the IADR/AADR annual meeting.

Special efforts will be made this coming year to enhance the current activities of TMD researchers to develop a working consensus for diagnostic criteria for temporomandibular disorders as a basis for data pooling in multi-center epidemiologic studies.

The Deputy Director is also taking the lead in developing a publication and analysis plan for the information on orofacial pain symptoms that was collected in the 1989 National Health Interview Survey. Analyses of the data are scheduled to begin in late October, with several papers projected for completion within the following six months.

Publications

DeNunzio MS, Hicks ML, Pelleu GB, Kingman A, Sauber JJ. A bacteriological comparison of ultrasonic and hand instrumentation of root canals in dogs. *J Endodontics* 1989;15:290-293.

Fleiss JL, Kingman A. Statistical management of data in clinical research in caries and periodontal disease. *Crit Rev Oral Biol Med* 1990;1:55-66.

Kingman A. Statistical methods in risk assessment of dental caries. In Bader JD (ed). Risk Assessment in Dentistry. Chapel Hill: University of North Carolina Dental Ecology, 1990;193-200.

Löe H, Drury TF. Future NIDR initiatives in risk assessment. In Bader JD (ed). Risk Assessment in Dentistry. Chapel Hill: University of North Carolina Dental Ecology, 1990;315-316.

Abstracts

Kingman, A. Assessment of examiner error in scoring periodontal status of adolescents, Abstract No. 627, IADR, Cincinnati, OH, 1990.

Kingman A, Bjarnason S, Plöger W. Surface-specific attack rates for 12 and 13 year olds in Iceland and the U.S., Abstract No. 119, ORCA, Ljubjlana, Yugoslavia, 1990.

Li S-H, Kingman A. Surface-specific attack rates in primary teeth in children from two national surveys, Abstract No. 576, IADR, Cincinnati, OH, 1990.

Presentations

Drury TF. Discussion of "Epidemiology of temporomandibular disorders" by Dr. Christian S. Stohler. Head and neck pain symposium: review of TMJ management guidelines. Clarion Hotel, Cincinnati, Ohio, March 7, 1990.

Kingman A. Methods for the standardization of rates: uses in the evaluation of cost patterns of grant applications at the NIDR. Invited seminar, NIDR, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00519-01 EPI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Measurement Error in Assessing Periodontal Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A — Chief Statistician, NIDR
Anerud, A — Visiting Scientist, EODPP, NIDR
Löe, H. — Director, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
EODPP

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.35	.35	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The reliability and measurement errors associated with the assessment of plaque, calculus, gingival bleeding and loss of attachment in two cohorts of subjects were investigated. Each type of measurement was replicated by the same examiner in two cohorts (29 Norwegians under office conditions, and 35 Sri Lankans under field conditions). All replicates were performed within a 24-hour recall period.

The results showed that these examiners were able to perform more reliably in an office setting than under field conditions. The examiners were best able to score gingival bleeding and loss of attachment, and were less consistent when scoring calculus and plaque. The estimated effect of measurement error for these indices in a longitudinal study would be rather small provided whole-mouth average scores are used to assess changes in disease levels because examiner differences explained a relatively small percentage of the variation in these scores.

PHS 6040 (Rev. 1/84) GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00520-01 EPI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Surface-specific attack rates in Iceland and the U.S.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A — Chief Statistician, NIDR
Bjarnason, S — Associate Professor, Göteborg, SWEDEN
Plöger, W — Chemist, Henkel, Duesseldorf, GERMANY

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical data from 12 and 13 year old Icelanders (n=1032) obtained in 1984 were compared with those obtained for 12 and 13 year olds in the 1980 and 1986 NIDR National Children's Surveys (n=3256 and n=3460, respectively) for the US population.

The surface-specific caries attack rates were consistently higher for the Icelandic population, in many instances 4 to 10 times as high. The notable difference was the strong inverse relationship observed between fluoride exposure and the level of smooth surface caries between the two groups. Also noticed was that the prevalence of dental caries is rapidly becoming a pit and fissure phenomenon in the US population.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00521-01 EPI

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Predictive value of salivary assays in predicting dental caries

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A — Chief Statistician, NIDR
Bjarnason, S — Associate Professor, Göteborg, SWEDEN
Plöger, W — Chemist, Henkel, Duesseldorf, GERMANY

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.05	.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Identifying subjects who are likely to develop high levels of dental caries based on results from simple salivary assays for microoganism counts was the focal point of this study. It is known that these microorganism assays are of limited value in areas with low caries prevalence. Therefore, we studied an adolescent Icelandic population whose mean DMFS scores were roughly 3 times that of their US counterparts.

The basic findings were that the microorganism assays were also of limited value in a high caries prevalence population, although their positive predictive values were slightly higher than those typically reported in a low caries prevalence population. They also proved not to be significantly better than using the initial DMFS scores for individual subjects, suggesting that they were of no cost-benefit value in targeting subjects at high risk for caries.

PHS 6040 (Rev. 1/84)

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00522-01 EPI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Statistical Issues for Gingivitis Trials of Superiority and Equivalence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, A Chief Statistician, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
EODPP

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.02	.02	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The purpose of this study is to present and discuss statistical issues as they arise in the design and analysis of gingivitis clinical trials demonstrating either superiority or equivalence. The problems of designing and interpreting data that are obtained from an equivalence trial are being investigated. There are a variety of statistical issues that are important when conducting studies in which no bona fide control or placebo group is included. Their implications for gingivitis studies will be the focus of this study.

OFFICE OF PROGRAM SUPPORT

The Office of Program Support conducts and directs a broad spectrum of scientific, technical, and administrative activities in support of Program needs. These activities are carried out within three functional workgroups: (1) a Biometry Unit, (2) a Data Services Unit, and (3) a Contract and Reports Unit. Cecelia B. Snowden—a Mathematical Statistician who joined the Program this past March—is Chief of the Office of Program Support, which also includes Drs. Li, Miller-Chisholm and Mirth; Mss. Gregg, Davis, Webb, Bock and Mr. Lee. Ms. Rodgers, who was with the NIDR for 22 years, left this past July.

BIOMETRY UNIT

The Biometry Unit within the Office of Program Support is involved in several research and consulting activities. The primary activities of this unit continue to be conducting research in methodological areas. Specific research examples would include the following: development of caries prediction methodologies, including procedures for estimating DMFS levels for a community based only on the knowledge of age-specific caries-free percentages; analysis of surface-specific attack rates in primary teeth in children from two National surveys; sialoachemistry in Bulimia Nervosa; analysis of the pattern of secretions from the labial minor salivary glands; and the effectiveness of halothane used with ultrasonic or hand instrumentation to remove gutta-percha from the root canal.

Consulting Activities

- Demonstration of sealant effectiveness in combination with fluoride modalities in Nelson County, Virginia
- Comparison of fluoride rinse and fluoride tablets in preventing dental caries in Springfield, Illinois
- Fracture of head and skull study from National Hospital Discharge Survey
- Tongue study to predict age of patients
- Ongoing consulting activities with Bethesda Naval Dental School.
- Tobacco and alcohol use patterns in U.S. school children.

- Prevalence of Oral Mucosal Lesions in U.S. school children.
- Smokeless Tobacco Associated Lesions and Tobacco Use.

DATA SERVICES UNIT

Staff continued computer programming support of the 20-year study of periodontal disease in Norwegian males and Sri Lankan tea laborers for the Periodontal Diseases Section of the Epidemiology Branch.

Staff prepared the school rosters and data collection forms for examinations in Broken Bow, Neb. and Holdrege, Neb. In early April, three staff members also traveled to Broken Bow and Holdrege, Neb. to help with the recording of the data obtained through the oral examinations, as well as with the training of one new examiner and six contracted personnel. In early Fall, staff will travel to Illinois.

Data processing—including code development, coding, data entry, data editing, and tabulation of basic statistical estimates for selected sociodemographic categories—also continued for the following studies:

- Nelson County, Virginia, study of dental sealants in combination with selected fluoride procedures
- Springfield, Ohio, study comparing the combined regimen of weekly fluoride rinsing and daily fluoride tablets with each procedure used alone
- China study of dental caries and periodontal disease indicators
- Nebraska - Fluorosis
- Illinois - Fluorosis
- Sri Lanka
- Guam study of dental caries
- Philadelphia, PA, study of dental caries and indicators of periodontal diseases
- Eastman study of the effects of prenatal fluoride on dental caries

Staff are also evaluating direct data entry to Personal Computers for data collected in field studies.

Training has been initiated for staff in the use of the Statistical Analysis System (SAS), WESVAR and SESUDAAN (Variance estimation procedures), Lotus, dBase, and Graphics packages to facilitate data entry, statistical analysis and to generate study reports.

CONTRACTS AND REPORTS UNIT

Research has continued on the development of the Intraoral Fluoride Releasing System (IFRS). This system is being developed by the NIDR in order to investigate the efficacy of continual topical fluoride for the prevention of dental caries. The IFRS consists of an Intraoral Fluoride Releasing Device (IFRD) and the method for retaining and protecting the IFRD in the mouth. The IFRS is designed to be attached to a molar tooth and to provide continual topical fluoride for six months without maintenance or adjustment. Clinical studies in adolescents recently completed by contractors at the Eastman Dental Center demonstrated that an appropriately designed IFRS can maintain significantly elevated concentrations of fluoride in saliva for at least six months without producing adverse effects and with excellent patient acceptance. A clinical protocol is presently being developed for evaluating the efficacy of the IFRS for the prevention of dental caries in children. It is anticipated that this contract-supported trial will begin in FY 1991 and take approximately four years to complete.

Contractors at the University of Kansas Center for Biomedical Research have completed the Phase I feasibility studies portion of their project for the development of an intraoral bioadhesive system for the delivery of an antifungal agent for the treatment of oral candidiasis. The *in vitro* data generated to date suggest that drug-releasing bioadhesive films should be feasible systems for the sustained delivery of antifungal agents to oral surfaces. After a review of Phase I results with contractor personnel, plans will be finalized for Phase II of the project, a scheduled two year period for additional formulation work to improve the delivery systems, *in vivo* drug release studies in animals, and evaluation of the efficacy of the systems for the treatment of oral candidiasis in an animal model.

Contractors at the University of Texas Health Science Center at San Antonio and the State University of New York at Buffalo have initiated procedures to merge clinical data from their collaborative pilot studies of periodontal diseases in older adults. Laboratory analyses of dental plaque, saliva, and serum samples from the study subjects are nearing completion and will yield data on the oral microbiology, immunology, and salivary biochemistry of older adults with and without periodontal disease. Additional data will be available from diet and psychosocial questionnaires administered to the study subjects. The project is scheduled to be completed in September 1990.

Contractors at the University of Alabama at Birmingham are entering the final year of a study of the transmission of *Streptococcus mutans* from mothers to children. This project is providing valuable basic data on the origin and stability of *S. mutans* infections in children as well as the period of greatest risk of infection. The data should help to describe children that have an increased risk of developing caries and to define the optimal time for preventive therapy.

The clinical phase of a small (30 patient) one year collaborative project with investigators in the Diagnostic Systems Branch and the Clinical Investigations and Patient Care Branch, NIDR, utilizing new techniques in digital subtraction radiography to investigate the effect of flurbiprofen, a non-steroidal anti-inflammatory agent, on the rate of alveolar bone loss in progressive periodontitis has been completed. The treatment codes for this double-blind placebo controlled study will be broken and the collected data will be analyzed in the coming months.

Support contracts administered by the Contracts and Reports Unit are currently providing assistance in the preparation of the NIDR Long-Range Research Plan, the NIDR Biennial Report, and the Research Agenda for Dental Public Health, the evaluation of the Dentist-Scientist Award, the development of a Personnel Operations Handbook for NIDR, the updating of the database for consumer mailings, and the planning of and publicity for the Kreshover Lecture.

The preparation of public use tapes for the National Survey on Oral Health in Employed Adults and Seniors is underway. Separate tapes will be created for employed adults and seniors. Documentation will include sample design, variance estimation, and estimation procedures for selected clinical findings.

The Westat, Inc. contract on the National Survey of Oral Health in School Children was modified in May 1990 to incorporate the calculation of sample weights, variances and documentation for the public use tapes. This modification will provide documented electronic records (public use tapes) which will (a) link related files for the residential history, tobacco and alcohol questionnaire, dental caries examination, periodontal examination, and oral lesion examination; (b) identify previously created derived variables and define data items from questionnaires; and (c) summarize frequencies for derived variables and questionnaire items. In addition, the project will include the development of generalized variance models and/or variance computations for selected statistics.

A collaboration has been initiated with investigators in the Clinical Investigations and Patient Care Branch, NIDR and at the American Dental Association Health Foundation Paffenbarger Research Center to investigate the feasibility of producing a tablet or lozenge for use in patients with xerostomia that will stimulate saliva flow and also provide minerals to repair or strengthen the tooth surface.

Discussions have been held with an investigator at the Eastman Dental Center concerning initiation of a dose-response study of enamel remineralization *in vivo* in the presence of different concentrations of fluoride in saliva. The fluoride would be provided by use of Intraoral Fluoride Releasing Devices. NIDR would be responsible for supplying the IFRDs and for regulatory liaison with the Food and Drug Administration, while the Eastman Dental Center would be responsible for clinical and laboratory activities.

Publications

Bhat M and Li SH. Episodes of Product-Related Traumatic Tooth Injuries Treated in Hospital Emergency Rooms: United States, 1979-87, Community Dentistry and Oral Epidemiology 1990;18:133-8.

Driscoll WS, Nowjack-Raymer R, Heifetz SB, Li SH and Selwitz RH. Evaluation of the Comparative Effectiveness of Fluoride Mouthrinsing, Fluoride Tablets and Both Procedures in Combination: Interim Findings After Five Years, Journal of Public Health Dentistry 1990;50:13-17.

Kleinman DV, Horowitz AM, Mirth DB. Dental technology in the U.S.: An overview for the practitioner. In Hardin JF, ed. Clark's Clinical Dentistry. Chapter 14, Philadelphia: J.B. Lippincott Co., 1989;1-29.

Shern RJ, Fox PC, Cain JL, and Li SH. A Method for Measuring the Flow of Saliva From the Minor Saliva Glands, Journal of Dental Research 1990;69:1146-9.

Presentations

Li SH and Kingman A. Surface-Specific Attack Rates in Primary Teeth in Children From Two National Surveys. Abstracted, AADR programs and abstracted of paper: Journal of Dental Research, 69: Abstr 576, 1990.

Roberts MW, Tylenda CA, Elin RJ and Li SH. Sialochemistry in Bulimia Nervosa presented at annual Pediatric Dentistry meeting.

Shern RJ, Fox PC and Li SH. Pattern of Secretions From the Labial Minor Salivary Glands. Abstracted, AADR programs and abstracted of paper: Journal of Dental Research, 69: Abstr 1542, 1990.

Other Conference Participation

The Office Chief organized and chaired session on "Legal and Ethical Issues in Sharing Large Social Science Databases" at the conference on Advanced Computing in the Social Sciences in Williamsburg, VA. and coordinated two PC-Hands on workshops at the SAS Users' Group International in Nashville, Tenn.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00282-11 OPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Refinement of the Intraoral Fluoride Releasing System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mirth, Dale B. Health Scientist Administrator NIDR,OPS

COOPERATING UNITS (if any)
Eastman Dental Center, Rochester, New York 14620

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Office of Program Support

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Intraoral Fluoride Releasing System (IFRS) is being developed by the NIDR in order to investigate the efficacy of continual topical fluoride for the prevention of dental caries. The IFRS consists of a small controlled-release delivery system for fluoride, the Intraoral Fluoride Releasing Device (IFRD), and the method for retaining and protecting the IFRD in the mouth. The IFRS is designed to be attached to a molar tooth and to provide continual topical fluoride for six months without maintenance or adjustment.

Clinical studies in adolescents recently completed by contractors at the Eastman Dental Center demonstrated that an appropriately designed IFRS can maintain significantly elevated concentrations of fluoride in saliva for at least six months without producing adverse effects and with excellent patient acceptance. These results, combined with earlier animal trials which demonstrated the efficacy of the IFRD in preventing experimental dental caries, suggest that the IFRS could be useful for controlling dental caries, especially in high-risk individuals. A clinical protocol is presently being developed for evaluating the efficacy of the IFRS for the prevention of dental caries in children.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00462-03 OPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Methods for Estimating Age-Specific DMFS and DMFT Scores in the U.S.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Li, Shou-Hua	Statistician (Health)	NIDR, EODPP, OPS
Kingman, Albert	Statistician (Health)	NIDR, EODPP, OD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Program Support

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Typically the prevalence of dental caries is determined by a full mouth examination for each subject. However, limitations of manpower or time may often preclude such a approach. Knutson showed that a screening examination using the presence or absence of caries could be used to predict DMFT scores for children. In this study we investigate how well DMFS scores for subjects under 35 years of age can be estimated by specific models based on age-specific prevalences, using data from the NIDR prevalence surveys (1987 children's survey and the 1985 adult survey). Data for adult older than 35 were not used, since the M component in DMFS is not caries specific for these adults.

Knutson in his original model studied the relationship between the age-specific mean DMFT and the age-specific caries prevalence. Here, we considered two models. One is Knuston's model with DMFS replacing DMFT. The second one is the linear regression model. The assumption for the linear regression model is that the prediction of DMFS can be expressed as a linear function of log of proportion of caries-free individual and age.

Both Knutson's model and the regression model can be used in both children and young adult separately to describe the relationship between caries severity (DMFS) and caries prevalence. The regression model is easier to interpret and estimate than the nonlinear Knutson model. The regression model also emphasized the need to adjust for age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00501-01 OPS

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Surface-Specific Attack Rates in Primary Teeth From Two National Surveys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Li, Shou-Hua	Statistician (Health)	NIDR, EODPP, OPS
Kingman, Albert	Statistician (Health)	NIDR, EODPP, OD

COOPERATING UNITS (if any):

LAB/BRANCH: Office of Program Support

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study was to investigate whether changes had occurred in attack rates in primary teeth during the 1980's. Data on decayed and filled surfaces (df) in 5-9 years old children from the 1979-80 and 1986-87 NIDR dental caries surveys were analyzed. The attack rate was defined as the number of decayed and filled surfaces per 1000 surfaces at risk.

The prevalence of df surfaces declined from an overall mean of 5.31 in 1979-80 to 3.91 in 1986-87. Surface-specific caries attack rates were found to be similar in both surveys. The rank order of the largest six attack rates were: occlusal surfaces of 2nd and 1st molars, distal surface of 1st molar, mesial of 2nd molar, lingual of upper 2nd molar and buccal of lower 2nd molars. The tooth-specific attack rates were, from high to low: 2nd molar, 1st molar, central incisor, lateral incisor and cuspid.

There was no appreciable difference reduction in caries attack rates between the occlusal and proximal surfaces of primary teeth from the 1980 to 1987 NIDR survey. This was in contrast to greater reductions in caries attack rates reported in proximal surfaces of permanent teeth between the 1971-74 HANES survey and the 1980 NIDR survey and also permanent teeth between 1980 to 1987 survey.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00503-01 OPS

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Documentation of Public Use Tapes- 1986-87 and 1979-80 Surveys of School Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Miller-Chisholm, Ann	Health Scientist Administrator	OPS,EODPP,NIDR
Snowden, Cecelia B.	Chief, Office of Program Support	OPS,EODPP,NIDR

COOPERATING UNITS *(if any)*

Westat Inc. Rockville, Md.

LAB/BRANCH

Office of Program Support, EODPP

SECTION

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a follow-up to the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this collaborative effort is to document the survey designs and produce public use tapes for both the 1986-87 and the 1979-80 surveys, and to provide clinical protocals and statistical methodologies for calculating national or regional estimates, weights and sampling errors. The documented tapes generated by this effort will be released for public use by the National Archives.

PHS 6040 (Rev 1/84) GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00504-01 OPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistical Analyses of the 1986-87 and 1979-80 NIDR Surveys of School Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Snowden, Cecelia B.	Chief, Office of Program Support	OPS,EODPP,NIDR
Miller-Chisholm, Ann	Health Scientist Administrator	OPS,EODPP,NIDR

COOPERATING UNITS (if any)
Westat Inc. Rockville, Md.

LAB/BRANCH
Office of Program Support, EODPP

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a follow-up to the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this collaborative effort is to develop generalized variance models for selected non-binary statistics common to both surveys, to calculate correlations between the two surveys and to measure design effects in order to establish optimal cluster sizes for future national surveys.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00505-01 OPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Public Use Tape Documentation-Survey of Oral Health in Employed Adults-1985-86

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Snowden, Cecelia B.	Chief, Office of Program Support	OPS,EODPP,NIDR
Miller-Chisholm, Ann	Health Scientist Administrator	OPS,EODPP,NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Program Support, EODPP

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.3	.2	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The National Survey of Oral Health in Employed Adults was conducted by NIDR in cooperation with Westat, Inc. during 1985-86 to monitor the oral health status of U.S. employed adults. The sampling frame for the survey, recommended by the Bureau of Labor Statistics, consisted of over four million business establishment profiles maintained by Dun and Bradstreet. The basic sampling frame was supplemented by the government facility frames from the U.S. Postal Service and the General Services Administration. The multi-stage sample was drawn from seven geographic regions by establishment size across the standard industrial codes (SICs) excluding military facilities and agriculture. Approximately 15,000 adults were examined representing almost 100 million employed persons aged 18-64. Clinical assessments were made for coronal caries, root caries and periodontal destruction. Demographic and health histories were collected for each person participating in the clinical examination.

The specific aims of this cooperative effort are to document the survey design for the sample of employed adults and to produce a public use tape including clinical protocols and the statistical methodologies for calculating national or regional estimates, weights and sampling errors. The documented tapes generated by this effort will be released for public use by the National Archives.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00506-01 OPS

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Public Use Tape Documentation - Survey of Oral Health in Seniors-1985-86

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Miller-Chisholm, Ann	Health Scientist Administrator	OPS,EODPP,NIDR
Snowden, Cecelia B.	Chief, Office of Program Support	OPS,EODPP,NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Program Support, EODPP

SECTION

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The National Survey of Oral Health in Seniors was conducted by NIDR in cooperation with Westat, Inc. during 1985-86 and provides the baseline for the oral health status of U.S. non-institutionalized persons aged 65 and older. The sampling frame was provided by the Area Agencies on Aging and the sample consisted of all older persons who attended selected multi-purpose senior centers within a designated 12 month interval. The sample of 5,886 seniors represented over 4 million older persons aged 65-103 who attend senior centers in the U.S. Clinical assessments were made for coronal caries, root caries and periodontal destruction. Demographic and health histories were collected for each older person participating in the clinical examination.

The specific aims of this cooperative effort are to document the survey design for the senior sample and to produce a public use tape including clinical protocols and the statistical methodologies for calculating national or regional estimates, weights, and sampling errors. The documented tapes generated by this effort will be released for public use by the National Archives.

PHS 6040 (Rev. 1/84)

GPO 812-018

MICROBIAL SYSTEMATICS SECTION

The Microbial Systematics Section (MSS) has three main areas of activity: (1) international communication of microbial and cell line clone data; (2) development of methods for computer management and analysis of such data; and (3) studies of microbial systematics and ecology. The Section has been active in all three areas this year. Dr. Krichevsky is Chief of the Microbial Systematics Section which also includes Ms. Walczak, Dr. McManus, Ms. Mercer, Ms. Chiu, and Mr. Kennedy.

International Communication

Various members of the MSS participate in the activities of the Microbial Strain Data Network (MSDN) which is a collaborative initiative of four components of the International Council of Scientific Unions (ICSU): Committee on Data for Science and Technology (CODATA), World Federation for Culture Collections (WFCC), International Union of Microbiological Societies (IUMS) and Committee on Biotechnology (COBIOTECH). The Chief, MSS, chairs the MSDN Committee of Management which is the policy and design oversight body. MSS staff are active on the Technical (i.e., Network operations and software development) and Central Directory (i.e., Network main database) Committees. The Information Officer, MSDN, works with the MSS Staff to design and implement the electronic mail, database management, and computer conferencing facilities of the MSDN. These facilities are now operational worldwide on the international telecommunications networks (PSS). MSS staff collaborated in design and as faculty members for MSDN training courses on use of microcomputers in interlaboratory communication, culture collection management, and strain data analysis. These courses are sponsored by the United Nations Environment Programme or research institutions in a particular country. Courses have been held in India, USA, Guatemaale, Brazil, USSR, Egypt, Germany and Spain.

The MSS actively participates in the Hybridoma Data Bank, an initiative of CODATA and the International Union of Immunological Societies. The Chief, MSS, is a member of the HDB Task Group. Staff of the MSS are responsible for much of the software and database design. The HDB also uses the electronic communication network described above to communicate among its nodes located at the American Type Culture Collection, Rockville, MD; at the Institute of Chemical and Physical Research (RIKEN), Saitama, Japan; Institute of Immunology, Dehli, India; National Research Council, Ottawa, Canada; and at the CERDIC, Nice, France.

Method Development

The MSS, in collaboration with many microbiologists around the world, has an ongoing project of developing a unified coding system for computer management of microbial information. The current version of this system in published in book form and is installed as a reference database on the aforementioned MSDN system. As such, the database forms the controlled vocabulary for the MSDN Central Directory of Collections of Strain Data. Further, the coding system is being accepted as an international standard for communicating strain data among microbiologists. The latest initiatives are in the areas of viruses and microbial genetics.

Design and programming of a comprehensive suite of computer programs—the Microbial Information System (MICRO-IS)—is a long term, ongoing project of the MSS. A main-frame version of the MICRO-IS is currently used extensively by the MSS for management of strain data. Additionally, the FDA and EPA use the system for managing and analyzing microbial data in their regulatory roles. The latest thrust of these efforts is development of a portable version of the MICRO-IS for installation on a wide size range of computers including personal computers, mini-computers, and main-frames.

A program for conversion of controlled vocabulary information in text records of the HDB into the highly compressed, table oriented MICRO-IS format has been implemented by the MSS for the HDB. The specifications (table structures) have been developed for transforming the data of the Hybridoma Data Bank into a relational model for ease of global editing and making special kinds of reports. A related project is the development of algorithms for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. Such facilities are required if we are to have any realistic chance of computerizing valuable archival records of phenotypic strain data.

A new program to display a numerical taxonomy similarity triangle in four distinct formats simultaneously is functional. The program allows the user to inspect the four views and choose any or all for full scale output. The program makes use of both color and three dimensional graphics to enhance the user's perception of groupings and taxonomic relationships.

Microbial Systematics and Ecology

The MSS continued its collaboration with the International Working Group on Mycobacterial Taxonomy to elucidate the taxonomic relationships within this genus of pathogens and saprophytes by providing computer analysis of the phenotypic data submitted by the cooperating reference laboratories. The latest findings are yielding new insights into the problem of the separation of the AIDS patient infective M. avium from the closely related non-infective M. intracellular. The latest analysis demonstrated at least one new distinct group of clinically important mycobacteria.

With staff of the EPA and ATCC, the MSS is establishing resources for use in risk assessment of release of genetically engineered organisms in the environment. These resources include accession and analysis of databases of phenotypic characteristics of microorganisms known to be used in genetic manipulation and biotechnological processes, analysis of problems in accession and standardization of such databases from diverse sources, and redefinition of taxonomic boundaries of such organisms. The redefinition is critical to the design of the computer registry for such organisms under the Toxic Substances Control Act.

Staff of the MSS are continuing analysis of phenotypic data on oral microbiota to improve taxonomy and identification criteria for these organisms.

Publications

Krichevsky MI, Sugawara H, and Fabricius B-O. Culture collections as information resources for biotechnology. In: Kirsop BE, Hawksworth DL, eds. Living resources for biotechnology vol. 2. Cambridge: Cambridge University Press, 1990;36-58.

Molitoris E, Marii MA, Joseph SW, Krichevsky MI, Fanning GR, Last G, El-Mishad AM, El Batwani YA, Cowell RR. Numerical taxonomy and deoxyribonucleic acid relatedness of environmental and clinical Vibrio species isolated in Indonesia, Int J Syst Bacteriol 1989;39:442-9.

Thorel M-F, Krichevsky MI, Levy-Frebault VV. Numerical taxonomy of mycobactin dependent mycobacteria and emended description of Mycobacterium avium subsp. avium, Mycobacterium avium subsp. paratuberculosis, Mycobacterium avium subsp. silvaticum subsp. nov., Int J Syst Bacteriol 1990;40:254-260.

Jong S-C, Ho HH, McManus C, Krichevsky MI. Computer coding of strain features of the genus Phytophthora, Binary 1989;1:187-193.

Krichevsky MI. Global communications among microbiologists: Microbial Strain Data Network with the Microbial Information System (MICRO-IS) as catalysts for data standardization. In: Rodgers J., ed. The first Canadian workshop on bioinformatics. Ottawa: Ottawa Carleton Research Institute, 1989;31-42.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE0044-20 EODPP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Handling of Microbial Strain Information by Computers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

McManus, Candace	Microbiologist	MSS, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, EODPP, NIDR

COOPERATING UNITS (if any)
See Attachment

LAB/BRANCH

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.56	1.25	1.31

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The MSS is developing a unified computer coding system for microbial information which is becoming an international standard for communicating strain data. The original bacterial system now includes the algae, yeasts, molds, protozoa, and hybridomas.

Strain data are being entered into computers to provide: data on specific organisms, identification of unknown isolates, definition of parameters of taxa, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Files of primary data on microorganisms found in the oral cavity and related types provide a resource for asking both ecological and epidemiological dental research questions. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

The MSS analyzes the phenotypic data submitted by the cooperating reference laboratories to the International Working Group on Mycobacterial Taxonomy to elucidate the taxonomic relationships within this genus. The latest analysis demonstrated at least one new distinct group of clinically important mycobacteria.

With EPA and ATCC staff, the MSS is building databases to aid risk assessment of release of genetically engineered organisms in the environment including features of microorganisms used in genetic manipulation and biotechnological processes and redefinition of taxonomic boundaries of such organisms.

COOPERATING UNITS: E. Baron, Wadsworth VA Hospital
F. Benedict, Food and Drug Administration
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
B. Kirsop, World Federation for Culture Collections
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
M. Newman, UCLA
S. Holt, University of Texas at San Antonio
V. Levy-Frebault, Pasteur Institute
D. Swartz, University of Maryland
A. Bussard, University of Nice
H. Sugawara, Institute for Physical and Chemical Research

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00250-13 EODPP

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Algorithms for Microbial Systematics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Walczak, Cynthia A.	Computer Scientist	MSS, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, EODPP, NIDR
Mercer, Paula	Computer Programmer	MSS, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, EODPP, NIDR

COOPERATING UNITS (if any): See Attachment

LAB BRANCH:

SECTION: Microbial Systematics Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.68	2.37	.31

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Microbial Information System (MICRO-IS) is an ongoing project to enter, retrieve, and analyze microbiological data for epidemiological, diagnostic, taxonomic, ecological, and regulatory uses. The long term goal is to establish a world-wide data network at a series of cooperating centers. A main-frame version of the MICRO-IS is currently used extensively by the MSS for management of strain data. The FDA and EPA use the system for analyzing microbial data in their regulatory roles. The latest thrust of this effort is development of a portable version of the MICRO-IS for installation on a wide size range of computers including personal computers, mini-computers, and main-frames. This version is now being distributed and accepted on a world-wide basis.

The programs for conversion of controlled vocabulary information in text records of the Hybridoma Data Bank into the highly compressed, table oriented MICRO-IS format were enhanced. The specifications (table structures) have been developed for transforming the data of the Hybridoma Data Bank into a relational model for ease of global editing and making special kinds of reports. A related project is the development of algorithms for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. Such facilities are required if we are to have any realistic chance of computerizing valuable archival records of phenotypic strain data.

A new program to display a numerical taxonomy similarity triangle in four distinct formats simultaneously is functional. The program allows the user to inspect the four views and choose any or all for full scale output. The program makes use of both color and three dimensional graphics to enhance the user's perception of groupings and taxonomic relationships.

COOPERATING UNITS: F. Benedict, Food and Drug Administration
E. Baron, Wadsworth VA Hospital
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
B. Kirsop, World Federation for Culture Collections
S. Socransky, Forsyth Dental Center
V. Levy-Frebault, Pasteur Institute
D. Swartz, University of Maryland
H. Sugawara, Institute for Physical and Chemical Research

EPIDEMIOLOGY BRANCH

The Epidemiology Branch plans, conducts, and directs a coordinated program of epidemiologic research on oral diseases and conditions, craniofacial diseases and disorders, and pain syndromes. This program includes studies designed to monitor the incidence and prevalence of oral diseases and conditions, as well as studies designed to explore the role and relative importance of major risk factors, and the natural history of oral diseases and conditions.

The Branch is formally organized into three sections: (1) a Caries Section, (2) a Periodontal Diseases Section, and (3) a Soft Tissue, Craniofacial Defects, and Pain Section. Dr. Carlos is Chief, Epidemiology Branch, and Acting Chief of the Caries Section, which also includes Ms. Brunelle, Ms. Wolfe, and Ms. Witt. Dr. Löe is Acting Chief, Periodontal Diseases Section, which, this past year, has also included Dr. Åge Ånerud as a Visiting Scientist. Dr. Dushanka Kleinman, Chief of the Soft Tissue, Craniofacial Defects and Pain Section, left the Branch in July to join the staff of the Surgeon General. Dr. Philip Swango was appointed Acting Chief of the section. The section also includes Dr. Bhat, Ms. Ruth Nowjack-Raymer, Ms. Bock, Dr. Gloriana Lopez, and Ms. Lettire. Ms. Baxter is the Branch secretary.

The Branch continues to benefit from the expert advice of Dr. Jens J. Pindborg in the area of HIV infection as well as in the area of oral mucosal tissue epidemiology. Ms. Susan Hayden was a COSTEP member of the Branch this past summer.

Study of the Natural History of the Oral Manifestations of HIV Infection

A major activity of the Branch continued to be a clinical study of the natural history of oral manifestations of HIV infection. Based at the Walter Reed Army Medical Center, the study has thus far enrolled and examined over 400 of a planned 1,000 HIV-positive subjects. Oral candidiasis has been observed in 14 percent and hairy leukoplakia in 12 percent. Both conditions were more prevalent as T4-lymphocyte counts decreased with advancing disease. The first analyses of the microbial constituents of subgingival plaque from infected subjects are in progress. These data will be used to study the relationship of several anerobic microorganisms to periodontal disease.

Caries Declines in Deciduous Dentition

A nation-wide epidemiologic study of school children, completed in 1987, had demonstrated a continued decline in dental caries in permanent teeth believed to be due, in large part, to the widespread use of fluorides. Further analyses during the past year showed similar results in deciduous dentitions. Caries in deciduous teeth of children aged 5-9 years was about 26

percent lower than in 1979-1980. Children who had always lived in optimally fluoridated communities had 23 percent fewer decayed or filled deciduous teeth than those from non-fluoride areas.

The Natural History of Periodontal Disease in Man

A seventh round of clinical examinations was completed in the longitudinal study of the natural history of periodontal disease in Sri Lanka. Continuing analyses of these data demonstrated that the presence of subgingival calculus was a significant risk factor for periodontal attachment loss in these subjects, but this relationship was not observed in a comparison population under observation in Norway. On the other hand, the amount of gingival recession was similar in the two groups, despite vast differences in the level of oral hygiene.

Oral Mucosal Pathology Among Hospitalized Veterans

A study of the prevalence and risk factors of oral mucosal lesions in a Veterans' Administration hospital was completed. Thirty-four percent of these adult patients had at least one lesion. About half of these were considered to require intervention and almost 16 percent were classified as pre-cancerous or disposing to cancer.

Oral-Facial Injury Epidemiology

Very few reliable data exist on the frequency and causes of oral-facial injuries. To begin to study these questions, a 6-month surveillance program was carried out in the emergency departments of the two major hospitals in an Illinois suburb. During this period, 496 persons were treated for oral-facial injuries. Injuries were most frequent in summer. Falls, assaults and vehicular accidents were the most common causes. This research is being expanded to include all medical and dental care providers, public schools and industrial first-aid stations. Collaboration continued with the National Center for Health Statistics in the conduct of the NHANES-III national survey. Activities included development of a plan for quality assurance of the oral examination data and design of computer software to process and analyze the results of replicate field examinations.

Other Activities

Staff members participated in several international activities related to the epidemiologic study and surveillance of oral manifestations of HIV-infection, including development of a collaborative protocol for standard, world-wide reporting of oral findings. Staff also served as faculty in a course designed to train oral health professionals from developing countries to recognize and document HIV-related oral lesions.

Branch staff continued to provide technical assistance and consultation to such organizations as the World Health Organization, American Dental Association, American Public Health Association and the National Center for Health Statistics.

Publications

Bhat M, Nelson KB. Developmental enamel defects in primary teeth in children with cerebral palsy, mental retardation, or hearing defects, Adv Dent Res 1989;3(2):132-42.

Bhat M and Li S-H. Episodes of product-related traumatic tooth injuries treated in hospital emergency rooms: United States, 1979-87, Comm Dent Oral Epid 1990; 18:133-8.

Brunelle JA, Carlos JP. Recent trends in dental caries in U.S. children and the effect of water fluoridation, J Dent Res 1990;69(Spec Iss):723-7.

Carlos JP, Wolfe MD. Methodological and nutritional issues in assessing the oral health of aged subjects, Am J Clin Nutr 1989;50:1210-8.

Ismail AI, Brunelle JA. Oral health of Hispanic Americans - summary of findings from HHANES. In: Hispanic HANES Chartbook, NCHS, 1990.

Kleinman DV, Horowitz AM, Mirth DB. Dental technology assessment in the U.S.: An overview for the practitioner. In: Harding JE, ed. Clark's clinical dentistry. London: Lippincott, 1989, Chap. 14.

Wilentz JS, Kleinman DV. Creating a national research agenda addressing the oral health of special patients. Special Care in Dentistry 1989;9:116-21.

Presentations

Bhat M, Flanders RA. Orofacial injury surveillance in Kankakee County hospitals, Illinois. Presented at the annual meeting of the Association of State and Territorial Dental Directors, San Diego, CA, April 1990.

Brunelle JA. Caries status in the U.S. and prediction of future trends. Invited presentation at the ORCA Satellite Symposium, Ljubljana, Yugoslavia, July 1990.

Brunelle JA. Effectiveness of fluorides - summary of findings from the two national surveys of U.S. schoolchildren. Presented at the ASTDD annual meeting and PHS Conference on Oral Health, San Diego, CA, April 3, 1990.

Brunelle JA. Caries attack in the primary dentition of U.S. children. Presented at the IADR/AADR, Cincinnati, OH, March 1990.

Kleinman DV, Swango PA. Oral health effects of tobacco use in U.S. teenagers, 1986-1987. Invited presentation to the NIH Prevention Coordinator's Committee, April 10, 1990.

Lopez, GM. Unmet oral health needs of the maternal and child population. Public Health Service Workshop on Oral Health of Mothers and Children, September 11-12, 1989.

Swango PA,, Kleinman DV, Levinson P, Niessen L, and Swisher L. Prevalence of oral soft tissue pathology in long-term extended care hospital inpatients. Presented at the IADR meeting, Cincinnati, OH, March 1990.

Swango PA, Kleinman DV. Overview and preliminary findings of oral manifestations of HIV infection in the Walter Reed study. Presented at the annual meeting of the Military Public Health Dentistry Special Interest Group, November 1, 1989.

Swango PA, Kleinman DV. Preliminary findings of oral manifestations of HIV infection in a military population. Presented at the monthly meeting of the National Capital Oral Diagnosis Study Group, October 10, 1989.

Swango PA and Kleinman DV. Preliminary findings of oral manifestations of HIV infection in a military population. Presented at a meeting of the Walter Reed Retrovirus Research Group, October 4, 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00399-06 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Periodontal Diseases in Adolescents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Carlos, James P.	Chief	EB, EODPP, NIDR
Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS (if any): Department of Oral Biology, SUNY at Buffalo, Buffalo, NY

LAB/BRANCH: Epidemiology Branch

SECTION: Caries

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.7	.7	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this continuing study is to determine the prevalence and progression of gingivitis, epithelial attachment loss, and bone loss in a group of adolescents residing in the U.S.

A longitudinal study is in progress of Navajo adolescents to investigate microbiologic, systemic and other factors that may contribute to the high prevalence of disease. Two hundred twenty-six subjects were examined in February 1986, 1987 and 1988 using the same clinical and radiographic techniques. Subgingival plaque samples were obtained from all first molars and analyzed for A. actinomycetemcomitans, B. gingivalis and B. intermedius. Analyses of first year data indicate that the combination of calculus, gingival bleeding and B. intermedius gave the most parsimonious explanation of the presence of attachment loss. Bite-wing radiographs from all three years have now been read for evidence and quantification of alveolar bone loss using image enhancement software (JAVA).

The proportion of subjects and sites infected with B. gingivalis had increased substantially by the last examination, while the prevalence of A. actinomycetemcomitans and B. intermedius remained fairly constant.

Multiple logistical regression analyses are in progress to study the joint effects of clinical and microbiologic variables on bone loss and attachment loss.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00410-06 EB

PERIOD COVERED
October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Natural History of Periodontal Disease in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Löe, Harald	Director, NIDR	PDS, EODPP, NIDR
Anerud, Åge	Visiting Scientist	EB, EODPP, NIDR
Kingman, Albert	Statistician	OPS, EODPP, NIDR
Silness, John	Visiting Scientist	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*
University of Texas Dental School, San Antonio, Texas
Department of Oral Biology, University of Buffalo, New York

LAB/BRANCH
Epidemiology Branch

SECTION
Periodontal Diseases Section

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study of the natural history of periodontal disease in Sri Lankan tea laborers and Norwegian males is continuing.
Future studies include (1) completing the analysis of the longitudinal impact of dental restoration in a gingival location and their relationship to the initiation and progression of periodontal disease. Also, (2) the study of gingival recession in a population who practiced mechanical oral hygiene daily and in a population where oral hygiene never was practiced will be finished and submitted for publication. (3) Stability of the gingival lesion and conversion of gingivitis to periodontitis are the critical issues in the quantitative destruction of the periodontium and can only be studied with some precision in longitudinal materials. These studies will be based on data from both populations. (4) Bacteriological and immunological studies of the two populations are also underway. The specific aims of these studies are to relate the presence and absence of selected periodontal pathogens and peripheral blood antibody titers to the rate of periodontal destruction in the two groups. (5) Studies of the pattern and rates of tooth loss over the twenty-year period in the Sri Lankan tea laborers will be initiated. (6) The problems of method errors and reproducibility in periodontal epidemiology which were studied during the previous period will be completed and the results published.
The two master data sets in SAS for the longitudinal surveys in Norway 1969-1988 are: LOE.NOR.AVO (All Valid Observations) and LOE.NOR.IAS (In All Surveys) are completed, for Sri Lanka 1970-1990: LOE.SRIL.AVO (All Valid Observations) and LOE.SRIL.IAS (In All Surveys).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00420-05 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Analysis of National Survey of Oral Health in School Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A. Statistician (Health) EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology Branch

SECTION: Dental Caries

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.40	.30	.10

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A national survey of the Oral Health of School Children was conducted during the 1986-87 school year. Approx. 41,000 children were examined by 13 trained and calibrated dental teams.

Analysis of DMFS indicated a 37% change in mean estimates compared to a similar study conducted in 1979-80. There was a lower level of disease at every age. Mean DMFS was also lower in all regions of the country; however, there were still regional variations as before. Approx. 50% of the children aged 5-17 were caries free in their permanent dentition. A monograph "Oral Health of U.S. Children, 1986-87" on caries levels was prepared.

Analysis of sealant observations was done. Only 7.6% of the children aged 5-17 had sealants present. More females than males had sealants. 11% of the 8-, 9-and 10-year-old children had sealants. The average number of sealants in children with sealants was 4.2 per child.

An estimate of the prevalence of dental fluorosis was made using Dean's Index on 2nd through 12th graders. 22% of children showed definite signs of fluorosis, 17% very mild, 4% mild, 1% moderate and 0.3% severe. Regions showed large variations.

Analysis of caries attack of primary teeth was done. Mean caries experience for children age 5-9 years was 3.91 dfs. This was a 26% decline from 1979-80 survey. The dfs was lower in every region except Region VII, with the greatest decline observed in Reg. I. Approx. 50% of the children had no dfs; 28% had more than 4 surfaces d or f.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00432-04 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Bone Loss in Adolescents Related to Periodontal Status in Adults

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Carlos, James P.	Chief	EB, EODPP, NIDR
Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS (if any): University of Umeå, Umeå, Sweden

LAB/BRANCH: Epidemiology Branch

SECTION: Caries

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.7	

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this investigation was to determine whether periodontal destruction, diagnosed in adults, can be predicted by examining bite-wing radiographs taken in adolescence.

This retrospective investigation was conducted in Sweden where a unique system of lifetime address registry and public dental care records are available for cross-referencing and locating subjects. The University of Umeå identified 250 subjects, approximately 30 years of age, for whom bite-wing radiographs taken at age 15 were still available, and who resided within a short distance from Umeå. Dental examinations were conducted on 250 subjects at four dental clinics in the surrounding county of Vasterbotten. Clinical components consisted of exam for evidence of gingival bleeding, calculus, pocket depth, and attachment loss at the buccal and mesio-buccal aspects of all teeth excluding third molars. Bite-wing radiographs were taken using an Eggen film standardizing device.

The radiographs taken at age 15 vary greatly in quality and angulation. Several computer assisted imaging protocols were explored to determine how the images can be improved to provide a suitable baseline for diagnosis of bone loss. The protocol selected employs a median filter and a SOBEL V convolution matrix.

Using this technique, 400 pairs of bite-wing radiographs have been diagnosed for bone loss, and the records prepared for analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00443-03 EB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prevalence of Oral Soft Tissue Pathology in a Long-Term Care Facility

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Philip A. Swango — STCDPS, EB, EODPP, NIDR
Dushanka V. Kleinman — STCDPS, EB, EODPP, NIDR
Linda C. Niessen — Perry Point VA Medical Center

COOPERATING UNITS *(if any)*
Perry Point Veterans' Affairs Medical Center
Perry Point, MD

LAB/BRANCH
EB, EODPP, NIDR

SECTION
Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is a cross-sectional survey to document the prevalence and risk factors of oral soft tissue pathologies occurring in patients admitted to the Perry Point VA Medical Center during a 24-month period. About 900 patients have been examined. Pathologies that cannot be definitively diagnosed by the examining dentist are referred to a consultant oral pathologist. The objectives of the study are to estimate the prevalence of oral mucosal conditions in the population, characterize the range and severity of the pathologies, and to assess treatment needs resulting from these conditions. Information is collected from existing records regarding risk factors such as dental prostheses, medical conditions, medications, and the use of tobacco and alcohol.

Findings to date show that 34 percent of the patients presented with one or more oral pathologic condition. Prevalence of oral lesions was greater in patients wearing removable dental prostheses (53 percent) than in those without prostheses (26 percent). Analyses for the effect of other risk factors are in progress.

PHS 6040 (Rev 1/84)

GPO 814-016

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00464-03 EB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural History of Oral Manifestations of HIV Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Philip A. Swango	STCDPS, EB, EODPP, NIDR
Dushanka V. Kleinman	STCDPS, EB, EODPP, NIDR
Philip Fox	CIPCB, IRP, NIDR
Joseph Zambon	SUNY at Buffalo
Edmond Tramont	Walter Reed Army Institute of Research
Charles Oster	Walter Reed Army Institute of Research

COOPERATING UNITS *(if any)*
Walter Reed Army Institute of Research

LAB/BRANCH
EB, EODPP, NIDR

SECTION
Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

United States Army personnel and dependents who have tested seropositive for the Human Immunodeficiency Virus (HIV) are given medical examinations and treatment at the Walter Reed Army Medical Center. Subjects are also invited to participate in a research protocol to study the natural history of HIV infection, conducted by the Walter Reed Army Institute of Research. An oral health research component conducted by NIDR is a part of this natural history study.

The oral component documents the prevalence and incidence of oral pathologic conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions are also characterized, and the role of oral manifestations as early predictors or markers of disease progression are studied. Areas of emphasis are mucosal pathologies, periodontal conditions, candidal infections, and salivary constituents. Results to date show an increase in mucosal pathology as T4 cell counts decrease with progression of HIV disease. The most commonly observed mucosal pathologies were oral candidiasis and hairy leukoplakia. Destructive periodontal disease has occurred in about 27 percent of subjects examined. Subjects are re-examined every six months, permitting longitudinal observation.

PHS 6040 (Rev. 1/84) GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00468-03 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Dental Caries in U.S. Children on Fluoridated/Nonfluoridated Water Supplies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brunelle, Janet A.	Statistician (Health)	EB, EODPP, NIDR
Carlos, James P.	Chief	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Epidemiology

SECTION: Caries

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.40	.35	.05

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Residential histories collected during a national survey of oral health of U.S. schoolchildren conducted in 1986-1987 were used to establish two groups: those children who always lived on a public water supply in a fluoridated community (N=8,165) and those who never lived in a fluoridated community (N=8,233).

Comparisons of the mean levels of Decayed, Missing and Filled Surfaces were made by age, sex and region of the country. Mean DMFS for children with lifelong exposure to water fluoridation was 2.8 compared to 3.4 mean DMFS for children who had never lived in areas with fluoridated water. Mean DMFS for mesial-distal surfaces was about one-third higher in children without water fluoridation. Both groups of children reported high use of supplemental and/or topical fluorides. Regional differences between groups varied greatly from 61% difference in Region VII to 6% difference in Region III. These findings were presented at a workshop "Mechanisms of Fluoride" and published in the Journal of Dental Research, Sp. Iss. Feb. 1990.

Mean dfs for deciduous teeth was computed for those children ages 5-9 who had a lifelong history of community water fluoridation or no history of water fluoridation. The mean dfs was lower in all age groups for children on fluoridated community water supplies, with a combined difference of 23% fewer dfs.

PHS 6040 (Rev 1/84)

GPO 914-919

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00469-03 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Distribution of Root Caries in U.S. Adults

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brunelle, Janet A. Statistician (Health) EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Epidemiology

SECTION: Caries

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.12	.12	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1985 the NIDR conducted a national survey of the oral health status of employed adults and seniors, in which measurements of root caries were made for the first time on a national sample of the U.S. population. Examinations were made on 15,132 employed adults representing 99.6 million persons. Visual tactile examinations were made for decayed and filled root surfaces. Each tooth was considered to have four root surfaces. No x-rays were taken.

Approximately 21% of the employed dentate population aged 18-64+ years had at least one decayed (D) or filled (F) root surfaces (S) with more males than females affected. The mean number of DFS was less than one for the employed dentate population. Less than half (47%) of the DFS were filled.

Distribution of the disease by tooth and surface type was analyzed. Buccal surfaces were the most frequently affected surfaces with decay or fillings occurring four times as often here as on any other surface. The distal and mesial surfaces are the next most frequent sites of root caries and the lingual surface is the least affected. However, the differences among these last three surface types is minimal. Root caries was most common in mandibular pre-molars, molars and maxillary cuspids. Lower incisors were rarely involved (<1% of the DFS occurred in these teeth). Analysis is continuing using multiple and logistic regressions to ascertain relationship of root caries to other variables measured in the survey. Manuscript on surface distribution is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00470-02 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Demographic and Environmental Correlates of Caries in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Carlos, James P.	Chief	EB, EODPP, NIDR
Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Epidemiology Branch

SECTION: Caries

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.8	.3	.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of the striking findings of the 1986-1987 NIDR Survey of Dental Caries of U.S. School Children was that approximately half the children ages 5-17 had never experienced caries in their permanent teeth. This study is exploring the relationship between a caries-free status and such variables as sex, ethnicity, geographic region and urban-rural residence. Controls are the approximately 1400 children of the same ages whose cumulative caries experience exceeds two standard deviations of the mean (mean DMFS) for that age group.

Preliminary analyses indicate highly significant differences among the two groups with respect to geographic region of residence. Therefore, the study was expanded to include data on ten trace metals obtained from drinking water samples from the schools attended by the subjects.

Because the probability of being in the high caries group was strongly correlated with age, separate logistical regression models were fit for each year of age. Phosphorus, silicon, strontium and molybdenum were significantly ($p < 0.10$) but weakly associated with caries status, though the relationship was inconsistent. Further models were fitted to test interactions among the independent variables.

The analyses were completed and are being summarized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00486-02 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Periodontal Health of 14-17 Year-Old School Children - U.S. 1986-87

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mohandas Bhat — Visiting Scientist, EB, EODPP, NIDR
Carla Bock — Computer Programmer, EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: EB, EODPP, NIDR

SECTION: Soft Tissue, Craniofacial Defects and Pain

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study analyzed the data on gingival bleeding, calculus, and periodontal attachment loss in 14-17 year-old children who participated in the National Survey of Oral Health in U.S. School Children conducted by the NIDR during 1986-87. The 11,111 children in the sample represent an estimated 13 million U.S. school children in this age group.

The prevalence of gingivitis was approximately 60% for children in this age group. The percent of examined sites with gingivitis per child, however, was less than 6%. There was an overall age associated decline in prevalence of gingivitis. Males, in general, and children in the category of "blacks and other races" showed higher prevalence of gingivitis. Prevalence of gingivitis was slightly higher at buccal than mesial sites and was most common in molar areas in the maxilla and incisor areas in the mandible. The prevalence of supragingival calculus alone was nearly 34% and subgingival calculus was approximately 23%. The percent of teeth with calculus per child was approximately 8 for the former and 4 for the latter type of calculus. There was a slight age-associated increase in prevalence of latter type of calculus. Males and children in the category of "blacks and other races" showed higher prevalence of both types of calculus. The Mean periodontal attachment loss, determined by probing with #2-12 probe, was 0.33 mm (0.26 mm for buccal and 0.40 mm for mesial surfaces) and varied very little by age, sex or race. The teeth most frequently affected by attachment loss of 2 mm or more were maxillary molars and bicuspids, followed by mandibular molars and cuspids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00511-01 EB

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Fractures of the Skull and Face among Native Americans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mohandas Bhat - Visiting Scientist, EB, EODPP, NIDR
Robert J. Collins - Chief Dental Services Branch, Indian Health Service
Carla Bock - Computer Programmer, EODPP, NIDR

COOPERATING UNITS (if any): Indian Health Service

LAB/BRANCH: Epidemiology

SECTION: Soft Tissue, Craniofacial Defects and Pain

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this study was to do a trend analysis of the the incidence of first-listed diagnosis of fractures of the skull and face (S&F) bones (ICD codes 800-804) from the Indian Health Service (IHS) Direct and Contract General Hospital Inpatient database for the fiscal years 1980 through 1988. This database is estimated to represent about a million Native Americans seeking treatment each year. This database is one of the few hospital discharge databases containg cause-specific injury data coded according to external cause of injuries (ICD-E codes).

The results showed that the incidence of fractures of all bones (ICD codes 800-829) for Native Americans generally declined during this 9-yr period from about 4,000 in 1980 to about 2800 in 1988. S&F fractures also showed a general decline from about 700 in 1980 to about 470 in 1988. During this period, S&F fractures averaged about 17% of all fractures and major causes of S&F fractures were: assault (35%), falls(24%), motor vehicle & road accidents (20%), and other accidents (8%).

PHS 6040 (Rev 1/84)

GPO 914-918

ANALYTICAL STUDIES BRANCH

The Analytical Studies Branch conducts research analyzing the relationships among patterns of oral diseases and disorders, the need for, and utilization of, dental care, dental education, dental research, and dental delivery systems. These functions are carried out by: (1) investigating the interrelationships among economic, social and personal characteristics, the distribution of various oral diseases, and dental treatment needed and utilized; (2) developing new models and theories which integrate social and epidemiological factors into models which explain the relations between oral disease and the social, economic, and personal characteristics of individuals; (3) conducting cost-effectiveness and cost-benefit analyses of various preventive and treatment methods; and (4) providing consultation and technical assistance in these subject areas to other Institute components, the NIH, PHS, and other Federal and nonfederal scientists and organizations.

Dr. Brown is Chief, Analytical Studies Branch, which also includes Mr. Oldakowski. Richard Oldakowski is a computer programmer. Ms. Linda Williams has been the secretary to the Branch Chief since November 1989. The Branch had a PHS Dental Co-Step, Mr. William Cruse, for the summer.

INTRAMURAL ANALYTICAL STUDIES

A variety of analytical research was conducted by Branch staff during fiscal year 1990. Several of the studies involved collaboration with university researchers.

Periodontal Studies

The periodontal health of employed U.S. adults is being studied in collaboration with Dr. Richard C. Oliver of the University of Minnesota. The collaboration has resulted in several publications and will result in several more. The overall description of periodontal conditions is complete and published. The association between sociodemographic variables and periodontal status is complete and has been submitted for publication. A study developing a model to predict groups at high risk to have periodontal destruction is also complete. Additional studies will soon be started.

Additional periodontal studies were conducted in collaboration with other NIDR staff. A study of the prevalence of juvenile periodontitis in U.S. children aged 14 to 17 years, using data from the 1986 NIDR Survey of Children, is completed. A study of variations in the periodontal health of older Americans among different sociodemographic groups is nearing completion.

Tooth Loss, Tooth Conditions, and Dental Caries Studies

Branch staff in collaboration with Dr. Philip Swango of the Epidemiology Branch completed a study entitled, "Trends in Caries Experience in U.S. Employed Adults from 1971-74 to 1985: Cross-sectional Comparisons". The paper has been accepted for publication in the Journal of Dental Research. Other analyses have been completed on the status of the dentition in U.S. adults, using data collected from a national household probability sample on measures of need for care in addition to usual measures of oral disease, and a manuscript will be submitted to a scientific journal. William Cruse, a summer dental COSTEP, assisted the Branch Chief in the completion of a study of the distribution of existing and needed dental prostheses in the U.S. in 1981. Analyses are complete and a manuscript is being prepared.

Dr. Lawrence Meskin collaborated with Branch staff to study tooth loss patterns. Analyses are complete. In addition, Branch staff collaborated with the Boston VA Outpatient Clinic to develop a risk model for tooth loss, using data from a panel of healthy males. Analyses are complete. Results will be reported in collaboration with Dr. Chauncey.

Dental Utilization, Need and Economics

Branch staff worked with Drs. Beazoglou and Heffley of the University of Connecticut to study the estimated savings in dental expenditures which resulted from prevention of dental disease. The study is complete and will be presented at the 2nd International Congress on Health Economics to be held in Zurich, Switzerland on September 10-14, 1990. The papers presented during the Congress will be published. Branch staff used longitudinal data to investigate the association between dental status and the utilization of dental services. Analyses are complete. A theoretical paper and an empirical paper will be submitted for publication.

Staff has collaborated with Dr. Mel Ringelberg, a dentist with the State of Florida, on the cost of water fluoridation among 44 Florida communities. Analyses are complete. Drs. Brown and Ringelberg will co-author two papers reporting the results of the analyses. One paper will report the costs of water fluoridation among the Florida communities. It has been submitted for publication. The second paper will report the results of the estimation of a cost function which will allow the prediction of costs based on population served by the water system and other explanatory factors. This is the first cost function that has been estimated for the costs of water fluoridation.

CONTRACTS

Partly because of their significance, and partly because they involve primary data collection through contracts, four analytical studies are discussed in more detail below.

A Microsimulation Model of Tooth Loss, Oral Conditions, and Dental Utilization and Expenditures

The Branch is developing a computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S. These forecasts will be developed in considerable sociodemographic detail. A feasibility study of alternative modeling methods indicated that development of a microsimulation model is both feasible and desirable.

Cornell University was awarded the contract to develop the model on May 15, 1990. Dr. Caldwell, a microsimulation specialist, will be principal investigator. Dr. Stephen Eklund of the University of Michigan will assist in the development of the oral disease and conditions portion of the model. Several noted dental specialists and modeling experts will be consultants to the project. Development of the model and the production of initial forecasts will take approximately two years.

Microsimulation will be the approach used. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental service use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. Policy experiments with the full model will be done both for past times and also for future times. As a framework for synthesizing research findings, the NIDR micro model would provide a vehicle for carrying out experiments in which the latest dental research could be applied consistently and systematically to key dental policy issues.

A Study of Utilization, Treatment Needs, Cost, and Dental Disease

The purpose of this study is to collect data to study the relationships between dental disease and other oral conditions, treatment needs, economic factors, and utilization of dental services.

In 1963, the Department of Veterans Affairs initiated an interdisciplinary and longitudinal investigation of the normal aging process. Participants consisted of 2,400 men with stable living and work conditions in the Boston, Massachusetts area. From this panel, 1,221 self-selected subjects between the ages of 25 and 75 volunteered for the Dental Longitudinal Study in 1968. These persons have received a complete dental examination every three years since 1968. The examinations included a radiographic survey and a comprehensive clinical examination documenting dental caries, periodontal status, missing teeth, and oral hygiene. This project is supplementing these clinical data with detailed data from the dental offices attended by the panel members over the past ten years. Utilization and cost data are being collected for approximately 728 panel participants. Data collection is approximately one-third complete and is scheduled for completion in December of 1990. Once these data are collected they will be integrated with the clinical data from the VA Dental Longitudinal Study. They will represent one of the richest databases ever assembled to analyze the relation between oral health measures, treatment needs, and the utilization of specific dental services.

Analysis of the data will be undertaken by the NIDR in collaboration with VA staff and expert consultants.

Collection of the data is over 80% complete and on schedule. To date, the response rate is over 90%. Complete utilization data over a 10 year period has been collected on 88% of the participants. Quality of the data is good. The utilization data will be integrated with existing data on oral health, general health, and socioeconomic attributes of the panel participants. A file of the integrated data will be available to the NIDR by December, 1990.

A Study of Alveolar Bone Loss and Aging Among Healthy U.S. Males

The project is a cross-sectional and longitudinal analysis of alveolar bone loss (ABL) in aging men. Study subject will be 700 adult male participants in the VA Dental Longitudinal Study (VADLS). Bone loss will be determined using existing radiographs, including (1) intraoral periapical, (2) cephalometric, and (3) hand-wrist radiographs. The VA-DLS has the most extensive longitudinal radiographic data base available with which to examine the factors associated with progressive alveolar bone loss. The existence of sequential radiographs permits a longitudinal analysis of the actual ABL experienced over a twenty year period at three year intervals. The study will be able to determine actual rates of ABL per site and per subject, while also controlling for any pre-existing ABL found at the baseline examination. Extensive dental and general health information is available for the subjects so relations between ABL and systemic conditions can be observed. Moreover, the unique opportunity exists to examine the relationship of ABL to changes in bone at non-periodontal sites in the same subjects.

Sequential full-mouth series of intra-oral periapical radiographs, obtained at three-year intervals, will be computer digitized. A two-dimensional transformation technique developed by Jeffcoat and coworkers will be used to determine alveolar bone loss over a 15 year period on all study participants. Frontal and lateral cephalometric radiographs were taken at three-year intervals on all participants for a 10 year period of the VADLS. These will also be computer digitized and measurement of bone density at various non-peridental sites will be calculated. Finally hand-wrist radiographs were obtained on all participants at three to five year intervals. These will also be digitized and changes in cortical plate thickness of metacarpal bones will be calculated. Computer digitization is important because it will produce quantity measures of bone loss that are comparable between the three types of radiographs. Measurement error will be reduced substantially.

The proposed project should lead to better understanding of the contribution of extra-oral factors to ABL. Furthermore, The relationship of ABL to age-related changes in bone at non-periodontal skeletal sites can be investigated, allowing study of the contribution of systemic changes in bone density to the intraoral changes in alveolar bone loss. Finally, the project will yield much improved estimates of the rate of ABL conditional on putatively important explanatory variables. This information is essential for the NIDR modeling project.

The study was approved for funding in December, 1989. The study will be conducted in collaboration with the Department of Veterans Affairs, Dental Outpatient Clinic, Boston, MA. An Interagency Agreement with the VA was awarded in June, 1990. Drs. Howard Chauncey and Raoul Garcia are principal investigators.

Determinants of Permanent Tooth Loss in the United States

Tooth loss remains an important problem in this country. Understanding its determinants is essential for the identification of individuals and groups at high risk for tooth loss and for effective interventions to reduce tooth loss among persons where it remains a problem. The objective of this project is to measure tooth loss and its determinants in the natural settings of dental offices and larger public clinics. The initial phase of the project is a pilot study to field test and refine a protocol for the full study. It is being conducted by the University of Connecticut. Information is being collected regarding the influence of disease/clinical conditions, economic variables, patient/provider characteristics, and attitudes on decisions to extract permanent teeth. The collected data will be used to assess the significance of factors contributing to the loss of permanent teeth in the U.S. With these data, a more complete model of tooth loss than is currently available can be estimated. The field portion of the pilot study will be completed in August, 1990. The results will be available in September, 1990. If the pilot study is successful, a larger, more general study will be proposed.

Other Activities

Branch staff participated in review and training of the dental examiners for the NHANES III study. Branch staff participated with other members of the Epidemiology and Oral Disease Prevention Program to study the epidemiology of the oral health of minorities. Several presentations were made at major dental meetings throughout the country. Papers were presented at the annual meetings of the American Association for Dental Research and the American Economic Association. A paper will be presented at the 2nd International Congress on Health Economics in Zurich, Switzerland.

Publications

Brown LJ, Oliver RC, Löe H. Evaluating periodontal status of U.S. employed adults. Epidemiology and Oral Disease Prevention Program, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892. J Am Dent Assoc 1990 Aug;121(2):226-32.

Oliver RC; Brown LJ, Löe H. An estimate of periodontal treatment needs in the U.S. Based on epidemiologic data [letter]. J Periodontol 1990 Jan;61(1):68.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00473-02 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Restorative and Treatment Needs in the U.S. in 1981

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson	Chief	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR
Cruse, William	Summer Co-Step	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treatment needs based on the existence of clinical conditions are important determinants along with socioeconomic variables of the demand for dental services and dental manpower. Little information is currently available on the extent of needs in the U.S. This study will result in a series of reports. One report will describe the types and the amount of treatment needed by the U.S. population in 1981. From a household probability survey of 7700 persons in the U.S., information was collected on the condition (sound, filled, missing, etc.) and the needed treatment (filling, crown, extraction, etc.) of each permanent tooth. Information was also collected on type of prosthesis (full, partial, fixed) present and needed for each permanent tooth. Selected findings follow: 43 percent of the U.S. population needed 1 or more restorations at the time of examination; 37 percent needed a posterior restoration while only 18 percent needed an anterior restoration; 9 percent needed an extraction; 2 percent needed endodontics; 4 percent needed an anterior crown; and 11 percent needed a posterior crown. The total cost in 1985 dollars to treat these conditions is estimated at slightly over 100 billion dollars. Close to 70 percent of the total is for the placement of a new prosthesis where none existed at the time of exam. Persons 55 years and older accounted for 30 percent of the cost while persons 35 to 54 accounted for 40 percent.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00475-02 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Utilization, Treatment Needs, Cost, and Dental Disease in Veterans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson	Chief	EODPP, NIDR
Chauncey, Howard H.	Associate Chief of Staff for R & D	VA

COOPERATING UNITS (if any): Veterans Administration Outpatient Clinic, Boston, Massachusetts

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Veterans Administration in 1963 initiated an interdisciplinary and longitudinal investigation of the normal aging process. Participants consisted of 2,400 men with stable living and work conditions in the Boston, Mass. area. From this panel, 1221 self-selected subjects between the ages of 25 and 75 volunteered for the Dental Longitudinal Study in 1968. These persons have received a complete dental examination every three years since 1968. The triennial examinations include a radiographic survey and a comprehensive clinical examination documenting dental caries, periodontal status, missing teeth, and oral hygiene.

This project is supplementing these clinical data with detailed utilization data from the dental offices attended by the panel members over the past ten years. The data collection is about one-third complete. Once these data are collected they will be integrated with the clinical data. The full dataset will be used to analyze the relation between oral health measures, treatment need as derived from those measures and the utilization of specific dental services. Field work will be complete and analysis will begin in early 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00477-02 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Condition of the Dentition in U.S. Adults - 1981

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson	Chief	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1981, the United States Public Health Service funded a survey, A Study of Dental Outcomes Related to Prepayment. Data were collected on a national probability sample of the U.S. civilian population. Calibrated dental examiners assessed the condition of each of 28 tooth spaces in the permanent dentition in individuals receiving an oral examination utilizing standardized criteria. The percent of all teeth that were sound ranged from 17% in the oldest age category to 67% in the youngest. Molars were least likely to be sound, and most likely to be satisfactorily filled, to need fillings, and to be missing than premolars or the anteriors. The percent of teeth needing a filling varied from three percent to seven percent. The percent of missing teeth increased with age from four percent in the youngest age group to 65% in persons 65 and older. The percent of sound teeth did not vary substantially with education; however, the percent of teeth needing fillings and the percent of missing teeth were negatively correlated with education. Biological factors are critical in the initiating stage of caries while sociodemographic factors may be more important in the treatment that carious teeth receive or that they do not receive. The intent of this project is to prepare a paper for publication. Analysis is complete and a report is being prepared for publication.

PHS 6040 (Rev. 1/84) GPO 814-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00494-01 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Longitudinal Analysis of Tooth Loss and Other Tooth Conditions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson	Chief	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS (if any): University of Colorado

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Longitudinal data from a panel of male veterans who have been examined every 3 years since 1968 and data from an experimental study of health insurance are being used to analyze the progression of tooth loss and the conditional probability of change in tooth conditions over time. Both data designate each tooth as missing or present. If present, a tooth is designated as sound, carious, or filled. If missing, the space is designated as replaced or not replaced. If replaced, the type of prosthetic replacement is recorded. These data will be analyzed to develop conditional probabilities of a tooth being lost over time. These probabilities will be conditioned on the status of the tooth at the previous examination and other putatively important explanatory variables. The panel of male veterans will allow several longitudinal examinations to be analyzed. The experimental data contains a wide array of potentially important explanatory variables which will allow the impact of economic and social variables on the probabilities to be assessed. Analyses are nearing completion. A report of the findings will be published.

PHS 6040 (Rev. 1/84)

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00495-01 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Relation Between Dental Conditions and Utilization of Dental Services

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH: Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.3	0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is now documented that dental caries in persons aged 5-17 years old are declining. These reductions have occurred for a sufficient number of years so several age cohorts of children who experienced reductions in caries are now adults. Some evidence suggests that young adults maintain these caries reductions. Less evidence is available regarding whether or not these caries reductions are maintained throughout life. Reductions in tooth loss also occurred in U.S. adults between 1974 and 1985. This continues a trend which was apparent in the 1960s. Reductions in the percentage of the population experiencing total tooth loss (edentulism) is especially pronounced.

This study is analyzing the impact of these changes in the extent of dental caries on the utilization of dental services. Longitudinal data from the Rand Corporation Health Insurance Experiment will be used to estimate the probability of subjects enrolled in the experiment attending a dental office over different time periods. For those subjects who attended a dental office, a demand function will be estimated to predict the amount of dental expenditures over these same time periods. The prevalence and extent of dental and periodontal conditions will be controlled. Analyses are nearing completion. Several reports will be published.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00496-01 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Determinants of Permanent Tooth Loss

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP, NIDR
Beazoglou, Tryfon	Assistant Professor	Univ. of Conn.
Crall, James	Assistant Professor	Univ. of Conn.

COOPERATING UNITS *(if any)*: University of Connecticut

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Notwithstanding significant reduction since the 1960s, tooth loss remains an important problem in the U.S. Its uneven distribution among adults makes this problem more severe for some individuals than the mean values of tooth loss indicate. While the role of disease in tooth loss in important, focusing on which disease causes the most tooth loss may obscure the complexity of the issue and underemphasize the influence of other factors. The overall objective of this Project is to measure tooth loss and its determinants in the natural settings of operating private dental offices and larger public clinics. The initial phase of the project will be a pilot study which will field test a protocol in a selected number of practices in Connecticut. The information from the pilot study will be used to refine and finalize the data collection procedures and the conceptual model of tooth loss for the full-scale study. Once the final protocol is set, the data to be collected will be used to estimate a more complete model of tooth loss than has been available to date and assess the relative significance of factors contributing to the loss of permanent teeth in the U.S. More specifically, the investigation will provide information regarding the relative influence of disease/clinical conditions, economic variables, and patient/provider characteristics and attitudes on decisions to extract permanent teeth.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00497-01 ASB

PERIOD COVERED: October 1, 1989 to May 31, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Forecasting Dental Health and Utilization Using A Microsimulation Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson	Chief	EODPP, NIDR
Caldwell, Steven	Professor	Cornell Univ.

COOPERATING UNITS (if any): Cornell University, Department of Sociology

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.25	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Branch is developing a computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S. These forecasts will be developed in considerable sociodemographic detail. A feasibility study of alternative modeling methods indicated that development of a model is both feasible and desirable.

Cornell University was awarded the contract to develop the model on May 15, 1990. Dr. Caldwell, a microsimulation specialist, will be principal investigator. Microsimulation will be the approach used. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental services use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. It will take approximately two years to develop an operational model. Policy experiments with the full model will be done both for past times and also for future times. As a framework for synthesizing research findings, the NIDR micro model would provide a vehicle for carrying out experiments in which the last dental research could be applied consistently and systematically to key dental policy issues.

PHS 6040 (Rev. 1/84) GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE000515-01 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Studies of Periodontal Health in Americans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP, NIDR
Miller-Chisholm, Ann	Epidemiologist	EODPP, NIDR
Oliver, Richard C.	Professor	Univ. of Minn.
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*: University of Minnesota, School of Dentistry

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.2	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The periodontal health of employed U.S. adults is being studied in collaboration with Dr. Richard C. Oliver of the University of Minnesota. The collaboration has resulted in several publications and will result in several more. The overall description of periodontal conditions is complete and published. The association between sociodemographic variables and periodontal status is complete and submitted for publication. A study developing a model to predict groups at high risk to have periodontal destruction is also complete. Additional studies will soon be started.

Additional periodontal studies were conducted in collaboration with other NIDR staff. A study of the prevalence of juvenile periodontitis U.S. children aged 14 to 17 years, using data from the 1986 NIDR Survey of Children is nearly complete. A study of variations in the periodontal health of older Americans among different sociodemographic groups is nearing completion. The study is using data from the 1985 National Survey of Oral Health in U.S. Employed Adults and Seniors conducted by the NIDR. Associations between periodontal health status and selected sociodemographic characteristics among U.S. seniors are being analyzed. Periodontal conditions included in the study are gingival bleeding, gingival recession, loss of attachment and pocket depth. Sociodemographic characteristics are age, gender, race, years of education, household income, dental insurance coverage, and the interval since the person's last visit to a dentist.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00516-01 ASB

PERIOD COVERED: November 1, 1989 to March 31, 1991

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Costs of Water Fluoridation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP, NIDR
Ringelberg, Mel	D.D.S.	HRS State Health Office

COOPERATING UNITS *(if any)*: Florida Department of Health and Rehabilitation

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Staff has collaborated with Dr. Mel Ringelberg, a dentist with the State of Florida, on the cost of water fluoridation among 44 Florida communities. Analyses are complete. Drs. Brown and Ringleberg will co-author two papers reporting the results of the analyses. One paper will report the costs of water fluoridation among the Florida communities. It has been submitted for publication. The second paper will report the results of the estimation of a cost function which will allow the prediction of costs based on population served by the water system and other explanatory factors. This is the first cost function that has been estimated for the costs of water fluoridation.

PHS 6040 (Rev. 1/84)

GPO 814-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00517-01 ASB

PERIOD COVERED: October 1, 1989 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: A Study of Alveolar Bone Loss and Aging Among Healthy U.S. Males

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP, NIDR
Chauncey, Howard H.	Associate Chief of Staff for R & D	VA
Garcia, Raul A.	Consultant Research Dentist	VA

COOPERATING UNITS *(if any)*: Veterans Administration, Boston, Massachusetts

LAB/BRANCH: Analytical Studies Branch

SECTION:

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project is a cross-sectional and longitudinal analysis of alveolar bone loss (ABL) in aging men. Study subjects will be 700 adult male participants in the VA Dental Longitudinal Study (VADLS). Bone loss will be determined using existing radiographs, including (1) intraoral periapical, (2) cephalometric, and (3) hand-wrist radiographs. The VADLS has the most extensive longitudinal radiographic data base available with which to examine the factors associated with progressive alveolar bone loss. The existence of sequential radiographs permits a longitudinal analysis of the actual ABL experienced over a twenty year period at three year intervals. The study will be able to determine actual rates of ABL per site and per subject, while also controlling for any pre-existing ABL found at the baseline examination. Extensive dental and general health information is available for the subjects so relations between ABL and systemic conditions can be observed. Unique opportunities exist to examine the relationship of ABL to changes in bone at non-periodontal sites in the same subjects and the contribution of extra-oral factors to ABL. Relationships of ABL to age-related changes in bone at non-periodontal skeletal sites can be investigated, allowing study of the contribution of systemic changes in bone density to the intraoral changes in alveolar bone loss. The project will yield much improved estimates of the rate of ABL conditional on putatively important explanatory variables.

DISEASE PREVENTION AND HEALTH PROMOTION BRANCH

The Disease Prevention and Health Promotion Branch (DPHPB) consists of two sections. Dr. Driscoll serves as Chief, Disease Prevention Section and as Acting Branch Chief. Staff of the Disease Prevention Section include Dr. Selwitz and Mr. Small. Ms. Furnia serves as Branch secretary. In addition, Ms. Nowjack-Raymer of the Soft Tissue, Craniofacial Defects, and Pain Section, Epidemiology Branch, continues to participate in three Disease Prevention Section projects in which she was involved while a member of the section. Dr. Gift is Section Chief, Health Promotion Section. Staff include Ms. Horowitz, Ms. Larach, and Mr. Zindel. The Branch also includes an assignee from the Centers for Disease Control, Dr. Corbin.

Staff of the Disease Prevention Section (1) plan and direct clinical trials, field studies and demonstrations of the effectiveness, feasibility and cost-effectiveness of accepted or new agents and procedures for the prevention of oral diseases through direct operation or contract procurement, (2) provide consultation, technical assistance, scientific issue resolution and other assistance to communities, organizations, institutions and individuals with regard to the efficacy, implementation and continuation of various oral disease prevention procedures and programs, and (3) participate in collaborative research efforts with other Institute staff members.

Staff of the Health Promotion Section (1) promote the use of established effective preventive and therapeutic procedures by both the public and health professionals, (2) promote the adoption of behaviors that are conducive to the improvement of oral health, (3) conduct research to (a) identify and evaluate new materials and strategies for promoting oral health and (b) evaluate and improve existing materials and strategies, and (4) participate in collaborative research and promotional efforts with other Institute staff members.

DISEASE PREVENTION SECTION (DPS)

Ongoing Clinical Research Activities

Although significant progress has been achieved in improving the oral health status of the U.S. population, many individuals, both young and old, still remain susceptible to dental caries. NIDR staff and others have studied various procedures or agents potentially useful for preventing or controlling tooth decay in children and in adults. One area of need is the identification of relatively low-cost methods of reducing both primary and secondary caries in at-risk individuals.

One such study, scheduled for funding in FY 1990, involves a competitive procurement designed to investigate the disease-preventive effects of topically-applied fluoride on root and coronal caries in adults. Few studies have been undertaken to assess the caries-preventive potential of self-applied fluoride preparations in an adult population. Participants will be selected with care based upon risk-assessment characteristics thought to be important in identifying an appropriate study population. Projected attrition levels of participants also will be considered in the selection of the study population. Subjects will be assigned to one of three groups, whose participants either will (1) rinse daily with a fluoride mouthrinse, (2) brush their teeth daily with a fluoride-containing gel or (3) serve as a control by using a suitable placebo rinse or brush-on gel. In addition, all participants will be instructed to follow their usual oral hygiene regimen. Participants' caries experience (DMFS) and level of gingival recession will be assessed at baseline and thereafter periodically for four years using standardized criteria.

In recent years there have been growing concerns that the ingestion of fluoride in both fluoridated and non-fluoridated areas may have increased significantly, and that this change has caused a concomitant increase in the prevalence of dental fluorosis. Factors contributing to a possible increase in fluoride ingestion include: (1) the processing of foods and beverages in fluoridated communities, resulting in products which are distributed to non-fluoridated areas (2) unsupervised use of fluoride dentifrices by young children, and (3) inappropriate prescription of dietary fluoride supplements. In response to these concerns, the NIDR carried out surveys in Illinois in 1980 and 1985 and in Iowa in 1982 to assess the prevalence of dental fluorosis and dental caries among children exposed to different concentrations of fluoride in their drinking water. Both Illinois surveys were carried out in the same communities, which had naturally occurring fluorides in their water supplies at concentrations of approximately one, two, three and four times that recommended as optimal for those areas. In contrast, the Iowa communities had negligible concentrations of fluoride in their drinking water. The collective findings of these surveys indicated that increases in dental fluorosis had occurred in both the optimal and above-optimal areas. Indications of similar changes in the fluoride-deficient Iowa communities, however, could not be detected. With respect to dental caries, DMFS scores in the optimal fluoride area averaged 38.1 percent lower than in the negligible fluoride area, and, in the higher-than-optimal areas, even greater caries protection was evident.

The need to continue efforts of monitoring possible changes in the prevalence of dental fluorosis must be afforded high priority. Additional research also is needed to further elucidate and define current interrelationships between dental caries, dental fluorosis and various concentrations of fluoride in drinking water. The continued availability of the same communities in Illinois and the identification of two communities in Nebraska with negligible concentrations of water-borne fluoride have afforded DPS staff an excellent opportunity to address these research needs. Examinations were completed this past April on approximately 300 schoolchildren ages seven through 16 in the two Nebraska communities. Each child was examined for dental caries using the DMFS index and for dental fluorosis using both Dean's Index and the Tooth Surface Index of Fluorosis. The data collected are being prepared for

statistical analysis. Examinations, using the same indices, are scheduled for this coming October in Illinois.

Section staff continue to be involved in research aimed at bringing the benefits of fluoride to residents of communities that have chosen either not to implement water fluoridation or have found fluoridation to be technically impossible or unfeasible. One such effort involves an eight-year clinical trial initiated in 1981 to compare the relative caries-preventive benefits of weekly fluoride mouthrinsing, daily fluoride tablet administration, and both procedures combined. Approximately 1,600 children in kindergarten and first grade residing in Springfield, Ohio were assigned randomly to one of three groups that 1) rinsed once a week in school with a 0.2 percent NaF solution, 2) chewed, rinsed, and then swallowed daily in school a 2.2 mg NaF tablet, or 3) carried out both procedures in combination. Interim two-year findings showed that the combined procedure produced a 33 percent lower increment in dmfs (primary teeth), and five-year findings showed a statistically significant 31 percent lower increment in DMFS (permanent teeth) compared with the fluoride mouthrinse procedure alone, which may itself have had an effect in lowering dental caries. The tablet procedure alone also showed a lower caries score than the mouthrinse procedure, but the difference was not statistically significant. Interim results, therefore, indicated that the combined fluoride treatment does provide additional benefit beyond that which may have been derived from fluoride mouthrinsing alone. Results from final treatment examinations conducted in May, 1989, recently have become available and final preparation of a manuscript will begin shortly.

It has been known that fluorides are most effective in inhibiting decay in smooth surfaces of teeth, whereas sealants have proven effective in preventing pit and fissure decay. Another study, initiated in 1983 in Nelson County, Virginia, provided the opportunity to investigate directly the combined use of certain fluoride procedures in conjunction with dental sealant therapy. The fluoride procedures, which had been ongoing for 11 years, consisted of weekly rinsing in school with a 0.2 percent NaF solution, daily ingestion in school of a 1 mg fluoride chewable tablet and home use of a fluoride dentifrice. A final evaluation of these procedures had shown a reduction in caries prevalence of 65 percent compared with baseline findings.

For the sealant procedure, newly erupted permanent teeth of children of selected ages received sealant therapy each year for a period of four years. Analyses of final results obtained in the fall of 1987, along with interim data, are almost complete. In addition to caries data, information has been obtained regarding sealant retention status, number of teeth treated, time required to carry out the procedure, salary of dental health professionals, and cost of materials. For example, during the last three years of the study, regarding 679 children for whom chair time at the unit was logged, on average 15.4 minutes were required to seal 11 tooth sites.

The reduction of gingivitis through the use of effective mechanical oral hygiene measures remains the best approach to the promotion of periodontal health to date. A number of studies have indicated, however, that it is difficult to motivate children to maintain good oral hygiene over long periods of time when an emphasis is placed on removing dental plaque.

Additional research has suggested that focusing on the elimination of gingival bleeding may produce better results than plaque control as a motivational tool for long term maintenance of optimal oral hygiene. To test this hypothesis, a two-year clinical trial was initiated in 1987 in York County, Virginia, in two randomly assigned groups of ninth and tenth grade school children. Children in one group received instruction for the self-assessment of gingival bleeding, whereas those in another group received the traditional instructions in plaque control. Children in both groups were supplied with soft toothbrushes, wooden toothsticks for interdental cleaning, and detailed instructions in their proper use. An oral prophylaxis and additional instruction were provided to participants at the end of the first year. A baseline and four follow-up examinations were conducted. Data analysis will indicate whether differences in periodontal health exist between the groups. If the expected benefit in favor of the bleeding approach can be verified and sustained, the results should be helpful in the development of more effective health education programs. Results are being analyzed, and a draft initial report is underway.

Fluoridation Consultation and Technical Assistance Activities

The section continued activities in support of public health agencies in states and cities involved in the implementation, extension, or promotion of community water fluoridation. During FY 1990, these activities have included: (1) provision of technical information for legislative and regulatory proceedings, (2) liaison with other health and technical entities (U.S. and foreign) for the development, synthesis, and exchange of needed information, (3) maintaining working contacts with professional groups in public health and in related fields, and (4) reviewing and preparing summary records of the deliberations and findings of judicial, regulatory, and legislative bodies concerning fluoridation. A staff member has continued the ongoing task of reviewing, consolidating, and retiring files on fluoride and health research and public issues, and arranging for the transfer to NLM of materials that can be considered historical. Again in 1990-91, a staff member will serve on the ADA's National Advisory Committee on Fluoridation.

HEALTH PROMOTION SECTION (HPS)

Ongoing Health Promotion Activities

Staff have been active in monitoring the field phase of the dental and orofacial pain components of the household interview of the 1989 National Health Interview Survey (NHIS) as well as that of the International Collaborative Study, II. The data from the NHIS are being edited, and analyses and publication plans are being made. Data should be ready for analyses by late summer/early fall, 1990. Staff are co-authoring the Series Report and arrangements have been made to collaborate with outside scientists on several analyses. Also, staff have been active in planning the 1990 and 1991 NHIS which are being developed to monitor the 1990 and Year 2000 National Health Objectives. The pretest for the 1991 NHIS was monitored by Section staff in June, 1990.

Staff have continued to be active in developing a research and action program for improving the oral health of adults and older Americans. A recent review of the status of the Research and Action Program was based on individual interviews with extramural, epidemiology, and intramural professional staff as well as a critique of coded projects. Based on this review, a new summary of the initiative, ongoing activities, and planned research directions has been prepared. A series of new actions are under way based on directives from the Institute Director. Efforts of particular interest to the Epidemiology Program are the recording of trauma, investigation of American Indian high risk adults, and improved understanding of the institutionalized populations. Section staff also are working with other staff in the Institute to facilitate planned projects related to osteoporosis, periodontal diseases, and oral physiology in Black populations, among others. One of these efforts involves the development of a collaborative activity with the Indian Health Service. An initial project will be an investigation of alternative treatment protocols for periodontal diseases with type II diabetic American Indians.

As part of the commitment to the National Institute on Aging in support of the IOM's "Health Promotion and the Second Fifty," the final chapter on oral dysfunctions has been completed for review by the committee and peers. The full publication will be presented in the summer, 1990.

The Section has increased its focus on research and health promotion activities among minorities. Staff revised the brochure on baby-bottle tooth decay which has been approved for publication in both English and Spanish. A contract is being awarded during the summer, 1990, to ROW Sciences, Inc. to develop a statement on epidemiological research needs within Black populations and to develop a detailed research protocol. Working groups of experts will be convened during the latter part of 1990 to develop these documents. Also, staff have been meeting with the National Dental Association to increase its involvement in the identification of oral health issues. Also, Section staff have been following up on other activities related to minority oral health by consulting with the Office of Minority Health, potential grantees, and other interested researchers. For example, discussions were held with investigators at the University of Michigan about their plans for a workshop on Black/minority oral health.

As a follow-up to a study initiated to inventory all existing national oral health and dental databases and to assess their analytical potential, several papers were developed including: application of secondary data analyses to policy and research, synthetic cohort approaches for the forecasting of tooth loss, description of behavioral and biological factors important to tooth loss, and lifestyle and socioeconomic factors related to oral health status and routine services. The full inventory has been made available to interested scientists and a summary of the inventory has been accepted for publication in the *Journal of Public Health Dentistry*. Analytical models and secondary data analyses have been accomplished as a result of these projects.

The Health Promotion Section continues to be active in analyses of existing data, working closely with the Analytical Studies Branch. Also, Section staff are collaborating with this Branch in developing additional investigations with the Boston VA.

Science transfer activity is ongoing. For example, staff provided consultation and assistance to health and educational agencies in the U.S. and abroad concerning oral health education and promotion and prevention of oral diseases. In collaboration with the Department of Veterans Affairs, Centers for Disease Control, Food and Drug Administration and the American Dental Association, staff developed and distributed an education package, "Infection Control in the Dental Environment." The acceptance of this package has been exceptional.

A staff member was instrumental in publishing, through the American Association of Public Health Dentistry's Oral Health Committee, recommendations for teaching how to prescribe dietary fluoride supplements. Staff, in collaboration with OPEC staff, developed clearance materials for an education leaflet on dental sealants. The leaflet will be prepared in English and Spanish.

Staff continues to supervise the distribution of the program's educational exhibits, films, and printed materials. The educational materials are used in school and other community-based activities by state and local health and education departments as well as in private practices. The distribution of the free-loan exhibits has been moved from Prospect Associates to the Section. The updated film, *Prescription for Periodontal Health*, has been placed in circulation.

Other DPHPB Activities

Staff have been active in working with other Institutes, associations, and agencies, providing health promotion and research expertise. A staff member continued to serve on, and was reappointed to, the ADA's National Advisory Committee on Fluoridation; another staff person has been appointed to the ADA's Caries Prevention Guide Ad Hoc Committee. Staff serve on several committees of the FDI: Oral Health Promotion Working Group, Marketing Working Group, Scientific Program Committee, and the Periodontal Health Services Working Group. Staff helped organize a symposium on health promotion for the 1990 FDI meeting. Several staff have consulted with universities and health departments regarding education, promotion and disease preventive regimens. Serving as chairperson of AAPHD's 1990 Annual Session, a staff member has organized several symposia related to health promotion and primary prevention. Staff have served as reviewers for manuscripts submitted for publication to numerous journals including the Journal of Dental Research, Social Science and Medicine, Health Education Quarterly, Journal of Public Health Dentistry, Public Health Reports, Community Dentistry and Oral Epidemiology and Advances in Dental Research. Staff also have participated in contract and grant reviews within the NIH community, and contract and grant reviews for others outside NIH, including OTA, ODPHP, and the American Fund for Dental Health.

Staff also are active in the broader NIH community. One staff member is NIDR's representative to the NIH Women's Health Issues Committee, the BID Prevention Coordinator's Committee, an NIH committee to plan the Christopher Columbus Quincentennial Conference on Aging, and is President of R&W. Other staff members serve as NIDR's representative on such bodies as the CCATT. Branch staff have been working with the National Cancer Institute on several initiatives. For example, staff participated in a working group at the National Cancer Institute to revise *How to Help Your Patients Stop Smoking—A National Cancer Institute Manual for Physicians* to make it suitable for the dental professional.

A staff member has been serving on a Subcommittee of the PHS Committee to Coordinate Environmental Health and Related Programs (CCEHRP). The Subcommittee is collecting, evaluating, and summarizing available information on the health benefits of fluoride use as part of the CCEHRP's broad examination of information concerning health risks and health benefits. The CCEHRP report, expected this Fall, will incorporate the Committee's documented findings, further discussion of the recent report by the National Toxicology Program on their bioassay of the carcinogenicity of sodium fluoride, and updated analyses by the NCI of epidemiological data on osteosarcoma in humans.

Staff work has continued on the nearly completed National Oral Health Objectives for the Year 2000. The American Association of Public Health Dentistry has published the oral health document which was prepared by the working group in Spring, 1989. This represents a more comprehensive approach to oral health than will the edited chapter on oral health which will be in the final Year 2000 Objectives. Since the last report, the objectives for the Year 2000, and supporting documentation, have been reviewed and revised by staff at the Office of the Assistant Secretary, the co-leads, and working group members. One staff person has served on a committee which planned a national conference in Washington to announce the release of the finished objectives in September 1990. Concurrently, staff also have been participating in the development of an improved edition of Community Model Standards for Preventive Services that will provide direction to communities in implementing programs to achieve better community health.

The OASH-level PHS Oral Health Coordinating Committee that has been established to serve as an administrative umbrella organization for a PHS effort toward advancing a national adult oral health promotion initiative had its first meeting on May 25, 1990. Also, an RFP has been issued to develop a mechanism to encourage national coordination of oral health efforts through a task force mechanism.

A staff member serves as co-director of the Future of Dental Public Health Project being pursued by the AAPHD and the Dental Health Section of the APHA. Recommendations will be developed covering several major areas of present concern. The availability of a review draft is anticipated early in the Fall.

During December 1989 and January 1990, several DPHPB staff served on an ad hoc NIDR Working Committee on Fluoridation and Fluorides to discuss issues and develop questions

and answers pertaining to fluoridation and fluorides for Congressional hearings, and to develop priorities for fluoride research. A comprehensive report depicting the Committee's findings and recommendations was submitted to the Director, NIDR.

Publications

Corbin SB. National oral health objectives for the year 2000. Journal of Public Health Dentistry. Special Issue, Vol. 50, No. 2, 1990.

Corbin SB. What should dental public health be and how to get to the should? Journal of Public Health Dentistry. Special Issue, Vol. 50, No. 2, 1990.

Gift HC, Gerbert B, Kress GC, Reisine ST. Social economic and professional dimensions of the oral health care delivery system. Annals of Behavioral Medicine, Fall 1990.

Mecklenburg RE, Christian AG, Gerbert B, Gift HC, et al. How to help your patients stop using tobacco. USDHHS, NIH-NCI, 1990.

Driscoll WS, Nowjack-Raymer R, Heifetz SB, Li SH, Selwitz RH. Evaluation of the comparative effectiveness of fluoride mouthrinsing, fluoride tables, and both procedures in combination: Interim findings after five years. Journal of Public Health Dentistry, Vol. 50, No. 1, 1990.

Presentations

Corbin SB. John W. Knutson distinguished service award presentation. APHA Annual Meeting, Chicago, Illinois, October 23, 1989.

Corbin SB. Contemporary ethical issues in dentistry: AAPHD Annual Meeting. Honolulu, Hawaii, November 1, 1989.

Corbin SB. AIDS and dentistry: an interpretive portrait of a profession's response to a crisis. AAPHD Annual Meeting. Honolulu, Hawaii, November 2, 1989.

Corbin SB. Scientific update on fluoride and the public health: That was then; this is now. Special Symposium sponsored by IADR/AADR/ADA/AADS. Cincinnati, Ohio, February 7, 1990.

Corbin SB. The future of dental public health: Scope of dental public health. National Oral Health Conference. San Diego, California, April 2, 1990.

Gift HC. Keynote Address: Integrating oral health services into maternal, child and family care programs. HRSA and New York City Health Department Conference, January 1990.

Gift HC. Research and action program through interagency efforts. AADR VA Researchers Group, March 1990.

Horowitz AM. Primary prevention of oral diseases in maternal and child health. University of Maryland, College Park, Maryland, March 28 and April 6, 1990.

Horowitz AM. The role of health promotion in preventive dentistry. AADS, Cincinnati, Ohio, March 5, 1990.

Horowitz AM. Issues in oral health promotion research and practice. Scholars in dentistry lecture, University of Washington, Seattle, Washington, April 2, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00070-18 DPHP

PERIOD COVERED: October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Combined self-applied fluorides and sealants for caries prevention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR
Selwitz, Robert	Disease Prevention Research Specialist	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Disease Prevention and Health Promotion Branch

SECTION: Disease Prevention Section

INSTITUTE AND LOCATION: NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.64	.58	.06

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A self-administered dental health program was initiated in Nelson County, Va., a fluoride-deficient community in October 1972. Children in the County's schools, under teacher supervision, chewed and ingested daily a 1 mg F tablet and rinsed weekly with a 0.2% NaF solution. A fluoride dentifrice was provided for ad libitum use at home. Baseline DMFS examinations were made of 2,138 children in the County's elementary, junior high, and senior high schools. Follow-up DMFS examinations were conducted at two-to-three year intervals. Final examinations were conducted in 1983 when the full effectiveness of the fluoride program could be assessed.

In the fall of 1983, a sealant program was added to the ongoing fluoride program. Children who were 6, 7, 12, and 13 were eligible to have pit-and-fissure sealants applied. An initial screening to identify those tooth surfaces to be sealed was made in December 1983. Caries data (DMFS) from the September 1983 dental examination served as a baseline for those children who participated in the sealant phase of the study. In succeeding years, new groups of 6 and 12 year olds were enrolled. Treatments continued for four years. Interim dental examinations took place at the start of the third year of the study (September 1985) and final examinations were made in September 1987. Data analysis is nearly complete and preparation of final reports has been initiated.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00310-10 DPHP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR
Selwitz, Robert	Disease Prevention Research Specialist	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.99	.75	.24

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In September 1981, a study designed to compare a combined regimen of weekly fluoride rinsing and daily fluoride tablets with each procedure used alone was begun in Springfield, Ohio, a fluoride-deficient community. The approximately 1700 children attending 20 public and non-public elementary schools were randomly assigned to one of three treatment groups. The children in Group I dissolved and ingested daily a 1 mg F tablet; the children in Group III rinsed weekly with a 0.2% NaF solution; and Group II carried out both procedures. The assigned treatments were self-administered under the supervision of teachers who received in-service training.

Before the procedures were started, baseline examinations were conducted. First and second follow-up dental examinations were conducted in October 1983 and November 1986 respectively. Final treatment examinations were conducted in May 1989. Data from this exam are being analyzed and reports prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00439-04 DPHP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

An evaluation of different approaches to prevent gingivitis in teenage children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.52	.40	.12

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A study to evaluate two approaches to the prevention of gingivitis in teenage children was begun in York County, Virginia in April 1987. Baseline examinations for periodontal health, DMFS and gingival recession were conducted on 500 ninth and tenth graders. Questionnaires regarding oral hygiene methods and professional care practices were completed. Following the examinations, subjects were randomly assigned by grade to either a positive control or test group.

The control group received a manual for the self-assessment and control of plaque and the test group received a manual for the self-assessment and control of gingival bleeding. Small group and individual sessions for instruction in the self-assessment procedures were held to ensure that all procedures were understood.

Interim examinations to assess periodontal health were conducted in October, 1987 and in April and October, 1988. In May, 1988 each participant was given the opportunity to have an oral prophylaxis and all participants received individual instruction to reinforce the appropriate group methods. Assessments for DMFS and gingival recession were included along with the periodontal assessments on the final examination, conducted in April, 1989. Following data analysis, final reports will be prepared.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00444-04 DPHP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Oral Health Attitudes and Dentally Related Behaviors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen	Sociologist	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	ASB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Health Promotion Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.10	.10	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
- ☐ (b) Human tissues
- ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study uses existing survey and interview data to describe and analyze the associations among attitudes, behaviors and oral health. The data analyses are used in the oral health promotion plan, papers and publications in preparation and will assist in understanding and improving the oral health of individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00453-04 DPHP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Analysis of Existing Oral Health Data on National Surveys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen	Sociologist	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	ASB, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Health Promotion Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.11	.11	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The potential value of existing data sources for further analyses has been investigated utilizing a purchase order mechanism. National dental survey and oral clinical exams conducted over the past 30 years were identified and contacts wre made with sponsors and principal investigators to determine the status of data files. Reviews of publications and other mechanisms wre used to determine the appropriateness of additional work, with emphasis on possible trend analysis and interpretation for public policy. Professional services contracts have been issued to several scientists to develop analytic frameworks or collaborate in data analyses and publications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00463-03 DPHP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inventory of Existing Oral Health Education and Promotion Activities for Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen Sociologist EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Health Promotion Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.05	.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The potential value of existing oral health education and promotion activities for adults is being investigated utilizing a purchase order mechanism. Telephone calls to approximately 500 selected national and state organizations using a standardized interview format has provided a description of oral health education and promotion activities being performed. Copies of available oral health education and promotion materials have been provided and evaluations to determine more extensive use of materials and programs are being performed. A summary of the available programs and educational materials has been prepared as well as a summary of the evaluation.

PHS 6040 (Rev. 1/84) GPO 914-915

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00487-02 DPHP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Health Attitudes and Dentally Related Behaviors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen	Sociologist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Health Promotion Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Institute of Medicine (IOM) has undertaken a project on Health Promotion and Disability Prevention for the Second Fifty. One of the ten components is oral problems for this age group. NIDR has contributed funds to support the committee activities and provided staff to do data analyses and prepare the issue paper for the committee. The review paper has been prepared and is being considered by the IOM committee.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00523-01 DPHP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Prevalence of Dental Caries and Dental Fluorosis in Relation to Water Fluoride

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Selwitz, Robert H.	Disease Prevention Research Specialist	EODPP, NIDR
Kingman, Albert	Chief Statistician	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Disease Prevention Section

INSTITUTE AND LOCATION
NIDR,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.40	.5	.9

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [X] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is a follow-up of previous studies conducted by the NIDR in Illinois in 1980 and 1985 and in Iowa in 1982 to assess the prevalence of dental fluorosis and dental caries among schoolchildren exposed to different concentrations of fluoride in their drinking water. Both Illinois surveys were carried out in the same communities, which had naturally occurring fluorides in their water supplies at concentrations of approximately one, two, three and four times that recommended as optimal for those areas. In contrast, the Iowa communities had negligible concentrations of fluoride in their drinking water.

The need to monitor for possible changes in the prevalence of dental fluorosis continues to receive high priority. Additional research also is needed to further elucidate and define current interrelationships between dental caries, dental fluorosis, and various concentrations of fluoride in drinking water. The continued availability of the same communities in Illinois and the identification of two communities in Nebraska with negligible concentrations of water-borne fluoride have afforded an excellent opportunity to address these research needs. Examinations were completed this past April on approximately 300 schoolchildren ages seven through 16 in the two Nebraska communities. Each child was examined for dental caries using the DMFS index and for dental fluorosis using both Dean's Index and the Tooth Surface Index of Fluorosis. The data collected are being prepared for statistical analysis. Examinations, using the same indices, are scheduled for this coming October in Illinois.

GPO 914-916

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