National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1983

U.S. DEPARTMENT OF HEALTH
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Public Health Service
National Institutes of Health
National Institute of Dental Research

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National Institute of Dental Research
National Institutes of Health
Bethesda, Maryland 20205

Contents

Office of the Director

OFFICE OF THE DIRECTOR

National Institute of Dental Research

October 1, 1982 - September 30, 1983

In January 1983, Dr. Harald Löe, former dean of the University of Connecticut School of Dental Medicine, was sworn in as the fifth Director of the National Institute of Dental Research (NIDR). In this capacity, he is responsible for overall determination of Institute policy and research objectives, as well as administrative management.

In assuming the Directorship, Dr. Löe defined his goals for the Institute as preserving the strength of the NIDR intramural basic and clinical research program; expanding NIDR's partnership with universities through support of university-based and investigator-initiated grant applications; developing a support mechanism for the training of clinical dental scientists; and developing an effective system for science and technology transfer.

The Director has played an active role in working toward the completion of the Institute's Long-Range Research Plan: FY 1985-89, which delineates specific research goals and strategies of the NIDR. Dr. Löe chaired a staff meeting to review an initial draft of the Plan, and also met with the Long-Range Plan Coordinating Committee, the National Advisory Dental Research Council, and representatives from biomedical research, education, and practitioner communities to review the draft and to seek comments.

The Director presented the NIDR budget request before the House and Senate during the Congressional hearings in April and responded to questions about future directions of NIDR research.

In keeping with the FY 1983 budget restraints and the intent to implement the goals set forth in the Long-Range Research Plan, the Director initiated a plan for realigning NIDR resources with program objectives. This is a dual effort to both reduce overhead and target staff and funds in areas of high priority. The plan is being prepared for NIH approval.

The Director also brought together a special panel of consultants to evaluate the Dental Research Institutes and Centers (DRICs). The panel advised the Director about options for future action on the DRICs given budgetary restrictions faced by those centers as well as the research needs defined in the Long-Range Research Plan.

Within the Office of the Director, Dr. Löe established the position of Special Assistant for Research Manpower and Training to undertake a review of NIDR manpower and training activities in relation to the future need for clinical dental researchers. A special in-house advisory committee was appointed, a panel of outside consultants was established, and representatives of the NIH and the National Academy of Sciences were convened to aid in this effort.

Participation in the general governance of NIH as part of the Public Health Service and the Department of Health and Human Services is also required of the NIDR Director. Dr. Löe attended weekly and special meetings of the Bureau-Institute-Division Directors and meetings of the NIH Director's Office of the Director staff. In addition, he met with the Assistant Secretary for Health to discuss the role of the NIDR with respect to HHS prevention goals and progress.

The Director initiated weekly NIDR Executive Staff meetings. He has participated in EEO activities, encouraging the full utilization of minority and women staff, both within the Institute and as consultants to the NIDR. The Director initiated an expanded NIDR Awards Ceremony which honors those employees who have distinguished themselves during the past year and have contributed to the success of meeting NIDR's goals. The forum provides an opportunity for the Director to deliver a "state-of-the-Institute" analysis to all staff. Dr. Löe also instituted an Annual NIDR lecture and developed procedures for the selection of lecturers. The purpose of this lecture is to recognize contributions in basic and clinical research and to honor distinguished scientists who have made important contributions in areas that directly relate to the research interests of the NIDR.

Maintaining awareness of dental research activities in other countries and promoting an exchange of scientific information with the leaders of international dental centers is another important role of the NIDR Director. Such communication is of great importance to the conduct of research at the NIDR and can provide the basis for the establishment of international cooperative studies that may be of significant benefit to NIDR's research mission.

Nationally, Dr. Löe worked to enhance NIDR's relationship with the dental constituency through such professional organizations as the American Dental Association, the American Association for Dental Research, and the American Academy of Periodontology, and also encouraged staff to actively promote dental research at specialty organization and association levels.

In keeping with his information exhange policy, the Director addressed professional organizations and universities, both internationally and in the U.S., about the status of dental research, the mission of tne NIDR, and his specialty area of periodontal disease prevention and therapy. Presentations included:

The Danish Dental Association - Copenhagen, Denmark - "The Impact of Research on Dental Education"
International Conference on Oral Biology - Sydney, Australia - Chaired the final plenary session and presented a "Summary of the Conference"
International Association for Dental Research - Sydney, Australia - "The Scientific Basis for Periodontal Disease Prevention"
University of Michigan School of Dentistry - Ann Arbor, Michigan - Commencement Address, "The Impact of Research on the Future of Dentistry"
First Annual Meeting of the Dental Services Research Scholars Program - Chapel Hill, North Carolina - "The Mission of the NIDR and its Relation to Health Services Research in Dentistry"
Symposium: Clinical Geriatric Dentistry - Biomedical and Psychosocial Aspects - Chicago, Illinois - "Fourscore and More Years of Oral Health"
Lister Hill Lecture, Honors Convocation, University of Alabama School of Dentistry - Birmingham, Alabama - "Scientific Impacts on the Furture of Dental Practice"
Second Gordon Conference on Periodontal Diseases - Plymouth, New Hampshire - "Natural History of Periodontal Diseases: Disease Activity as a Basis for Understanding the Natural History of Periodontal Disease"
Cariology 1983 - Zurich, Switzerland - moderator, "Prevention and Therapy - Where From Here?"

The Director was also a participant at the annual meeting of the American Academy of Periodontology in Atlanta, Georgia.

Two universities conferred honorary degrees upon the NIDR Director during this fiscal year — a doctor of science degree was awarded by the Georgetown University School of Dentistry and a doctor honoris causa was presented by the University of Lund in Malmo, Sweden. At the latter ceremony, Dr. Löe addressed faculty and students on "The longevity of the human dentition." The Director also received the Daniel F. Lynch Award presented by the Dental Society of Greater Waterbury, Connecticut, and the International Lecturer of the Year Award, presented by the Academy of International Dental Studies.

Dr. Löe continues to serve as an editor of the *Journal of Periodontal Research*, a publication he founded, and also reviews state-of-the-science papers prepared for the NIDR Long-Range Research Plan and all manuscripts submitted by NIDR investigators for publication. He has been appointed a Clinical Professor in Periodontics at the Georgetown University School of Dentistry.

OFFICE OF THE SPECIAL ASSISTANT TO THE DIRECTOR

Activities related to planning, evaluation, international health, social and behavioral sciences consultation, prevention, and legislation are handled by the Office of the Special Assistant to the Director, as well as coordination of special assignments for the Institute Director and a variety of other services and consultation. During the past fiscal year the Office continued to employ the full-time services of a science writer who has been directly involved in the preparation, writing and design of the *NIDR Long-Range Research Plan:* FY 1984-89. Dr. David Suomi, Craniofacial Anomalies Branch, also joined this Office for ten weeks during the months of November and December to assist with the Plan and preparation of related meetings.

Planning

Activities related to the completion of the *NIDR Long-Range Research Plan:* FY 1984-1989 included coordination of meetings, preparation of design and layout, writing and editing of narrative material, collection and selection of photographs, and development of illustrations and charts. Three major meetings were organized including one for the NIDR staff to review the first draft (December 13-14, 1982), another for the Long-Range Plan Coordinating Committee to review a revised draft (January 17-18, 1983), and the last (June 10, 1983) with the National Dental Research Advisory Council together with the Long-Range Plan Coordinating Committee, Chairmen of the NIDR Advisory Committees, and representatives from the dental research, education and practitioner communities. The comments received from these meetings have been incorporated into the Plan. In addition to the above activities, presentations were made by staff at the NIDR Advisory Committee meetings regarding an update of the Plan.

The coordination of materials in preparation for the NIDR portion of the annual NIH research plan, FY 1985, was completed. An initial planning review session was held with the Director, NIH, on January 21, 1983 to prepare for the Appropriation Hearings and a draft research plan was submitted in May 1983. In addition, staff assistance was provided to the Budget Office in preparing the narrative required to accompany the various budget submissions. Staff also participated in the monthly NIH Planning and Evaluation Officers meetings.

Evaluation

A bibliometric analysis study of publications resulting from the NIDR Dental Research Institutes and Centers (DRICs) as compared to those from investigator-initiated research grants was initiated. Eight hypotheses will be tested to determine the attainment of selected DRIC goals and objectives. Together with the NCP Coordinator for Health Education and Promotion Activities, an RFP was developed and proposals solicited for the evaluation of oral health education and promotion activities of the National Caries Program directed towards educators of dental hygiene students. This study will entail a survey of educators directly involved in the teaching of caries-preventive procedures and techniques in all 201 U.S. dental hygiene programs to determine their need for improved education and promotion materials.

The FY 1984 Evaluation Plan was developed, reviewed in May 1983, and submitted in June, 1983. Two new proposed projects include an Evaluation of the NIDR Data Information Vocabularies and an Assessment of the Effectiveness of NIDR Training Programs in Preparing Dental Clinical Investigators.

International

Coordination of international activities included furthering NIDR participation in international projects and activities and reporting on such activities. The NIDR was represented at periodic meetings of the BID International Representatives coordinated by the Fogarty International Center. Briefing materials were prepared for foreign visitors who require meetings with the Director, NIDR.

Regular liaison is provided to the World Health Organization, as the NIDR is a WHO Collaborating Center for Oral Health. Staff support is given for the WHO/USPHS International Collaborative Study of Dental Manpower Systems in Relation to Oral Health Status and a staff meeting was organized at the NIH (April 20-23, 1983) to obtain reactions to the final report. Comments were solicited by the WHO on a project related to traditional dentistry in India, the WHO International Manpower Deployment Project, Human Performance Institute activities, and the WHO Collaborative Centers programs located in Thailand and in Nigeria.

Staff served as panelists on review of contract proposals for the Evaluation of the Senior International Fellowship Program, Evaluation of the US/USSR Scientific Exchange Program, and as a review of one round of fellowship applications for the Fogarty International Center. Additionally, staff contributed to the preparation of briefing materials for the U.S.-Nigeria Task Force Meeting, February 1983, and the World Health Assembly meetings, May 1983.

Consultation was provided to the International Dental Federation in the development of scientific programs for the Congresses to be held in Tokyo and Helsinki. Staff served as chairperson for the International Relations Committee of the International Association for Dental Research and as consultant to the American Dental Association's Council on International Relations.

Social and Behavioral Science

Regular consultation was provided to the Pain and Behavioral Studies Program in the formulation of plans for the Program's activities. Staff supported the co-editing tasks involved in the forthcoming publication Social Sciences and Dentistry: Volume II. Staff also collaborated with the National Caries Program on the development of social epidemiological questions for the forthcoming survey of adult oral health needs. Assistance with planning for data needs and project strategy was given on the NIDR Clinical Investigator Project. Liaison was provided to the NIH Health and Behavior Committee. In addition, editorial review was solicited by, and provided to, the following journals: Social Sciences and Medicine, Journal of the American Dental Association, and the Journal of Dental Education. Consultation was given to the Robert Wood Johnson Foundation on needs for dental research and to the University of Connecticut School of Dental Medicine on a project related to work loss and dental disease.

Staff coordinated the monthly meetings for Behavioral Scientists in Dental Research of the D.C.-Baltimore metropolitan area, represented the organization at meetings of their Executive Committee as Councilor, and represented them at the General Assembly of the Federation Dentaire Internationale.

Prevention

Staff coordinated prevention activities for the Institute. Activities included participation in monthly meetings of the NIH Prevention Coordinators; preparation of materials requested for the monthly DHHS Disease Prevention Calendar, health promotion activities, health risk assessment activities, disease prevention-related research and activities, long-term health care research, and research related to the Department's Objectives for the Nation; and participation in a working group to refine the definition of disease prevention-related research.

This year, NIDR staff met with the Assistant Secretary for Health to discuss PHS progress made toward the Fluoridation and Dental Health objective. The Centers for Disease Control was the lead agency, and other participants included the Health Resources Services Administration and the Chief Dental Officer, PHS. This meeting resulted in revision of several specific objectives for 1990. In addition, the Evaluation Officer served as a member of the Prevention Committee, American Public Health Association, and assisted the President of the American Association of Public Health Dentists in planning activities related to the prevention of periodontal diseases.

Legislation

Monitoring of legislative activities of interest to the Institute and coordination of Institute responses were provided. Mrs. Rose McLaughlin received training in Legi-Slate and now serves as the Institute's representative.

Special Assistant to the Director

Editorial and writing assistance were provided for the NIDR Director's presentations about dental health services research, geriatric dentistry, foods and nutrition, as well as commencement addresses, reports to Congress, reports to the National Advisory Council, briefing materials for the NIH Director, and the Assistant Secretary for Health and the Secretary, DHHS, and selected internal and external correspondence. Action item minutes of weekly NIDR executive staff meetings were also written and distributed. In addition, the Special Assistant served on the Research Panel for the American Dental Association Committee on the Future of Dentistry. She coordinated the reactions of the NIDR staff and consultants on the preliminary reports of that Committee.

Other Activities

The Special Assistant provided advice and consultation to faculty of the Kennedy School of Government and the School of Public Health, Harvard University, on proposed projects on health science policy research. She also served as a member of the Health Science Policy Working Group of the Harvard Division of Health Policy Research and Education. Additional activities included serving as a panelist in reviewing candidates for Grants Associate Program and Health Scientist Administrator for NIH Office of Personnel Management; member electorate of Nominating Committee-Dental Section for the American Association for the Advancement of Science; and reviewer of the Department of Community Dentistry, University of Washington School of Dentistry.

The Evaluation Officer also served as a reviewer of the Department of Community Dentistry at the University of Washington; coordinated the NIDR response and dental questions proposed for the FY 1984 Health Interview Survey Supplement on Health Promotion for the National Center for Health Statistics, served as reviewer for the Abstracts 1983 meeting of the American Public Health Association's Dental Section; served as liaison to the Assistant Secretary for Health's Task Force on Women's Health; served on the Ad Hoc Strategic Planning Committee for the Chief Dental Officer, PHS; and served as immediate Past-President, Chair-Nominating Committee and Chair-Research Award Committee for Senior Women Dental Students of the American Association of Women Dentists.

Publications

Cohen, L.K. "Behavioral and Social Research Support from the National Institute of Dental Research," *J. of Dental Education*, Feb. 1983, Vol. 47, No. 2.

Cohen, L.K. "Theory and Practice of International Comparative Study in Collaboration with Japan" in *Dental Health Care in Japan*, edited by Masao Onisi and Motoi Morimoto. Tokyo, Japan: Ishyaku Publishers (Dental Outlook, Special Issue), 1983.

Cohen, Lois K. Book review of Social Science Research and Decision-Making by Carol H. Weiss with Michael J. Bucuvalas, NY: Columbia Univ. Press, 1980, XIV, 332 pp, *Health Services Research* Vol. 18(1), Spring 1983, p. 100.

Presentations

Cohen, L.K. "Socio-dental Sciences and the WHO International Collaborative Study of Dental Manpower Systems in Relation to Oral Health Status." School of

Dentistry, University of Groningen, The Netherlands, Oct. 5, 1982.

Cohen, L.K. "A History of the Socio-dental Sciences." "The WHO International Collaborative Study of Dental Manpower Systems in Relation to Oral Health Status." WTA Foundation on Post-Graduate Education of the Netherlands Dental Society, Amsterdam, The Netherlands, Oct. 6, 1982.

Cohen, L.K. "Dental Health Needs and Dental Delivery Systems Around the World." School of Dentistry, University of Detroit, Oct. 22, 1982.

Cohen, L.K. "Behavioral and Social Science Research for Oral Health." International Conference on Dental Hygiene Research, University of Manitoba, Winnipeg, Canada, Oct. 1, 1982.

Cohen, L.K. "Sharing Challenges for Global Oral Health." Commencement Address, School of Dental Medicine, Harvard University, Boston, MA, June 8, 1983.

Cohen, L.K. "Market and Community Responses to Changing Demands from the Workplace." Continuing Education Course on Dentistry and the Workplace, University of Melbourne, Australia, July 29, 1983.

Cohen, L.K. "Health Policy Research" Lunch and Learn, International Association for Dental Research, Sydney, Australia, August 2, 1983.

Cohen, L.K. "International Dental Care Delivery Systems," Seminar for Clinical Investigations Branch, Intramural Program, NIDR, September 1, 1983.

Kleinman, D. V. "The NIDR Long-Range Research Plan - Research Objectives Related to the Dental Clinician." NIDR Dental Clinic Staff Seminar, February 17, 1983

Kleinman, D. V. "Research Advances in Preventive Dentistry." Georgetown University School of Dentistry, April 7, 1983.

Kleinman, D. V. "Mercury Hazards in Dentistry: A Review of Recent Research Findings." NIH Prevention Coordinators Meeting, April 12, 1983.

SPECIAL ASSISTANT FOR MANPOWER AND TRAINING

In April 1983, Dr. Preston A. Littleton, Jr., was appointed Special Assistant for Manpower and Training. This position was established in the Office of the Director to provide leadership to the review of NIDR manpower and training activities in relation to future needs for dental research personnel, and to keep the Director apprised of developments affecting dental research manpower.

Dr. Littleton is responsible for planning and initiating a study to examine the problems and issues associated with the preparation of competent dental clinical researchers. This one year study will include the documentation and evaluation of past NIDR training activities, an analysis of the changes and trends in dental education, and an assessment of the current supply and future demand for dental clinical researchers. Recommendations will be developed on how to increase the effectiveness of the current NIDR training support programs and/or the specification of new mechanisms designed to better meet future manpower needs. The findings of this study about dental clinical researchers will complement the *NIDR Long-Range Research Plan.*

Although the Director, NIH, has given high priority to increasing the overall number of clinicians pursuing research careers, it appears that the impediments confronting researchers in dental medicine may be more severe than for researchers in medicine. This is thought to be partially related to the changes that are occurring in the structure and finance of dental and advanced dental education and the characteristics of the currently authorized National Research Service Act training programs.

A detailed protocol for the study of dental clinical researchers has been developed and application has been made to support some aspects of the study using funds set-aside for NIH evaluation. An internal NIDR advisory committee has been appointed and a panel of expert consultants has been identified to assist in the conduct of the study. The first meeting of the ad hoc consultant panel was held in July. Following discussions with the National Academy of Sciences, general agreement has been reached concerning their collaboration on some aspects of the study. Liaison has been established with the major dental associations and the input of the concerned groups and organizations will be sought throughout the study. Formal presentations and discussions have already been scheduled for upcoming meetings of the American Association of Dental Research and the American Association of Dental Schools.

OFFICE OF SCIENTIFIC AND HEALTH REPORTS

The Office of Scientific and Health Reports is the focal point of the National Institute of Dental Research for disseminating information about the Institute's research

programs and activities. Dr. Kenneth C. Lynn, Acting Scientific and Health Reports Officer, continues to provide leadership to the OSHR in conducting a comprehensive information program aimed at increasing awareness of current research highlights, special areas of emphasis in oral and dental health, and health prevention. The OSHR reaches audiences that include the scientific community, dental and health professionals, dental students, the media, health educators, Congress, and the general public.

During FY 1983, the Office underwent a change in personnel when Hilah B. Thomas, a medical science writer in the OSHR, retired from the Federal Government, ending a career of nearly 23 years of service. She had been with the OSHR since 1966. Mrs. Thomas was replaced by Pat Sheridan, a science writer formerly with the NIADDK.

Media

A major portion of OSHR's work involves arranging extensive coverage of NIDR activities by media representatives from television, radio, newspapers, and magazines. This past year, the OSHR responded to over 165 telephone calls from different media contacts requesting information about oral and dental diseases and NIDR research advances.

Several events in particular generated widespread publicity during FY 1983. The NIDR opened the first multidisciplinary pain clinic in the United States devoted exclusively to research. After the OSHR issued a press release about the clinic, numerous inquiries were received about the activities of the clinic and the types of patients being sought by NIH scientists for their studies of acute and chronic pain. Channel 5 Metromedia News aired a segment about the clinic that included an interview with Dr. Mitchell Max, clinical coordinator of the pain clinic. Articles about this new NIH facility also appeared in the Washington Post and Public Health Reports. Dr. Ronald Dubner, chief of the Neurobiology and Anesthesiology Branch, was interviewed for an article that was published in the Journal of the American Dental Association, entitled "Research Holds Promise for New Techniques in Pain Prevention," and for an article that appeared in the newsletter of the American Dental Association about the pain clinic.

Publicity about a new dry mouth consultation service offered by Dr. Bruce Baum, NIDR clinical director, and Dr. Philip Fox, an oral surgeon and staff scientist in the clinical investigations section, also created considerable media interest. The OSHR published stories about dry mouth and related oral conditions in the NIH Record and News and Features from NIH which resulted in numerous calls from newspapers and magazines, including the Journal of the American Medical Association, and an article in the Washington Post.

As work on the intraoral fluoride-release device progresses, the OSHR has been active in arranging media coverage. Drs. Dale Mirth and Michael Cole of the National Caries Program appeared on ABC News and Metro Media News of New York to discuss the device, its present stage of development, and future application. The Office also provided the Encyclopedia Britannica with text and a photo of the fluoride-release device for use in the 1984 Yearbook of Science and the Future.

Other topics featured on television during FY 1983 included periodontal disease and the research conducted by former NIDR investigator Dr. Paul Keyes (Walter Cronkite and Health Beat, N.Y.); sealants and juvenile periodontitis (Cable TV Health Network); bonding (Channel 2, Baltimore); and three segments on general dental subjects (Good Morning America, N.Y.). Dr. Kenneth Brown (Laboratory of Developmental Biology and Anomalies) and his work with mice and predisposition to malformation was filmed by Channel 7, Washington, D.C. Many telephone calls and letters of inquiry continued to be generated by the August 1982 CBS-TV Universe program on periodontal disease.

During February, National Children's Dental Health Month, the OSHR helped to draw attention to the lifetime benefits of proper care for children's teeth by publishing articles related to this subject. The Office prepared three Search for Health columns on nursing bottle mouth syndrome, snack facts, and malocclusion. Search for Health columns are sent to 300 editors across the country for use in local newspapers. The NIH Record also included an item about Childen's Dental Health Month.

The NIDR was featured as the cover story in the March issue of the *Journal of the American Dental Association*. The story, entitled "NIDR 1983: New Directions for Improving the Nation's Dental Health," highlighted research conducted by the Intramural Research Program, the Extramural Programs, and the National Caries Program, and provided information about dental grants and contracts and dental research data. The OSHR was instrumental in arranging for color photographs to be taken of NIDR research, scheduling interviews with scientists, providing background material and text, and reviewing and editing the final write-up.

The role of NIDR in maintaining an effective relationship with print and broadcast media was the subject of a talk presented by Sue Burroughs, OSHR public affairs specialist, at a seminar sponsored by the

American Association for Dental Research during their March meeting in Cincinatti, Ohio. The purpose of the seminar, entitled "Media Relations: Building a Positive Image for Dental Research," was to establish a network of specialists who can present accurate state-of-the art comments on research-related issues.

Throughout the year, the OSHR assisted free-lance writers and staff of national magazines in preparing articles pertaining to NIDR research. The OSHR furnished background material and photographs for articles and revised copy for such periodicals as *Glamour, People, Better Homes and Gardens, Newsweek, Vogue, Consumer Reports, Reader's Digest, Science 83, Working Woman,* and *American Health.*

Items of interest about current NIDR intramural and extramural research are regularly submitted to the American Dental Association. Subjects that would attract the attention of the general medical profession are submitted to the *Journal of the American Medical Association (JAMA)* for use in their "From the NIH" column. This year, OSHR staff prepared two articles — "A Hormone-like Epidermal Product Stimulates Immune Reactions" and "Sensitivity to Pain Greater in a Clinical than a Laboratory Setting" — for use in *JAMA.*

Staff also prepared 10 press summaries of papers being presented at the March meeting of the American Association for Dental Research, and eight press summaries of papers presented at the August meeting of the International Association for Dental Research.

Text was composed for four radio spot announcements — Save Your Baby's Teeth, Oral Cancer, Mouth Ulcers, and Fluoridation — to be distributed by the NIH Audiovisual Branch.

In addition, the Office regularly submits numerous articles on NIDR activities and research accomplishments to the NIH Record, News and Features fron NIH, and Search for Health columns.

Publications

To meet the increasing demand for information about NIDR research and health prevention measures, the OSHR issued two new fact sheets and an administrative publication during FY 1983 and reprinted three other publications.

The NIDR Fact Sheet has been useful for visitors because it provides an overview of the Institute's three component programs. The fact sheet titled *Xerostomia* has been sent in response to requests for information about dry mouth, and has also been distributed to patients at the NIDR Dental Clinic and the dry mouth consultation service.

The administrative publication concerned the Intramural Research Program and included a detailed description of the eight laboratories and branches plus training and employment opportunities. This leaflet serves as a companion to the newly revised and reprinted (March 1983) Grant and Contract Research Programs of the NIDR.

Numerous requests for the popular publication *Snack Facts* required reprinting of this leaflet. *Snack Facts* is designed for children and encourages them to enjoy snacks that will not promote tooth decay. *Seal Out Dental Decay* was also reprinted. In order to continue to provide health education information to the general public, the OSHR requested permission to reprint *RX for Sound Teeth* and *Periodontal (Gum) Disease.* Permission was denied by the DHHS Deputy Assistant Secretary for Public Affairs. The OSHR plans to appeal this decision, based on the fact that to date, 1,100,000 copies (87,000 copies distributed this fiscal year alone) have already been distributed and the public demand for these leaflets has accelerated.

A full-color brochure describing the NIDR, the scope of dental science, and progress being made by the Institute in the fight against oral and dental diseases is presently being prepared and will be published in FY 1984.

The OSHR continued to assist the National Caries Program and the Extramural Programs with clearing, editing, designing, printing, advertising, and distributing several publications about their program activities. *Fluoride to Protect the Teeth of Adults,* a new leaflet, was cleared and printed, as well as two new posters about the use of fluorides for adults and two new sealant posters. *Dental Treatment Needs of U.S. School Children 1979-80,* the second in a series of five publications dealing with dental caries in U.S. school children, was also published this fiscal year.

During FY 1983, announcements about Institute publications appeared in the Consumer Information Center Directory and other periodicals, numerous magazines, and in newsletters of state dental societies. This publicity generated requests for 766,280 of these publications from dental health professionals, members of Congress, State health departments, dental schools, nursing schools, hospitals, state, county, and community health agencies, coordinators of health fairs, and the general public. Most of these publications were mailed by the OSHR through a contract mailing service. The Consumer Information Center in Pueblo, Colorado, distributed 25,200 copies of RX for Sound Teeth.

OSHR staff also provides editorial and printing assistance to the Office of the Director, the Intramural Research Program, and the National Caries Program in preparing their Annual Reports. Assistance with the editing, artwork, and printing of other miscellaneous publications, such as the NIDR Awards Booklet and invitations and programs for the NIDR Director's swearing-in ceremony was also given.

The OSHR provided clearance for 215 manuscripts and 125 abstracts written by Institute scientists and administrators during FY 1983. The OSHR also continued to obtain departmental clearance and provided editorial services as required for all other Institute publications.

NIDR Research News, a newsletter prepared by OSHR staff, was issued three times this past year and contained 24 science articles, as well as additional items about the availability of Institute publications and appointments and awards of special note. *NIDR Research News* is sent to a mailing list of 1,570 science writers and editors of state and county dental journals who use these items in informing their readers about NIDR research and program activities. Dentists, members of dental societies, universities, members of the National Advisory Dental Research Council, and the NIDR Programs Advisory Committee also receive this publication.

NIDR Abstracts, which contained 173 summaries of scientific papers published by NIDR investigators, was issued three times during FY 1983. Six hundred and four copies of this publication, reporting research findings to the scientific community, are sent to libraries of dental schools and universities, members of the Institute's council and advisory committees, U.S. and foreign researchers, and the dental section of the Pan American Health Organization.

Editorial, Public Inquiries, and Other Activities

OSHR staff was actively involved in preparations for the FY 1983 Congressional Appropriations Hearings. The Office assisted with compilation of a briefing book, composition of the Director's general and opening statements, clarification and editing of transcripts from both House and Senate hearings, and preparation of the Committee reports for both houses of the Congress.

Staff also prepared several speeches for the Director about dental health services research, geriatric dentistry and research, and nutrition research and the NIDR Long-Range Research Plan, as well as an article on NIDR's role in international dental research that was published in the International Health News. Minutes of the NIDR Advisory Council meetings were also edited.

During the past year, the Office contributed to several NIH and DHHS reports, including the NIADDK special reports to Congress on arthritis, cystic fibrosis, diabetes, and digestive diseases; and the NICHD inventory of research related to maternal and child health.

On request from the OMB, the OSHR submitted plans for keeping publication costs at a consistent level for FY 1983 and FY 1984. Staff also prepared budget reports, audiovisual reports, the Annual Report, and weekly reports of significant Institute activities for inclusion in reports to the DHHS Secretary.

Annual revision of portions of NIH publications describing NIDR programs was continued. This resulted in the updating of the *NIH Almanac*, the *NIH Publications List, NIH Extramural Programs, Scientific Directory and Annual Bibliography*, the Fogarty Center's *Annual Report of International Activities for FY 1982*, the NIH *Information Index*, and *Medical Sciences Report*.

Staff also updated and edited portions of the DHHS/ Department of Agriculture publication on nutrition, and edited the PHS Report article on the NIDR pain clinic.

As sponsor of one of the NIH Lectures this past year, NIDR was in charge of handling publicity for the event. OSHR staff arranged for appropriate coverage in the NIH Record, prepared opening remarks for the Director of Intramural Research, and was responsible for the artwork, printing, and distribution of tent cards, flyers, and posters announcing the lecture — "Information Processing in a Simple Sensory System: Bacterial Chemotaxis" — delivered by Dr. Daniel E. Koshland of the University of California.

Upon the arrival of the NIDR Director in January, staff assembled and updated information for the Director's briefing book and edited a speech given by the Director of NIH at the swearing-in ceremony.

The Office continued to be responsible for all NIDR exhibits and their scheduling, storage, maintenance, and repair. During FY 1983, these exhibits were shown at 17 meetings, including health educator, public educator, and dental professional meetings. The OSHR also participated in an exhibit and distributed publications at the Interagency Meeting on Health Promotion through the Schools, sponsored by the DHHS Department of Education.

Tours of the NIDR laboratories were arranged for 124 American and foreign dental students, visiting researchers, and dental practitioners. Arrangements were also made for NIDR staff to speak to the visitors about specific research activities of the Institute.

OSHR staff responded to 1,485 telephone calls and 6,796 written inquiries from professional associations, Federal, State, and county health agencies, journals, Congress, dentists, health professionals, and the general public. These requests were for information on all phases of dental research, including periodontal disease, caries, temporomandibular joint dysfuntion, the herpes simplex virus, mouth ulcers, dry mouth, and oral-facial malformations.

Staff also arranged for photographic services for special events such as the NIDR awards ceremony, and for the videotaping of several television programs that highlighted NIDR research advances.

To express their appreciation to NIDR's OSHR and other NIH information offices who provided materials for their Library of Health series, Time-Life Books, Inc., made a donation to the NIH Preschool fund this past year.

Fluoridation Specialist

The Fluoridation Specialist, Mr. John Small, continued activities in support of public health officials in states and cities defending community water fluoridation against legal actions initiated by persons or organizations opposed to fluoridation. This support has included developing communications among legal personnel involved in all recent cases, assisting in the preparation of affidavits and technical documentation, locating and briefing expert witnesses, transmitting current research findings and legal decisions to involved officials, acting as a liaison to provide information or expertise to other Federal agencies, and providing assistance to newspersons or journal writers publishing information on the proceedings and outcomes of cases.

There were six significant court actions during FY 1983:

A local court in Alton, Illinois, decided a case concerning fluoridation in December 1982, finding the State mandatory fluoridation law unconstitutional. The State has appealed that ruling to the Illinois Supreme Court. The State's attorney is preparing for a hearing before the Supreme Court in early 1984. Fluoridation in Alton has continued pending a final decision.

In Clinton, Indiana, in December 1982, a local court found in favor of the City's action to fluoridate, and fluoridation of the water supply began on January 4, 1983.

In Charleston, S. C., in November 1983, a local court upheld the City's decision to fluoridate, and fluoridation began on December 6. The plaintiffs have appealed this decision; a ruling is pending.

In December 1982, the U. S. Supreme Court upheld decisions of Ohio courts requiring that the cities of Cuyahoga Falls and Akron comply with the State law requiring fluoridation of water supplies.

In Houston, Texas, opponents of fluoridation have appealed the local court's decision made in February 1982 upholding the City's power to fluoridate the public water supply. Fluoridation continues in Houston while the appeal is pending.

In June 1983, the Court of Sessions, Scotland's highest court, issued an opinion that ruled against the initiation of fluoridation in Glasgow for technical legal reasons concerning the existing water supply laws. In the same opinion, the Court fully upheld the safety and the effectiveness of fluoridation, and its suitability as a public health measure. All of the plaintiff's claims regarding alleged adverse effects (cancer, birth defects, etc.) were specifically rejected. Because of the great interest in this case, and its obvious applicability to similar court cases and appeal actions, detailed summary information on this case was sent to all of the "fluoridation" mailing key addressees. In addition, 20 copies of the complete 390-page decision were sent to health officials and attorneys in localities having active cases or appeals, or the expectation of legal action.

All activities in connection with legal actions or community action concerning fluoridation are carried out in full cooperation with the dental public health staff of CDC.

During FY 1983, the specialist has been a member of a PHS committee on the legal defense of fluoridation that is preparing a draft plan of action to be reviewed and acted on by the Surgeon General and representatives of other involved organizations.

From April through June of 1983, Mr. Small served on an ad hoc committee appointed by the Director of the Clinical Center responsible for reviewing the recent medical literature and reports on ongoing research relevant to the adverse health effects of excessive fluoride intake. The committee reported their findings to the Surgeon General.

Mr. Small served on the planning committee for, and participated in, a conference on "Fluoridation: Litigation and Changing Public Policy", held at the University of Michigan on August 9-10, 1983. The conference was held to educate and assist state health and legal personnel in resource development and development of tactics for countering legal challenges to public health programs.

In May 1983, Mr. Small participated in a national conference in Boston, Massachusetts, sponsored by the CDC Dental Disease Prevention Activity, for a review and discussion of the status, progress, planning, and funding of state and Federal fluoridation activities.

As requested, the specialist provided consultation, contacts, and documentation to several state health departments in support of their actions concerning antifluoridation publicity, legal actions against fluoridation, new statewide promotional activities, and promotion of fluoridation in specific large communities.

The specialist provided background information, answers to specific questions, and document sets to writers and editors at several publishing houses and news services, including CBS-TV News, NBC-TV News, the New York Times, Schnell Publications (Chemical Business), The Rodale Press, the American Dental Association, and many state dental and medical journals.

Three mailings of new documents of special interest to dental public health workers and advocates were sent to all persons on the "fluoridation" mailing key (about 120 addressees in 13 countries). CDC distributes these and other documents to U.S. addresses. Our combined mailings provide information to nearly 300 persons.

In October 1982, Mr. Small presented a paper on the world status of community water fluoridation at the Vienna, Austria, meeting of the Federation Dentaire Internationale.

In August 1983, Mr. Small was appointed to a one-year term as a member of the National Fluoridation Advisory Committee. The Committee is sponsored by the ADA Council on Dental Health and Health Planning.

In September 1983, Mr. Small participated as an invited subject specialist in a meeting of the AADR Science Information Committee that was held at Fairleigh-Dickinson University to plan responses to attacks on fluoridation and on use of fluorides in school health programs.

THE FINANCIAL MANAGEMENT OFFICE

The Financial Management Office (FMO) is the financial component of the Office of the Director, NIDR, and serves as principal advisor to the Director on all financial matters relating to the Institute's programs and activities. The FMO provides managerial support during the appropriations process and tracks obligations throughout the year in order to provide the financial expertise and data that are required to complete the NIDR's program plans for the fiscal year.

During FY 1983, the FMO continued to manage the fiscal year budget for the NIDR. The FMO maintained payroll records, generated monthly personnel status and program expenditure reports, tracked the funding of grants, requisitions, and purchase orders, and worked with Institute administrative staff to ensure that budgeted amounts were not exceeded, and that reprogramming actions were initiated in areas where additional funds were required. The FMO apportioned funds by quarter to fund planned activities and, at the end of the fiscal year, reconciled all accounts and balanced the books.

The FMO monitored the Institute's trans-NIH activities including research studies in the areas of diabetes, arthritis, nutrition, disease prevention, and acquired immune deficiency syndrome (AIDS). The FMO prepared special reports and forecasts, and responded to requests for program and financial data from Congress, the Office of Management and Budget, and other Federal and non-Federal agencies.

While the FMO was managing the 1983 budget, it was also forecasting future requirements for enactment of the 1984 appropriation. This effort included formulating zero-based budgets for prospective fiscal years; preparing budgetary estimates by mechanism and program area; reflecting changes from prior years; and accompanying the Director to Congressional hearings and summarizing programmatic decisions in financial terms. The budget resulting from this process served as the operating plan for the current fiscal year.

In addition to financial duties, in FY 1983 FMO staff enhanced office operations with extracurricular activities including course work and committee work on office automation and automated reporting methods. In order to meet increasing demands for dissemination of financial data, FMO staff cooperated with the NIDR Word Processing Committee to develop new methods of using word processors to aid in data storage and retrieval. One FMO staff member, on detail to the Office of Management and Budget in the Executive Office of the President, assisted in preparation of the President's 1984 Budget. In addition, the entire office staff assisted an NIH management intern and stride program trainee with budget assignments providing valuable training in the formation, execution, and analysis of the budget of the NIDR and the Federal Government.

For its efforts in FY 1983, the FMO received a group cash award for superior performance.

PERSONNEL AND MANAGEMENT ANALYSIS SECTION

The Personnel and Management Analysis Section (PMAS) is the focal point for both the personnel and management analysis functions of the Institute. Personnel management activities encompass staffing and placement (including merit promotion), classification and pay management, employee relations, and employee development and training. Management analysis activities include providing staff advisory service and assistance on organizational and procedural problems and serving as the central clearance and management point for Consultant Services, Conference Management, Contracting Out of Commercial/Industrial Type Product/Services, and Records Management.

During FY 1983, the first full cycle of the Employee Performance Management System (EPMS) was completed. This appraisal system applies to all Institute civil service employees who are not covered by the Merit Pay and SES/SSS systems. The cycle was in effect from October 1, 1981 to December 31, 1982, with final appraisals given in February, 1983. The appraisals are used for many personnel-related activities, such as career promotions, merit promotions, within grade increases and quality increases.

During the past year, the personnel staff, together with program managers, has commented on a number of proposed changes in personnel regulations. The most notable among these involved the Performance Based Incentive System. Several of these changes may be put into effect in late 1983, although they will be somewhat modified from what was originally proposed.

The Institute continued to have an active employee incentive awards program. Program chiefs, as well as Institute employees, were encouraged to submit names of nominees for awards so that supervisors could continue to recognize the excellent quality of their staffs. Again this year, NIDR SES/SSS level staff was recognized for their contributions through bonuses. These award activities culminated in another well-attended Annual Awards Ceremony.

Staffing activities received considerable attention during FY 1983. The NIDR continued its efforts through an intensive program to help employees from the DHHS and Public Health Service adversely affected by budget and personnel ceiling cuts. In addition, the loss of a senior intramural branch chief generated considerable staffing activity. This position is at the Senior Executive level and has involved a nationwide search. Such a search entails the establishment of the position and search plan (approved at the Department), a search committee, and communication with over 150 professional societies and dental schools.

The Staff continues to collaborate with the NIDR EEO Officer and the NIDR EEO Advisory Committee on matters of joint concern. They actively participate in Advisory Committee meetings to keep the EEO community informed about Institute personnel policies, procedures, and activities. The staff also works closely with the NIDR EEO Officer and with managers in assuring the feasibility and legality of personnel activities related to affirmative action and EEO concerns. Again this year, minority and women summer hiring goals were exceeded through the cooperative efforts of managers, EEO, and the PMAS.

One new NIDR Policy and Procedure was issued — "NIDR FEORP Implementation." "NIDR Leave Policy" is in preparation.

Several reports which require Institute-wide response were coordinated by PMAS. Both new and recurring requests were covered. These reports included the Information Technology Systems Budget (the ADP Plan), NIH Organization and Functions Manual, Organization and Functions Manual, Hardware Systems Narratives, Annual Survey of Records Holdings, Annual Report-Copying Equipment, Inventory of Word Processing Equipment, the Biennial Inventory of Controlled Substances, conference management, and consultant services estimates.

Other areas of management analysis activity included completion of the reorganization of several laboratories in the Intramural Research Program. Included were: the abolishment of the Laboratory of Biochemistry and the Laboratory of Biological Structure; the establishment of the Laboratory of Oral Biology and Physiology and the Mineralized Tissue Research Branch; and approval of a new Functional Statement for the Intramural Research Program as well as revision of Functional Statements for the Diagnostic Systems Branch and the Neurobiology and Anesthesiology Branch. The use of NIH computer facilities was increased to ease the workload in the PMAS and was accomplished primarily through the extended use of WYLBUR. The PMAS also served as the coordinator of the survey of needs and the selection, justification, and purchase of word processing equipment for the Office of the Director.

During FY 1983, PMAS staff continued to participate in trans-NIH activities. These included membership and active involvement in the DPM Committee on the Continuing Education of Personnel Management Specialists, the DPM Professional Personnel Program Series, the NIH Administrative Training Committee, and the NIH Office Technology Task Force.

DENTAL RESEARCH DATA OFFICE

The Dental Research Data Office (DRDO) provides scientific and technical information on current dental research through the collection, analysis, storage, and retrieval of subjective and statistical data. While the purpose of the office is primarily to serve NIDR staff, its services are available to outside requestors as well. Recognized as a unique source of information on dental research activities, the DRDO is listed in published directories of information centers.

Printed Reports

Six printed reports are published by this office: *National Institute of Dental Research Programs; Dental Research in the United States and Other Countries; National Institute of Dental Research Annual Report; Selected List of Technical Reports; Trainees and Fellows Supported by the NIDR; Graduate Training,* and the *Council Orientation Handbook.* The office also assisted in the publication of the *NIDR Long-Range Research Plan.*

Great advances have been made in utilizing the computerized type setting capabilities of the Government Printing Office. For several years, the DRDO has used this service to publish *Dental Research in the United States and Other Countries* and the *NIDR Annual Report Index*, using magnetic tape provided by the Smithsonian Science Information Exchange (SSIE) and the Division of Research Grants (DRG). Now, in addition, the office produces its own tapes from coded WYLBUR data sets. The FY 1982 NIDR Annual Report and the NIDR Long-Range Plan are being published through this method. The resulting reports are both more attractive and less expensive to produce.

Beginning with FY 1982, costs have also been lowered by changing the format of the Annual Report to one complete volume, reducing the total number of pages. A unified schedule of reporting for the whole Institute will enable the Annual Report to be a more timely and useful product.

A number of options have been explored for collecting data for *Dental Research in the United States and Other Countries.* Information on current non-NIDR research projects, formerly supplied by the Smithsonian Science Information Exchange, is now collected by the National Technical Information Service and made available through several vendors. The DRDO has affiliated itself with the Federal Library Committee (FEDLINK) of the Library of Congress. A Consolidated Interagency Agreement with FEDLINK enables the DRDO to use FEDLINK's contract with DIALOG Information Services, Inc. to obtain direct online access to the old SSIE data base. The Management Information Service of NIDR will compile and process the material.

Selected List of Technical Reports in Dentistry is prepared from a Selective Dissemination of Information (SDI) subscription with NTIS. Periodically, lists of these reports are distributed to NIDR staff and other interested persons. The titles of all reports received during one fiscal year are compiled in the yearly publication. This year's publication has been modified to include NTIS price codes and to employ a more attractive and space-saving multiple column format.

Ad Hoc Requests

In addition to these printed publications, the DRDO responds to *ad hoc* requests for information on Institute research activities. Percentage breakdowns of request sources are: NIDR staff, 40 percent; non-NIDR Government personnel, 48 percent; and non-Government requestors, 3 percent. Primarily, the type of material requested relates either to different research subjects, to NIDR funding history and practices, or to the impact of research activities on certain groups (minorities, handicapped, Pacific Island populations). These requests may be recurring or one-time. Examples of research subjects are arthritis, cancer, cystic fibrosis, diabetes, digestive diseases, drug development and testing, fluoride, herpes simplex, immunology, maternal and child health, nutrition, trauma and burns. While DRDO indexing and retrieval methods provide the base for preparing reports, responses also may involve collaborative efforts with other NIH or NIDR organizational units, such as the Management Information Section (NIDR), the Office of Scientific and Health Reports (NIDR), NIDR Program/Laboratory/Branch Chiefs, and the NIH Division of Research Grants.

Privacy Act and Freedom of Information Act

The number of Privacy Act (PA) requests received during FY 1983 increased slightly compared to FY 1982. Requests were for pre-Council grant summary statements and for Clinical Center patient records. All Privacy Act Systems of Records were reviewed this year, and a model Job Element was drafted for all NIDR employees with PA responsibilities who are under the Employee Performance Management System (EPMS). The three NIDR System's Managers in the EPMS now have a PA Job Element in their Performance Plans.

Freedom of Information Act (FOIA) requests increased approximately one-third over the amount received during the previous year. The majority of requests have

been for successful grant or contract applications. Two sizable, comprehensive requests have been received.

One of the largest FOIA requests received during the year could set a precedent at the NIH for the release of medical and dental records. The matter has been studied by Legal Counsels and Freedom of Information Officers at the NIH, PHS, and DHHS levels. Preliminary decisions to deny access to patient records will most likely be appealed and a ruling at a higher level required.

Freedom of Information Act billing practices have been changed this year to allow greater recovery of costs incurred in handling requests. In accordance with NIH policy, the DRDO is also requiring payment prior to sending the material. A few cases have been closed because of the requestor's failure to send advance payment.

Impact of Automation and Technology

Office automation and computer technology continue to have an impact on DRDO services and procedures. The office is using the DIALOG network to retrieve current research information for publication purposes. In addition, with the physical separation of the Dental Research Data Officer from documents in the Westwood Building, and in the absence of the Technical Information Assistant, it has been necessary and advantageous to have online availability of frequently used inhouse data sources, such as the "Keyword Directory," "Clinical Trials," "New Projects," and "Master.Detail" listings.

The use of National Library of Medicine Online Retrieval Services has expanded as more NIDR personnel become aware of the inhouse ability to access MEDLINE and other NLM databases. This office performs literature searches for NIDR staff and also advises staff members wishing to perform their own searches.

Finally, WYLBUR is used to prepare, format, and store reports; to transmit material between buildings; and to retrieve and modify data supplied by the Management Information Section. The computerized method of typesetting has led to our requiring each NIDR program area to submit its portion of the Annual Report on WYLBUR.

Collateral Staff Activities

The Dental Research Data Officer is a participant in two of the Institute's evaluation plans. He serves as the Project Monitor on a project to evaluate the NIDR Data Information Vocabularies and is on the Internal Advisory Group for Assessing the Effectiveness of NIDR Training Programs in Preparing Dental Clinical Investigators.

The Technical Information Assistant continues to serve on the NIDR Word Processing Committee, and this year was also a member of the NIH Safety and Health Fair Committee.

EQUAL EMPLOYMENT OPPORTUNITY PROGRAM

The NIDR EEO Program continued its work in the areas of support to minority schools, reports and analyses of the Institute's profile, the assignment of collateral EEO duties, the recognition of EEO accomplishments, and the presentation of monthly informational seminars. Added emphasis was placed on the Institute's Civil Rights Program through the preparation of a detailed study of support to Historically Black Institutions. The Institute also prepared its first multi-year Affirmative Action/Federal Equal Opportunity Recruitment Plan (AAP/ FEORP). The Institute increased its recruitment activity directed toward minorities, women, and the handicapped and began collecting data on the race and sex of applicants. EEO functions were continued during FY 1983 under the direction of Garland N. Martin, Jr., Acting EEO Officer.

Discrimnination Complaints

The Institute has had no informal or formal complaints of discrimination since FY 1980. The NIDR EEO Counselor, at the direction of the NIH Division of Equal Opportunity, provided counseling for other NIH Institutes. The EEO Officer, EEO Assistant, and Counselor provided assistance to employees whose concerns involved career counseling, job applications, leave, training, safety, and supervisor/employee relations.

Training

The EEO Officer produced a series of monthly informational seminars on subjects of special interest to women, minorities and the handicapped. An estimated 600 NIDR/NIH employees attended the nine programs which ran from September through May. The series will continue into FY 1984.

The Institute's Office Support Staff Training Activities Group developed a training session for employees as a part of the Secretaries' Week Program of the NIH. The session, "Short Cut to a Long Life," was presented by Louis Chacos of Montgomery College.

The EEO Officer presented a number of seminars outside the NIDR during FY 1983. "Career Pathways" and "Surviving the 80's" was presented to the FDA's Federal Woman's Program Advisory Committee and a workshop entitled "Finding the Opportunities in a RIF Environment," was presented at the Federally Employed Women's (FEW) annual regional training meeting. "The Role and Responsibility of an EEO Officer," was presented to the NIH Contract Compliance Committee.

The NIDR EEO Officer, EO Assistant, and Counselor received EEO training in FY 1983.

Federal Contract Compliance (EEO) training was provided to an NIDR Project officer. The NIDR EEO Officer/Contract Compliance Coordinator, Contract Officers, Contract Specialists and Project Officers have all completed contract compliance training.

The NIDR continues its support of the NIH Minority Research and training programs through the MBRS, MARC and the NIH Extramural Grants and Associate Programs.

Multi-year Affirmative Action/Federal Equal Opportunity Recruitment PLAN

The EEO Officer, in cooperation with NIDR management, developed a multi-year Affirmative Action Plan for use through 1985. A Federal Equal Opportunity Recruitment Program was also developed, and the combined AAP/FEORP plans were implemented in FY 1983. The plans were based on a work force profile, an underrepresentation analysis, and the identification of priority targets by the NIH and the NIDR.

The Institute established goals for each targeted occupational series and barriers to the elimination of underrepresentation were identified. Strategies were developed by the Institute for overcoming these barriers and an implementation process for FEORP was established. A personnel directive outlining these implementation procedures was prepared by the Institute's Personnel and Management Analysis Section and was distributed to NIDR managers and supervisors. As part of the FEORP plan, the Institute began collecting data on the race and sex of job applicants using OPM Form 1386.

Preparation of a similar AAP/FEORP plan specifically directed toward handicapped individuals and disabled veterans is underway.

Civil Rights

The EEO Officer serves as the Federal Contract Compliance Coordinator for the Institute. All contracting and project officers in the Institute have completed training in Contract Compliance and are presently administering the EEO Check List for non-construction contracts in accordance with Executive Order 11246.

The EEO Officer instituted an annual Civil Rights Report for the Director, NIDR. Other special reports prepared for the Director, NIDR, include a study of funding of Historically Black Institutions by the NIDR, as well as a study of the institutions' applications for NIDR funding. The EEO Officer, in cooperation with the Dental Research Data Officer, prepared a report on NIDR funding directed toward the problems of the handicapped.

The NIDR continues to participate in NIH-directed programs including the Extramural Associates Program, the NIH Consultant File on Committees/Advisory Groups, MBRS, MARC, NIH Visiting Professor Program, Small Disadvantaged Business Program, Small Grants Programs, and an NIDR-directed program to increase the participation of Historically Black Dental Schools. The latter program is new this year and is directed by the NIDR Planning Officer in cooperation with the EEO Officer.

Recruitment and Selection

All NIDR recruitment and selections involving NIDR FEORP-targeted series are carried out in accordance with the provisions the Institute's FEORP Implementation Plan. Eleven series in four occupational classes have been selected as targeted series for the elimination of underrepresentation of minorities and women at the NIDR.

The NIDR EEO Officer continued to extend the list of contacts at minority and women's schools through the addition of a number of hispanic and native Americans. The Institute sent over 500 packets of information to these contacts concerning the NIDR mission and the summer program of the NIDR. The computerization of the mailing list in FY 1982 aided in this mailing.

The EEO Officer, in cooperation with the Office of Scientific and Health Reports, conducted a number of tours of the Institute's research facilities for groups of minority and women college students.

Other Program Activities

The assignment of collateral duties in EEO to the NIDR staff includes the appointment of the EEO Counselor, the Delegate and Alternate to the NIH Women's Advisory Committee (WAC), the Delegate and Alternate to the Handicapped Employee Committee, and Representatives to the EEO Advisory Committee. The Counselor, Delegate and Alternate to the WAC, the Alternate to the Handicapped Committee and half of the members of the EEO Advisory Committee were appointed in FY 1983.

The NIDR Delegate to the WAC received the NIDR EEO Achievement Award. The Delegate to the NIH Handicapped Employee Committee received the DHHS Handicapped Employee of the Year Award and was nominated for the Outstanding Federal Employee of the Year Award. The EEO Officer received the EEO Advisory Council Award.

Publication of the NIDR EEO Report begun in FY 1983 was discontinued with the retirement of the EO Assistant in February 1983. Publication is expected to resume with the hiring of her replacement.

The Institute's EEO Officer, Scientific Director and a number of NIDR scientists participated in the MBRS Symposium held in Washington, D.C. The Institute sponsored an information booth which provided facts on the Institute's grants program, intramural and caries research programs, job opportunities, and dental care.

The NIDR EEO Officer was selected to chair the Executive Board of the NIH EEO Advisory Council and to serve on the NIH Director's Select Committee on EEO. In addition, the EEO Officer serves as a member of the NIH Contract Compliance Committee, the NIH EEO Awards Committee, the NIH Statistical Committee, and the NIH AAP/FEORP Plan Review Group.

MANAGEMENT INFORMATION SECTION

The Management Information Section (MIS) is responsible for the collection and dissemination of data on research studies supported by the National Institute of Dental Research.

Innovative computerized technology has enabled the MIS staff to continue the development and enhancement of the Research Projects Management System (RPMS), a collection of separate files dealing with grants, contracts, dental research subprojects, and intramural projects. The system has been designed to provide "real time" information retrieval on various research efforts supported by the NIDR. This information is used in preparing fiscal and programmatic reports which answer public and Congressional inquiries; in preparing budgetary and management decisions; informing administrative officials of expenditures; and keeping the NIDR staff informed of research activities within the Institute.

The Division of Financial Management continues to supply the MIS with monthly magnetic tapes which contain accounting information concerning obligation of funds for the entire Institute. The MIS accesses these files to provide NIDR management and budget officials with reports for budget tracking and reconciliation purposes.

MIS staff trained the NIDR Budget Office on the use and maintenance of the Full Time Equivalency (FTE) Tracking System. This computer-based system provides timely personnel ceiling balance information and fiscal year projections on a recurring basis. Designed, developed, and previously maintained by MIS staff, the FTE system can now be accessed by individuals trained in its procedures. Transferring the activities of FTE's day to day operations has enabled MIS staff to broaden and pursue other activities.

This year, MIS staff developed a computerized training system for the NIDR Personnel Office. This system provides information on training received by NIDR employees. Data is collected from training forms using a series of "user friendly" programs designed by MIS. The system enables the user to generate reports which illustrate the time, dollars, and training subject for each employee.

The NIDR Word Processing Committee has continued their activities in assessing the word processing needs of the Institute. The committee holds monthly business meetings and vendor demonstrations which assist management in the selection of Word Processors in terms of cost savings, productivity, facilities, and personnel. These efforts have resulted in the acquisition of nine Word Processors for the Office of the Director and nine for Extramural Programs.

MIS staff continues to work in conjunction with DRG and the NIDR Grants Management Office on the in-house generation of grant award statements and the obligation of funds locally. This method has proven to be an extremely efficient and cost-effective manner of producing award statements. The time savings realized represent a reduction from approximately three days to fifteen minutes. The cost to produce an award, once estimated at three hundred dollars, is now three dollars for computer time.

A member of the MIS staff has become involved in the NIH Office Technology Task Group (OTTG), a committee established to identify and define office automation issues at NIH. This committee develops procedures and guidelines for the effective acquisition, integration, and dissemination of office technology throughout NIH.

National Caries Program

NATIONAL CARIES PROGRAM

National Institute of Dental Research

October 1, 1982 - September 30, 1983

REPORT OF THE ASSOCIATE DIRECTOR

The National Caries Program was formally established in late 1971 in response to a Presidential initiative to expedite the elimination of dental caries as a national public health problem. It was designed, therefore, to conduct targeted research and development with all mechanisms available to NIH and to transmit information to the public about caries prevention so that useful techniques would be rapidly put into practice.

Science administrators interested in program structure and operations for planned "R & D" at NIH are referred to a history of the Program available from my office. It was prepared for the 1982 Annual Congress of the European Organization of Caries Research (ORCA) and describes the relationship of organizational structure to implementation of targeted research and development, methods of planning, coordination of activities and priority setting, and numerous special issues such as evaluation and use of project support mechanisms in targeted programs.

The following report describes the stage to which NCP was able to develop several major approaches to caries prevention, but by no means are all of NCP's FY 1983 activities described. For some approaches the earlier history and relevance of the activity is delineated. These accounts are vastly incomplete but should provide some insight to those wishing to assess the potential of targeted programs.

Just prior to the start of the report year Dr. William Bowen, Chief of the Caries Prevention and Research Branch (CPRB) left the NCP to assume a research position at the University of Rochester. To help fill the leadership void created by the departure of Dr. Bowen, several steps were immediately taken: Dr. Michael Cole was made Acting Chief of the Etiology Section, Dr. Horace Stiles was transferred to Chief of the Preventive Methods Development Section, and Dr. Ralph Frew was appointed Acting Chief of the CPRB (as well as continuing as Assistant to the Associate Director) and was asked to devote as much attention as possible to making administrative matters run smoothly in the Park 5 laboratories.

Furthermore, to identify exactly the research priorities of sections and individuals within the frame of NCP overall plans, all NCP professional staff were convened in a 2-day retreat at Harpers Ferry coincident with the start of the fiscal year. Priorities, coordination of efforts, and reporting were major issues on the agenda. In addition, four major research areas (vaccine development, intra-oral fluoride slow-release technology, measurement of food cariogenicity, and caries of tooth roots) were reviewed intensively and tasks within each area were assigned to staff. The implementation of these tasks as well as progress in other research areas will be reviewed below.

One of the issues that came into sharp focus at Harpers Ferry was the increased importance of dissemination of current information, not only in my office but throughout the NCP, on the projects on which various groups of staff were working. There were over 60 contracts and direct operations projects, many of which are technically complex. In addition, there were uncounted numbers of NCP projects such as conferences, development and distribution of health educational materials, generation of major reports, planning of new contracted research, liaison with other Federal and professional entities, and about 120 research grants and other awards relevant to the Program. Needs for program information were twofold: information about research projects and timetables, and information about staff involvement in their various activities.

After the meeting, Dr. William Rogers was asked to devise a computer-based management information system to maintain this information on a current basis. A netted system based on micro-computers operating in word processing mode using simple indices has been designed and the micro-computers have been ordered. These devices also will be used for program analysis and computation of research data,and because they are easily transportable, they will be tested for use in the field for direct recording of clinical and epidemiological observations.

Vaccine Development

At the Retreat considerable time was spent discussing with staff and representatives from the pharmaceutical industry the unusual opportunity for caries prevention which might be provided by vaccination. The best role for the Program in this area was discussed in depth relative to new information on: 1) mechanisms of the secretory immune system (as described for Strategy I), 2) an impending clinical trial of caries preventive trans-

dermal vaccination in England, and 3) untoward reactions in animals hyperimmunized with crude mixtures of *S. mutans* antigens. These discussions led to a conclusion that a caries vaccine would not be acceptable unless there was complete assurance of its safety. In addition, staff believed that an acceptable vaccine against caries would have to be quite effective over a long period of time, be simple and painless to deliver and, of course, be inexpensive.

These considerations led to a decision to explore thoroughly the potential for achieving useful caries immunity through the secretory immune system and a staff committee was appointed at Harpers Ferry to implement this course of action. During the year the committee developed a plan comprised of research objectives and detailed approaches to these objectives. Probably the most important of the planned activities is to carry out a clinical trial in adult volunteers of intragastric immunization with *S. mutans* in whole cells. This trial will be of sufficient quality to establish as clearly as possible at this time whether practical immunization is potentially feasible by the intragastric route using this source of antigen. Dr. Michael Cole has prepared an application to the FDA for an IND (an exemption allowing the investigational use of a new drug) for use of *S. mutans* in the planned clinical study. A request for proposals (RFP) also was advertised to continue development of information on interaction of specific *S. mutans* antigens and adjuvants with components of the mucosal immune system. Other projects are being implemented to increase research momentum in this extremely promising area.

Root Surface Caries

A second major topic at the Harpers Ferry Retreat was caries of the tooth roots. When caries prevalence data from the 1980 NCP and earlier NCHS surveys are compared it is clear that dramatically increased numbers of tooth surfaces and teeth now are being retained into adulthood. The root surfaces, because of loss of gingival attachment with age, will be at risk to caries throughout adult life but there is little information on the etiology or epidemiology of caries under these conditions, of attack rates to be expected, or on possible approaches to prevention for individual or public health use. It was agreed by staff that caries in adults could become a problem of significant proportions and that a balanced program should be initiated to measure and characterize it. At the end of these discussions individual staff were asked to develop plans for research in the above areas. Dr. Ann Miller was asked to be responsible for exploring the feasibility of a national survey of caries (and possibly other oral diseases) in adults.

During the year Dr. Miller has discussed possible ways to conduct such a survey with staff of the Administration on Aging, the Department of Labor Statistics, the National Center for Health Statistics, and the NIH Institute on Aging. She has determined that obtaining a completely representative national sample of the adult population would be an impractical undertaking but that a survey would be feasible if limited to working adults. This sample frame would be acceptably reproducible and can be supplemented with other samples representing particular populations, such as the elderly. Discussion of these directions within NIDR, with consultants, and with the Programs Advisory Committee led to agreement to survey the working population and seniors on a national basis and to measure periodontal disease in addition to root and coronal caries. In anticipation of clinical research in these areas, NCP staff visited Dr. Stamm at McGill University to become familiar with measurement of root caries, worked extensively with other consultants on proper use of periodontal disease indices, and commenced review of data recording and analytical techniques used in field studies of these diseases. It is projected that a sample frame for the survey will be drawn by the contractor and that the actual survey will commence within a year. As indicated in project reports such as Z01-00369, NCP's laboratory staff also commenced "gearing up" during FY 1983 for research in this area.

It also should be mentioned that in March groundwork commenced on a clinical trial to establish whether fluoride mouthrinsing could be used to prevent caries in adults. This study is being carried out through contract (N01-DE-32400) with SUNY at Stony Brook.

Diet and Caries

A third issue reviewed at Harpers Ferry was the continuing lack of substantial information concerning the effect of diet on caries. It was pointed out by Dr. Johnson (as described for Strategy III) that the level of fermentable carbohydrate in the diet remains at astonishing levels and that the habit of snacking is pervasive in the United States. Though many snack foods, when eaten at frequent intervals, cause massive caries in animal models, almost no information is available from clinical trials on the relative cariogenic challenge to humans caused by individual dietary items, or on characteristics of dietary items or idiosyncracies in eating that might modify the underlying challege due to carbohydrate content. There are a number of important questions in this area. One concerns the relative caries risk contributed by various parts of the American diet. Another is the balance that can be assumed to exist between the cariogenicity of the diet on the one hand

and the protection provided by factors such as fluoride on the other hand.

The major factors impeding the rapid development of answers to such questions is the impropriety of conducting clinical experiments in which caries may be induced by the food item being tested. Therefore, to obtain indirect but dependable information on the relative cariogenicity of individual foods the NCP has proposed that data on acid production in plaque when the food is consumed as a meal in clinical studies be supplemented by data on caries actually induced when fed to animals for several weeks. Reasonable as the approach appears, its technical implementation has been slow. The animal model, briefly described in Strategy III, requires both complete standardization of all variables (such as age of rats) and large inputs of labor to obtain reproducible data on the rank order of cariogenicity of a small number of foods. Data are accumulating slowly. On the other hand, the measurement of acid production in the mouth also has problems but these are concerned with fabricating devices equipped with sensors and data transmittors that function reliably *in vitro*. NCP's role is to promote reseach on dietary effects by developing these techniques to be a widely accepted way to measure relative cariogenicity. This requires both detailed information on the precision, reliability and use of these techniques and the ready availability to scientists of suitable intraoral devices or of their components.

Subsequent to these discussions the work scope of the contract with Eastman Dental Center (N01-DE-12434) was modified to establish more exactly the specifications of the animal test. Results obtained through this contract are now quite similar to those obtained by NCP staff. Also, the device for intra-oral measurement of pH has been brought closer to being a reliable, commercially available research tool. During the year Dr. Roald Shern has demonstrated a prosthetic device mounted with four pH sensors with which plaque pH can be measured at different oral sites during consumption of a snack. Use of field effect transitors for intra-oral pH measurement appears to have considerable potential and a grant was recently awared to Dr. Lauks at the University of Pennsylvania to explore this possibility.

Two other developments are noteworthy in this area. One of these is that the NCP's longitudinal epidemiological study to correlate current food intake patterns with development of caries is now entering its second year of data collection. This difficult and highly important study is being conducted by Dr. Brian Burt at the University of Michigan. The other notable development is the FDA clearance of Searle Pharmaceutical Company's sweetener, Aspartame, for use in soft drinks following last year's clearance for use in beverage mixes and as a table-top sweetener. Aspartame has been found non-cariogenic in NCP animal tests and large scale use in beverages could make significant inroads on caries prevalence.

Intra-Oral Fluoride Slow Release

Some of the prospective techniques for caries prevention on which the NCP has worked have needed only measurement of acceptance and cost before being promoted into widescale use. School-based fluoride mouthrinsing is an example of a technique that was at this stage of development. Other prespective techniques such as vaccination were at the stage of early animal or *in vitro* studies. The history of others can be traced back to phenomena or relationships observed in NCP studies of caries etiology. One of these is the intra-oral fluoride slow-release device.

The concept for the device originated in large part from observations in the early 1970s by Dr. Rachel Larson that caries in rats could largely be prevented by quite small amounts of topical fluoride if the fluoride was provided frequently. Contracts were employed in the period 1974 to 1977 to explore whether devices could be made to release small amounts of fluoride continuously in the mouth. One of these contracts (with Southern Research Institute) provided prototypes consisting of small capsules of fluoride-saturated hydrogel within a diffusion-limiting membrane. This design appeared promising and a time-consuming but essential series of development and evaluation steps followed. These were conducted both by contracts and by direct operations. Performance, safety, and anti-caries effectiveness data from animal studies was submitted to the FDA and clearance was received in 1979 to conduct a small-scale, short-term trial of the device in adult volunteers. At that point, a sufficient quantity of devices to conduct anticipated clinical trials was procured from Southern Reseach Institute. Based on results from the study in adults the FDA has given approval for a short-term trial in children to test performance, durability and acceptability of the device and a contract was recently awarded to the University of Maryland for this study. If clinical results continue to be favorable we plan to carry out a full-scale clinical trial in children to measure effectiveness in preventing caries. This trial, conducted by our own staff, could commence as early as late 1984.

The device has wide potential application, including prevention of caries that occurs during orthodontic treatment, assisting in prevention of caries and (with further development) maintenance of oral hygiene in handicapped individuals.

Clinical Trials of Fluoride-Based Techniques

During FY 1983 NCP staff continued a series of clinical trials to establish the best procedure when several variations of the treatment have been published or are in use. This has been carried out through direct operations or contracts using side-by-side comparison of procedures under double-blind conditions. In past years studies have been carried out on school water fluoridation, fluoride tablet programs, fluoride mouthrinsing, fluoride dentifrices, and professionally applied topical fluoride treatments. Variations in procedure that have been compared include frequency of treatment, period of treatment, concentration of fluoride, prior tooth cleaning, and nature of the fluoride agent. In FY 1983 the Program is comparing a dentifrice containing Na-MFP with one containing only NaF and comparing NaF-MFP dentifrices at 2,500 ppm and at 1000 ppm fluoride. These determinations are particularly important because, despite the fact that most individuals in the U.S. use fluoride-based dentifrices, we have little knowledge of optimal dentifrice characteristics.

In further attempts to increase the effectiveness of simple fluoride-based modalities NCP staff are continuing their assessment of combinations of treatments such as: 1) weekly fluoride mouthrinsing plus daily ingestion of a fluoride tablet, and 2) weekly fluoride mouthrinsing plus daily use of a chewable fluoride tablet and home use of a fluoride dentifrice.

Pit and Fissure Sealants

The fluoride-based techniques described above have their greatest effect in preventing caries of the smooth surfaces of the teeth They are much less effective in protecting pits, fissures or grooves such as those on the occlusal surfaces of the molar teeth where 50 percent of all caries activity may occur. Adhesive sealants can be used to exclude bacteria and food constituents from the pits and fissures of these surfaces but it is necessary to seal the surfaces soon after eruption, to apply the sealant meticulously, and to recheck the surfaces periodically for sealant loss. Through many years of investigation of the use of sealants NCP staff have concluded that, for the approach to become widely used, it will be necessary to reduce the cost of these repeated examinations and treatments.

Our staff has calculated, none the less, that by combining sealant treatment with fluoride supplementation, upgraded dental hygiene and dietary practice it should be possible to eliminate most dental caries in children. Staff has calculated that if such a program were delivered by preventive dentistry aides in school settings to reduce cost, that overall program costs relative to near-total elimination of caries might be highly attractive. To explore this possibility we have contracted (N01-DE-22439) with Forsyth Dental Center to determine effectiveness and cost, including training, of a program using preventive dentistry aides and assistants supervised by a dental hygienist to deliver the above procedures in schools. This 3-year clinical trial got underway in FY 1983. Its results could have far-reaching implications for future school-based programs. In addition, a Consensus Development Conference on pit and fissure sealants has been planned for December, 1983.

Treatment Needs

In December 1982 we published "Dental Treatment Needs of United States Children," a report summarizing data on restorations, extractions, tooth replacements, crowns, pulpal treatment, and gingival treatment needed by the 40,000 children participating in the 1980 National Dental Caries Prevalence Survey. Findings indicate that approximately 30 million United States school children (62.8 percent) had no need for restorative treatment at the time of the survey. On the other hand, many children did need restoration, indeed, the data indicate a need for approximately 32 million restorations in the permanent dentition of school children. The report, produced primarily by our Biometry Section, provides the most comprehensive information that is available on dental treatment needs of United States children.

The Future

Data from several sources indicated that caries prevalence in school-age children has decreased recently. When one weighs the factors that can affect caries prevalence — use of preventive techniques such as those mentioned above, life style, diet, environment and others — it seems probable that prevalence in this age group will decrease further if recently established preventive programs can be maintained and expanded to reach larger segments of the population. But caries will still be present at unacceptable levels in the U.S. population. It will increase as a public health problem that is difficult and costly to solve in older age groups and it will persist as an acute problem for children in areas or situations underserved with preventive programs. To continue the eradication of the disease will require a high technology attack on *S. mutans* and perhaps other microorganisms. This will require vaccines, non-virulent competitors of *S. mutans*, chemotherapeutics aimed specifically at *S. mutans*, and other approaches.

Already some of these approaches have been found effective in *in vitro* tests and in animal models. Continued development of these techniques through stages of pre-clinical and clinical testing, and eventual promotion into

use will require long-term careful support of programs and coordination of many disparate activities. Management of this "R & D" process in a timely and cost-effective manner is the central problem for the eventual eradication of caries.

STRATEGY AREA I. Combatting the Microbial Agent

Bacterial Surface Components Involved in Adherence and Aggregation

Few details are known concerning the initial attachment of oral streptococci to the tooth surface. Grantees studying this important reaction report that attachment appears to be highly specific, involving interaction between certain surface components of the bacterium and salivary glycoproteins. Recently they have found evidence that the receptor of *S. sanguis* responsible for adherence to saliva coated spheroidal hydroxyapatite (SC-SHA) is proteinaceous since it is heat labile and trypsin sensitive. Though the suspected receptor is rather firmly bound to cell surface components (e.g., polysaccharides) partial purification appears to be feasible using SHA columns and elution with increasing molarities of phosphate buffer. The streptococcal receptor appears to be a high molecular weight protein with a pI of 5.5. It is hoped that once the identification of these receptors and the nature of their binding to salivary proteins and other bacteria is understood, strategies for controlling these reactions will evolve.

A number of laboratories supported by NCP have isolated and partially purified protein components from the surface of *S. sanguis*, which competitively blocks *S. sanguis* adherence to SC-SHA. One grantee, for example, removed a putative adherence receptor from cell walls of three strains of *S. sanguis* by sonification. This soluble supernatant (named ABC for Adherence Blocking Component) was used in competitive adherence assays against whole cells of several *S. sanguis* strains and other streptococcal species (e.g., *S. mutans*, *S. mitis* and *S. salivarius*). Analysis of the data demonstrated that the ABC of *S. sanguis* competitively inhibited the adherence of the other bacteria. This suggests a common proteinaceous adherence receptor between *S. sanguis*, *S. mitis* and *S. mutans* associated with adherence to SC-SHA.

Further evidence for a proteinaceous surface structure involved in adhesion comes from studies by several grantees of *S. salivarius*, *S. mitis* and *S. pyogenes*. These bacteria appear in electron micrographs to attach themselves to epithelial cells by means of a "fuzzy coat," also referred to as fimbriae. A fibrous structure also has been found on the surface of *S. sanguis* FW213. Trypsin and other protein solubilizing agents remove the fimbriae and impair the ability of the organism to adhere to hydroxyapatite. Recent studies on the effects of carbohydrates on the adhesion of *S. sanguis* FW213 to SC-SHA, suggest that the fimbriae are not glycoprotein.

A basic premise of the models proposed to describe adhesion of oral bacteria to surfaces is that the binding reaction involves specific receptors. Specific binding can be distinguished from nonspecific binding by an assay in which nonradiolabeled bacteria compete with radiolabeled cells for adherence to particulate substrates. Preliminary data obtained by a grantee indicates that *S. mutans* does not compete with itself for SC-SHA under the conditions of the assay and that 50% of the binding of *S. mutans* to SC-SHA occurs via nonspecific mechanisms. Analogous experiments using *S. sanguis* Challis showed that *S. sanguis* readily competed with itself for binding sites and approaches saturation. There appears to be a small but detectable level of nonspecific binding, about 5%, for *S. sanguis*. Kinetic experiments reveal two rates of attachment of *S. mutans* and a single rate for *S. sanguis*. These three separate pieces of evidence form the basis of a proposal that *S. sanguis* and *S. mutans* adhere to SC-SHA surfaces by both specific and nonspecific reactions, and that interference with nonspecific binding influences the high affinity specific attachment reaction.

There are numerous contributions from various laboratories supporting the concept that non-glucan, non-peptidoglycan components of the *S. mutans* cell wall are involved in the interaction between the bacterium and the saliva coated tooth. One example is the effect on the interaction when *S. mutans* is carefully treated with reagents that would react with surface proteins if these were present. Thus it was found that acetylation and succinylation procedures affected the adherence of two strains of *S. mutans* (6715 and 10440) to SC-SHA and SHA beads in differing ways. Adherence to SC-SHA was enhanced for both strains upon acetylation, whereas adherence to SHA was depressed with moderate levels of acetylation. Succinylation enhanced adherence of *S. mutans* 6715 to SC-SHA; however, the adherence of *S. mutans* 10440 was depressed. Adherence profiles to SC-SHA of the two succinylated strains also differed.

Another grantee is studying the possibility that salivary proteins, by preferentially aggregating bacteria, prevent cell adherence to teeth. The experimental approach is to introduce dispersed *S. mutans* bacteria and clean vertical squares of human enamel simultaneously into a saliva milieu. The rates of bacterial adherence and aggregation are monitored continuously by measuring scattering of laser light and changes in zeta potential. Results indicate that adherence and aggregation are competitive

processes dependent on charge-altering factors such as pH, salivary protein/bacteria concentration ratios, the bacterial growth stage, and fluoride treatment of the enamel, and provide evidence for an important role of electrostatic forces in bacterial aggregation and adherence to the tooth surface.

Glucosaltransferases and Oral Bacteria

During FY 1983 there was definite progress in establishing the mechanism of action and properties of glucosyltransferase (GTF), the enzyme of *S. mutans* and a few other oral bacteria that converts dietary sucrose to sticky long chain polysaccharides used for bacterial attachment to smooth enamel surfaces. During the year an enzyme-glucose intermediate was isolated and characterized. The intermediate is a peptide-glucose unit that occurs transiently when glucose from sucrose is transferred to a growing polysaccharide chain. Evaluation of the specificity of the active site of the GTF enzyme has involved the synthesis of 19 sucrose and fructose analogs to help establish the steric requirements of the active site. Thus far, none has inhibited the glucan synthesis reaction.

Upon close examination, the reactions that synthesize glucans and anchor them to the bacterial surface are turning out to be quite complex. *S. mutans*, for example, produces a variety of exocellular proteins which are involved in the synthesis, modification, degradation and binding of glucans, including: several isozymes of glucosyltransferase synthesizing either water-insoluble or water-soluble glucans from sucrose; an endodextranase; an inhibitor of endodextranase; and several dextran-binding proteins resembling lectins. NCP grantees are isolating these proteins by affinity chromatography. Also monoclonal antibodies are being prepared to various GTF isozymes. These are being used for further purification of the isozymes and dextran-binding proteins and also for analyzing the roles of individual enzymes and proteins in cell adherence and plaque formation.

Although purification of GTF activities has been extensively pursued by grantees, homogeneous GTF preparations (judged by rigorous chemical criteria), have yet to be obtained and thus the question of whether one or more distinct GTF activities are produced by each microorganism remains unanswered. One approach to answering the question is to isolate the GTF genes from *S. mutans* by recombinant DNA techniques. A grantee has recently cloned *S. mutans* GS5 GTF genes into *S. milleri* to determine the number of these genes on the *S. mutans* chromosome. Characterization of the GTF containing clones will help determine whether or not a single GTF enzyme is capable of synthesizing both water-soluble and insoluble as well as adherent glucans. The chimeric plasmids isolated from these clones will also be utilized to transform mutants of strain GS5 which are defective in glucan synthesis. Such an approach will be useful in further characterizing the mechanism of adherent glucan synthesis by *S. mutans*.

The GTF enzyme has attracted so much scientific interest that last year NCP sponsored a three day workshop covering the area. The meeting titled, "Glucosyltransferases, Glucans, Sucrose and Dental Caries," covered the properties of GTFs, their substrates and products; purity of the enzymes and new approaches for their isolation and purification; role of host, including anti-bodies, and bacterial products that modulate GTF activity; and synthesis and testing of GTF inhibitors for possible use in preventing the sucrose-dependent adherence of oral streptococci. One hundred and fifty scientists attended the meeting.

Antimicrobial Factors Associated with Saliva

In addition to immunoglobulins, saliva contains a number of antibacterial factors such as lactoperoxidase, lysozyme, lactoferrin and glycoprotein agglutinins. The existence of these factors relative to possible roles in oral ecology is extremely intriguing and continues to attract grantee attention.

The mechanism of action of salivary lysozyme on microorganisms is being examined by several grantees. One scientist has recently shown that bacteria deplete lysozyme from saliva and that different bacteria do this to different degrees. Thus the greatest mean depletion (60-70%) was observed with *S. sanguis* (biotype I), serotype *b S. mutans*, and fresh isolates of *S. salivarius*, while relatively low mean depletion was noted with *S. mitus* (15%) and biotype II *S. sanguis* (ca 30%). Additional work indicated that lysozyme depletion by *S. sanguis* depended upon the concentration of bacteria, the time of incubation, and the ionic strength of saliva, but was independent of pH over a range of 3.9 to 8.3. The data indicate that depletion is due to the adsorption of lysozyme by the organism, and that adsorption is mediated by anionic bacterial surface components and modified by salivary constituents which interact with oral bacteria.

During the year another grantee has reported electron microscope studies of lysozyme's effect on *S. mutans*. The EM pictures showed gelled protoplasmic masses being released from cells when sodium thiocyanate is added to reaction mixtures. Evidence suggests that at neutral pH, autolytic enzymes (e.g., trypsin) may be activated by lysomzyme-inorganic anion (e.g., bicarbonate, fluoride, chloride, and thiocyanate) treatments to produce a hole in the protective surface large enough to cause an expulsion of intracellular macromolecular substances. At low pH, only

bicarbonate, but not fluoride, chloride, or thiocyanate, can effect lysis of *S. mutans* cells previously treated with lysozyme and a protease. In the reaction, lysozyme and the protease appear to act synergistically.

Lysozyme binding components of bacteria have been detected by a grantee in saline extracts of both BHT and LM7 strains of *S. mutans*. Binding components were purified by affinity chromatography on Sepharose to which lysozyme had been coupled. Purified components from strain BHT reacted with anti-polyglycerolphosphate serum, suggesting a lipoteichoic acid molecule. Binding components may play important roles in determining whether bacteria are sensitive (*S. mutans* BHT) or resistant (*S. mutans* LM7) to the antibacterial activity of lysozyme.

The salivary peroxidase system that generates the antibacterial substance, hypothyocyanate ($OSCN^-$) consists of the peroxidase enzyme, the thiocyanate ion (SCN^-), and hydrogen peroxide (H_2O_2). Recent results obtained by a NCP grantee indicate that there is a significant source of H_2O_2 within the human parotid gland and that peroxide producing oral bacteria are not a requirement for the generation of significant levels of $OSCN^-$ in the mouth. The grantee has established that $OSCN^-$ in parotid saliva is critically dependent upon actitivy withing the pH range of 6.5 - 7.0 and upon the relative and absolute concentrations of H_2O_2 and SCN^-.

Whole saliva from some caries-resistant individuals exhibits antimicrobial activity toward the oral streptococci. NCP grantees have established that this activity is associated with specific anionic proteins that either have direct antimicrobial effects or inhibit or the microbial metabolism of protein substrates. The relationship of the inhibitory anionic proteins of whole saliva, the inhibitory proteins (apparently different) of parotid saliva, and known salivary antimicrobial factors such as peroxidase remains to be established.

Also, it should be noted that saliva is a major source of amino acids, peptides, and proteins required for growth and metabolic activities of oral streptococci. Again, caries-active and resistant individuals exhibit differences in these nitrogeous substrates. Thus, for instance, a grantee has found that salivary secretions from caries-active individuals contain anionic proteins, which serve as nitrogenous growth substrates for *S. mutans* and *S. sanguis*, whereas salivary secretions from caries-resistant individuals contain electrophoretically similar constituents, which do not serve as growth substrates.

Another class of salivary molecules, bacterial agglutinins, bind to the bacterial surface and cause aggregation. The aggregation presumably leads to clearance of the organisms from the oral cavity. An interesting agglutinin was recently identified in whole saliva by an NCP supported grantee. This protein is immunologically identical with serum fibronectin and can be isolated from saliva by affinity chromatography. Serum fibronectin agglutinates *S. mutans*, and when incubated with hydroxyapatite and saliva, is incorporated into artificial pellicles. Salivary fibronetin also has been detected in artificial pellicles by use of monospecific antibody to fibronectin. Studies on the interaction of bacteria with fibronetin suggest that this complex protein may contain distinct binding sites for different bacteria. Although binding regions of the fibronetin molecule for *S. pyogenes*, *S. mutans*, and *S. pneumoniae* have not been isolated, data suggest that the binding of fibronectin to these bacteria is mediated by lipoteichoic acid (LTA). The data further suggests that the fatty acids of LTA exposed on the surface may play a central role in the adherence of *S. mutans* to the tooth pellicle.

Anti-Caries Agents and their Affect on the Metabolism of Plaque Bacteria

The search was continued in FY 1983 for agents that might be used therapeutically against cariogenic microorganisms and plaque. During the year, one grantee synthesized several sucrose analoques, carbohydrate derivatives, amino sugars and pyridine analogues and found some to be effective inhibitors of glucosyltransferase (GTF), sugar uptake, or acid production by intact *S. mutans*.

Another grantee has been studying the properties of periodate-oxidized dextran, and found it to be a potent *inhibitor* of soluble, but not cell-bound, GTF. Interestingly, the oxidized dextran seems to have little effect on *cell-adherence* and *plaque-forming* properties of *S. mutans*. Several lower molecular weight enzyme inhibitors also have been developed that are able to penetrate dental plaque and decrease the synthesis of insoluble -glucan and the production of *acid* by plaque microorganisms. The inhibitors, including modified oligosaccharide substrates, are being tested for their effects on extracellular glucosyltransferases that metabolize *sucrose*, and on intracellular enzymes that metabolize *maltodextrins*.

The inhibitory effect of fluoride on the metabolism of plaque microorganisms is of increasing interest to a number of NCP-supported grantees. Though fluoride inhibits glycolysis in a large number of streptococci, different strains exhibit different sensitivities toward it. Results of recent studies confirm that fluoride inhibits streptococcal enolases. This presumably brings about a reduction in the intracellular concentrations of phosphoenolpyruvate (PEP) and adenosinetriphosphate (ATP), a reduction of sugar transport into the cells, and

a diminution of the rate of acid production. Furthermore, fluoride can inhibit the membrane ATPase activity of the cells and may, thereby, have immediate and profound effects on the intracellular pH of the cells.

Regulation of Secretory Immunity

During the year, NCP grantees continued to probe the apparently common mucosal immune system in which ingested antigens sensitize lymphoid cells (both T & B) in gut-associated lymphoreticular tissue (GALT), which subsequently results in IgA responses in secretory tissues including salivary and mammary glands. Studies from one laboratory have shown that lipopolysaccharides (LPS) from indigenous Gram negative gut microflora influence the inductive events of antigen stimulation of GALT in an LPS responsive host. Specifically, LPS affects T lymphocytes in GALT. This supresses GALT responses to T dependent antigen, and ultimately results in oral tolerance. LPS nonresponsive hosts appear not to have these LPS-induced T suppressor (Ts) cells and exhibit elevated IgA responses to T-dependent antigens. Furthermore, when T cell populations of mouse spleen and Peyer's Patches (PP) were stimulated (through oral administration with sheep red blood cells) and assessed for helper and suppressor activity, LPS responsive mice exhibited largely suppressor activity while non-responsive mice primarily exhibited helper activity. Scientists believe that Ts cells from responsive mice probably originate in PP, and after homing to the peripheral tissues, such as salivary glands, mediate systemic unresponsiveness by suppressing IgA (and other isotope) responses. These mechanisms suggest gut bacterial endotoxin might give rise to precursor Ts cells which can be induced by antigen to become mature Ts cells and suggest an important role for T cells in the induction and/or regulation of IgA immune responses to *S. mutans* antigens.

Studies in other NCP-supported laboratories have shown that oral presensitization leads both to stimulation of salivary IgA responses and suppression of serum antibody responses, possibly through concomitant effects on isotype specific T helper and suppressor cells. Possibly suppresive effects on the immune system therefore must be considered in developing an orally administered vaccine.

Soluble antigens, administered intragastrically, induce relatively weak salivary immune responses. Therefore, an NCP grantee has been exploring the use of insoluble antigens and found that particulate forms of DNP-BGG (a model antigen) induce much greater salivary IgA antibody responses than do soluble forms of DNP-BGG. Results from further experiments suggest that the optimal approach to induction of SIgA at mucosal surfaces may involve systemic priming with soluble antigen followed by oral administration of a particulate form of the same antigen.

A major concern in the development of a vaccine for human use is the cross-reactivity of certain *S. mutans* antigens and mammalian tissues. Recent investigations have shown that at least some serotypes of *S. mutans* share antigens with cardiac muscle, skeletal muscle and kidney glomeruli. Indirect immunofluorescence assays indicate that sera from rabbits, hyper-immunized either intradermally or intravenously with disrupted *S. mutans* contained antibodies reactive with human heart and kidney components. In many of the rabbits immune deposits of C3 and IgG, IgM or IgA and fibrinogen were seen in the kidneys within the glomeruli and in the heart myocardium, and focal deposits of *S. mutans* antigen were detected in the glomeruli and in the kidney interstitium. These and other pathological results clearly establish the need for extreme caution in developing an anticaries vaccine.

It is also possible, as one NCP grantee suggests, that cariogenic bacteria, resident in the mouth, establish a condition of immunologic tolerance to the bacterial antigens. This grantee has established that the secretory immune response is restricted in the quantity of antibodies that it can generate against oral antigens and that the antibacterial response in the gut activates regulatory systems that restrict it further.

During the year 90 grants, 5 contracts and 27 direct operations projects were active in Strategy Area I representing 67 percent of National Caries Program Research projects and 64 percent of funds expended in support of projects.

STRATEGY AREA II. Increasing the Resistance of the Teeth

Use of fluoride in water supplies, dentifrices, mouthrinses, dietary supplements and in other forms continues to be the most effective way to prevent dental caries. In the United States, recognition of fluoride's outstanding preventive attributes has come about largely through research, development and promotional efforts of the NCP. That these efforts are beginning to have a marked effect is evidenced by several recent reports, which have indicated a decline in the prevalence of dental caries in the United States. For example, findings published in FY 1982 from the NCP-sponsored National Dental Caries Prevalence Survey, compared to a survey by the National Center for Health Statistics in 1971-3, indicate that in that period the prevalence of dental caries among United States school children had decreased about 32 percent.

The NCP particularly emphasizes those regimens that are cost-effective and easy to implement in wide-scale programs. Accordingly, the self-administration of fluorides as mouthrinses, dietary supplements and dentifrices received considerable attention in both direct operational and contract-supported research in FY 1983.

Further progress toward control of dental caries with fluorides is likely to come from the use of combinations of fluoride procedures that are believed to work largely by different mechanisms of action. For example, certain combinations of systemic and topical fluorides have been shown to produce additive benefits. To determine the total effectiveness of a combination of some of the most feasible methods of self-administering fluorides, the NCP began a long-term study in October 1972 in Nelson County, Va., a fluoride-deficient community. Children in the County's schools, under teacher-supervision, chew and ingest daily a 1 mg. F tablet and rinse weekly with a 0.2% NaF solution; a fluoride dentifrice is provided for *ad-libitum* use at home. Follow-up examinations have been conducted at two-to-three year intervals to determine the effectiveness of the program as increasingly larger segments of the participants become exposed to the F treatments since entering school in Kindergarten. The most recent follow-up examinations were conducted in 1980 after eight years of treatments. Overall findings showed that continuous participants in grades 1-9 (ages 6-14) had 49 percent fewer DMF surfaces in 1980 than did their cohorts in 1972. In mesiodistal surfaces the benefits were particularly striking, an 86 percent caries inhibition. The final exam inations are scheduled for September 1983. Early in 1984, a sealant program will be added to the ongoing fluoride program. Newly erupted teeth of children in selected grades will be sealed with a pit and fissure sealant. The program will run for five years. The combined preventive program has the potential of virtually eliminating dental caries in Nelson County.

Although the findings of the Nelson County study are highly encouraging, no specific claims of additive effects of the fluoride procedures can be made because all study participants received all of the preventive regimens. Consequently, a longitudinal study to evaluate the caries-preventive effects of dietary fluoride supplements and weekly fluoride mouthrinsing when each method is used alone as well as in combination was initiated by the NCP in September 1981 in the fluoride-deficient community of Springfield, Ohio. The procedures are carried out in school under teacher-supervision. Treatments will continue for a minimum of eight and nine school years for first grade and Kindergarten children, respectively. The first follow-up dental examinations will be conducted in October 1983.

Recognizing the need for current information on the relation between water fluoride concentration and the prevalence of dental fluorosis and dental caries, the NCP conducted a cross-sectional survey in April 1980 of 807 schoolchildren in four areas of Illinois that had naturally-occurring fluoride in their drinking water at concentrations of 1, 2, 3 and 4 times the recommended optimal for the respective areas. Also, in April 1982, 317 children from four communities in Iowa with negligible concentrations of fluoride in their drinking water were examined for comparison with the children in Illinois. Dental caries was assessed with the DMFS index and fluorosis was measured with Dean's Index and with a newly developed Tooth Surface Index of Fluorosis (TSIF). Findings analyzed to date show that the mean caries score was approximately 38 percent lower in the optimal fluoride area compared with the areas with negligible concentrations of fluoride in their water supplies. Mean caries scores in all three above-optimal fluoride areas were significantly lower than those in the optimal area but were not significantly different from one another. The prevalence of dental fluorosis was negligible in the nonfluoride area and characteristically low in the optimal fluoride area. Significant increases in the prevalence and severity of fluorosis occurred in the above-optimal fluoride areas, with the condition being most pronounced in the 4-times optimal area. The new TSIF Index served exceptionally well and should prove to be more sensitive and useful for assessing dental fluorosis than the index of Dean.

Because epidemiologic data on dental fluorosis have almost wholly been derived from studies of children's dentitions, data on the long-term sequelae of dental fluorosis in adults are lacking. Specifically, it is not known if severely fluorosed enamel, hypomineralized to the point of showing pitting and susceptibility to abrasion in children, eventually leads to premature loss of teeth, dysfunction of the masticatory system or other adverse effects on the oral health of adults. In order to gain information in this regard, the NCP has contracted with the University of Michigan to compare the oral health of lifetime adult residents of an area with severe endemic dental fluorosis with that of residents in an area with optimally fluoridated water. Dental caries, tooth loss, and attrition are some of the variables that will be compared. In addition to its epidemiologic value in refining our knowledge of the lifelong effects of dental fluorosis, the study should help to provide much needed and timely data on whether excessive fluoride levels in water supplies pose any hazards to the oral health of adults.

The fluoridation of a school's water supply at 4-1/2 times the concentration considered optimal for community fluoridation in the same geographic area has been shown to reduce the prevalence of dental caries by

about 40 percent. It is presumed that school water fluoridation and community water fluoridation act in similar ways to produce their cariostatic effect. Therefore, because studies have shown added caries-preventive benefits from a procedure of fluoride mouthrinsing in optimally fluoridated communities, there is reason to believe that a similar result can be achieved by combining fluoride mouthrinsing with school water fluoridation. To test this hypothesis, the NCP is planning to implement a four-year study among 600 children in grades K and 1 and 600 children in grades 6 and 7 who have consumed fluoridated water at school from the earliest grades. Subjects will be randomly assigned to one of two groups that either rinse weekly with a placebo solution or with a 0.2% NaF solution. This study design will permit early detection of added effects of fluoride rinsing. Both school water fluoridation and weekly fluoride mouthrinsing in school are feasible, economical, safe, well-accepted by school personnel and students and, once in operation, have minimal need for the direct services of professional dental personnel. If the procedures are shown clinically to impart additive caries-preventive benefits, this knowledge will be important to public health officials who establish programs for caries prevention in areas lacking a central water system.

Contract-supported clinical trial activities in FY 1983 include the following:

Final results were reported from a three-year clinical study conducted by investigators from SUNY at Stony Brook to determine the effect of prior tooth-cleaning on the efficacy of semi-annual, professionally applied acidulated phosphate fluoride (APF) treatments. Findings showed that the effect of APF gel treatments was not influenced by prior tooth cleaning, whether performed professionally (pumice prophylaxis) or by the subjects (supervised brushing and flossing).

First-year follow-up examinations were conducted in a three-year study to determine whether the efficacy of a fluoride dentifrice can be improved by raising the fluoride concentration from 1000 to 2500 ppm. The study will also determine whether a combination of sodium fluoride and sodium monofluorophosphate (MFP) confers greater protection than a formulation of MFP alone. Interim findings are not yet available.

A contract was awarded for a clinical study of the effect of dietary fluoride supplements used during pregnancy to prevent dental caries in deciduous teeth of offspring. This five-year trial will begin in mid-1983.

A study of the effectiveness of fluoride mouthrinsing in inhibiting caries development in adults was also begun during the fiscal year. This trial will assess caries increment in both coronal and root sufaces of teeth.

The projects highlighted in this section represent a major part of the overall NCP effort in "Increasing the resistance of teeth." During the year, 9 direct operations and 6 contracts were carried out in Strategy Area II. Including 31 grants in this area, the total coordinated activity amounts to approximately 25 percent of NCP research projects and 26 percent of funds expended in support of projects.

STRATEGY AREA III. Modify the Diet

Despite public awareness of the dental risks of frequent intake of sugary foods, per capita consumption of total cariogenic sugars has been fairly constant over the last few years at over 120 pounds per year. The total includes cane and beet sugar, dextrose, corn syrup, high fructose corn syrup, maple syrup, sorghum, molasses, and honey. Among them, consumption of cane and beet sugar has declined while that of corn-derived sugars has increased.

It is expected, however, that comsumption of cariogenic sugars may change significantly in the near future, reflecting the introduction of Aspartame and possible changes in the FDA acceptance of saccharin. Aspartame, a synthetic sweet dipeptide introduced by Searle Pharmaceutical Company, was recently approved by the FDA for table-top use and use in dry mixes and in canned soft drinks. Concern has been expressed by several scientists about possible metabolic effects of high intake of Aspartame's hydrolysis products, the natural amino acids aspartic acid and phenylalanine, and methanol, so usage of the sweetener will be monitored carefully. In 1983, the FDA was petitioned for approval of the use of acesulfam and cyclamate as sweetening agents. These non-cariogenic sweeteners and others currently are in use in foreign countries. Examples are: cyclamate in Canada; acesulfam and Thaumatin (from an African berry) in the United Kingdom, and sweeteners derived from plants such as Thaumatococcus and Stevia in Japan. In addition, in various parts of the world, local plants and plant products are used for sweetening purposes. Examples are the use of Lo Han Kuo fruits from the *Mormodia grosveneri* tree in South China, and the use of the *Lippia dulcis* in Mexico.

Research work sponsored by NCP has characterized several sweeteners of natural origin. In the past, the protein, monellin, from the berries of an African plant was studied by researchers at the Monell Institute in Philadelphia. Also, analogues of dihydrochalones derived from citrus fruit were synthesized and characterized by a research group at the Dynapol Corporation in California. During FY 1983 scientists at the University of Illinois have continued isolating and characterizing a variety of novel sweeteners derived

from plants. Scientists at the University of California and at Research Triangle Institute worked on dipeptide sweeteners, both to develop new sweet dipeptides, and to learn what characteristics of these molecules invoke sweet taste. Scientists at the NCP laboratories in Rockville, Maryland, continued to screen sweeteners for cariogenicity, using rats as the test animals. Sorbitol, xylitol, Aspartame, and acesulfam were shown to be noncariogenic. In addition, testing of acesulfam gave evidence of cariostatic effects.

Also during FY 1983 scientists in NCP laboratories and scientists at the Eastman Dental Center have continued developing an approach for studying cariogenic characteristics of foods. In this research rats are fed their essential nutrition by intubation, and receive test snacks by mouth by way of a feeding machine, so that in a reproducible way only the test food comes into contact with the rats' mouths. Using this approach scientists found peanuts to be uniformly low in cariogenicity and found increasing cariogenicity in the order of corn starch (raw), milk chocolate, potato chips, beet or cane sugar and cupcakes.

The NCP has also contracted with the University of Michigan for a prospective study of the relationship of diet to caries development in school children. Detailed dietary histories are being taken at regular intervals and will be correlated with the development of caries over a three year period of time. Following data acquisition, caries incidence will be correlated with diet components and dietary habits such as frequency of snacking.

During the year 3 grants, 4 contracts, and 3 direct operations projects were active in Strategy Area III, representing 6.5 percent of National Caries Program research projects and 7.4 percent of funds expended in support of projects.

STRATEGY AREA IV. Improved Delivery and Acceptance of Caries Preventive Procedures

The NCP makes extensive use of educational and promotional activities to stimulate use of new methods of caries prevention by both health professionals and the general public. In general, these activities include presentation of lectures and seminars, organizing and sponsoring conferences, providing scientific exhibits at national meetings of health and educational organizations, and producing and distributing brochures, pamphlets, movies and other educational materials concerning the appropriate use of fluorides and fissure sealants.

Often in these activities the NCP collaborates with other Government and non-government agencies. Thus in March the NCP was involved in the Interagency Meeting on Health Promotion Through the Schools that was organized by the Department of Health and Human Services and the Department of Education to increase cooperative planning of this activity. Copies of NCP's educational materials for school-based caries prevention were distributed at the meeting and descriptions of NCP school-health promotion activities were included in the organizer's *Inventory of Federal School Health Promotion Activities.*

The NCP also was involved in a multi-agency activity distributing educational materials on dental health to all Head Start programs, community health centers, rural health clinics, and Indian Health Service clinics in the United States. The major Federal components involved in the project were the Division of Maternal and Child Health, Bureau of Health Care Delivery and Assistance (PHS), and the Administration for Children, Youth, and Families (OHDS). Of the ten publications included in the distributed packet, eight were produced by the NIDR. The response to the distribution was excellent and provided the opportunity for direct NCP staff contact with hundreds of these programs.

During FY 1983 NCP staff organized and/or participated in a number of conferences and programs at national and regional meetings of professional organizations to disseminate up-to-date information on caries prevention. Particularly noteworthy was a forum, "The Use of Fissure Sealants in Public Health Programs", which was sponsored by the Dental Health Section at the 110th Annual Session of the American Public Health Association held in Montreal. The proceedings of this forum was published in the Summer, 1983 issue of the *Journal of Public Health Dentistry.*

Last year a day-long symposium, "Dental Caries Prevention: An Update', sponsored by the NCP, was held during the 1982 National Convention of the American Dental Hygienists Association. The papers presented at this meeting were published in a special issue (May, 1983) of *Dental Hygiene*, which is the official publication of the ADHA and has a circulation of over 30,000.

Also during FY 1983, the Program sponsored the second in a series of conferences titled "Dental Caries Prevention in Public Health Programs". This series is designed to provide participants with current research information on effective caries preventive methods and to discuss ways to use this information to improve oral health. Two papers from the 1982 Conference concerning the importance of transmitting information on caries prevention to students of dentistry and dental hygiene were published this year in the *Journal of Public Health Dentistry*, and were distributed to attendees at the

conferences and all dental hygiene programs in the U.S. In September of 1983, the Program will hold its third conference of the series. The attendees will be educators who are responsible for teaching community or preventive dentistry to dental hygiene students.

There is little definitive information about the teaching of caries prevention to dental hygiene students, though dental hygienists are an important source for the public of information on preventive practices and provide an important stimulus to carry out these activities. To obtain information on this area NIDR has advertised for a contract to survey the teachers of dental hygiene in all 201 schools where dental hygiene is taught. Data will be collected on the types of preventive information taught to the student, on curriculum approaches, and on the needs of the educators for resource materials.

In FY 1983 NCP staff also were active participants at annual meetings of the National Dental Association, Pennsylvania Public Health Association, American Academy of Pedodontics, American Association of Public Health Dentists and the American Public Health Association. Presentations also were made for staff and students at many dental and dental hygiene schools.

In last year's report it was noted that the NIDR had contracted with the American Dental Association Health Foundation to examine the current role of dentists and physicians in the adoption of various proven caries preventive methods by the public. The data from the mailed questionnaires have now been collected and processed and are being analyzed in preparation for the final report. From the preliminary analysis of this data, several important findings are apparent, including the following:

- Dentists believe that it is possible to prevent most dental caries and they regard the use of fluorides in water supplies as the single most effective preventive measure.
- Dentists and pediatricians generally prescribe dietary fluoride supplements for child patients who reside in areas without fluoridated water supplies.
- The value of pit and fissure sealants for the prevention of occlusal decay has yet to be recognized by a large number of practicing dentists and physicians.
- A majority of dentists and pediatricians believe that a school provides the best setting in which to provide oral health education to chidren and to provide caries and gingivitis preventive treatments.
- Most dentists and pediatricians regard the television advertising of sugary snack foods as harmful to the oral health and general nutrition of children and favor the requiring of a health-warning label on such foods.

The findings from this study will, needless to say, be very important to health planners in their efforts to educate health professionals and the public on the best methods to attain and maintain good oral health.

New posters also were published this year after almost two years spent in obtaining clearance from the Department for their development. The posters, "Fluorides Aren't Just for Kids—" and "Adults Need Fluoride, Too—" and an accompanying leaflet, "Fluoride to Protect The Teeth of Adults," will help to dispel the misconception among adults that only children benefit from the appropriate use of fluoride and are part of an effort to expand NCP's health promotion efforts from school-based, self-applied fluoride regimens to other needed areas.

The posters and the accompanying leaflet are suitable for use in all types of dental and medical offices and health centers, worksites, and schools. They are brightly colored so that they will attract attention, create an awareness among adults of their need for fluoride, and remind children that they will never outgrow their need for nature's most effective mineral for preventing tooth decay. Although these materials were not available until recently they have been extremely well received. To assist health professionals in increasing their knowledge on the subject, a paper entitled "The Use of Topical Fluorides to Prevent Dental Caries in Adults: A Review of the Literature" by a member of NCP staff was published in the September 1983 issue of the *Journal of the American Dental Association.*

Recently, permission was finally granted by the Department to publish two other, much-needed posters. These will promote the benefits to be obtained from combined use of fluorides and dental sealants and will be available in FY 1984.

Each year the short NCP films on caries prevention are widely shown as indicated by the tables that follow covering FY 1983. These films are primarily designed for use by health professionals. What has been urgently needed in addition are audiovisual materials to educate the lay public about caries prevention. Recent surveys indicating, for example, that a large proportion of the public does not understand why fluoride is added to municipal water supplies amply document this need. Though NCP staff have tried for many years to obtain clearance to develop such materials they have been thwarted, most recently by a Department moratorium. This year, therefore, staff sought financial support from the private sector and were indeed fortunate to receive means to develop two films about the use of fluoride. These films, oriented for child and adult audiences, will be available early in FY 1984 and are expected to have extremely wide national, as well as international, use.

Also in FY 1983 NCP distributed close to 400,000 publications and posters as itemized in the tables that follow. Most of the requests were State, County, and local health department bulk orders which are encouraged by the NCP to help decrease Federal distribution costs.

It should be noted that NCP's personnel staffed its large scientific exhibit on school-based self-applied fluoride regimens for a total of 34 days at meetings of the following orgainizations: the American School Health Association, the National Asocition of Education for Young Children, the American Public Health Association, the Public Library Association, the National Catholic Eduction Association, the National School Boards' Association, the U.S. Public Health Service Commissioned Officers' Association, the National Education Association, the American Dental Hygienists' Association, the National Dental Association, and the National Rural Primary Care Association.NCP's 11 free-loan table-top exhibits on school-based self-applied fluorides were used by educators, health professionals, and the public in 67 settings for a total of 488 days. These small exhibits and free educational materials were used at health fairs, scientific workshops, Head Start screening clinics, teachers' meetings, school board meetings, and at dental, dental hygiene, and public health association meetings.

NCP's health promotion and education staff also gives high priority to providing technical assistance and advice to local, State, and national groups on establishing and maintaining supervised, school-based, self-applied fluoride regimens. Consultation most generally is provided by phone and letter.

In addition to the activities described above in the Office of the Associate Director, 3 contracts and 1 direct operations project were active in FY 1983 in Strategy Area IV. One of the contracts (NOI-32445, costing $60,000) was for an evaluation supported by the NIDR, Office of the Director. The projects represent about 2.6 percent of NCP's total grant, contract and direct operations projects and 4.4 percent of funds expended in support of projects.

NATIONAL CARIES PROGRAM

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00070-11	Herschel S. Horowitz	Combined Self-applied Fluorides for Caries Prevention in a Non-fluoridated Area
Z01 DE00112-10	Roald J. Shern	Screening of Anticaries Agents
Z01 DE00113-10	Roald J. Shern	Short-term Clinical Trials of Antiplaque Agents
Z01 DE00147-09	Dale B. Mirth	Lectins in the Study of Plaque & Caries Development
Z01 DE00154-09	Stanley A. Robrish	Chemical Products and Energy Requirements of Plaque
Z01 DE00190-08	Joseph E. Ciardi	Extracellular Macromolecules & Virulence of Cariogenic Streptococci
Z01 DE00222-07	Michael F. Cole	Immunoglobulin, Antibody and Proteins in Plaque Fluid
Z01 DE00234-06	Roald J. Shern	Develop Method of Intraoral Telemetry of Various Ions
Z01 DE00243-06	Stanley A. Robrish	Growth Energetics and Interaction of Plaque Microorganism
Z01 DE00262-05	Roald J. Shern	Study of an Intraoral Device
Z01 DE00274-05	Joseph E. Ciardi	Role of Host Saliva on Implantation of *S. mutans*
Z01 DE00277-04	Herschel S. Horowitz	Prevalence of Dental Caries & Fluorosis
Z01 DE00281-04	Dale B. Mirth	Analysis of Oral Fluids Using High Performance
Z01 DE00282-04	Dale B. Mirth	Anticaries Evaluation of an Intraoral Fluoride Release Device
Z01 DE00292-04	Janet Ann Brunelle	Analysis of National Dental Caries Survey

Z01 DE00295-04	Christopher W. Kemp	Effect of Fluoride Pulse on Mutans
Z01 DE00310-03	Herschel S. Horowitz	Evaluation of Fluoride Mouthrinsing & Fluoride Tablets
Z01 DE00319-03	Roald J. Shern	Simple Microdiffusion for Measuring Fluoride in Biological Samples
Z01 DE00324-03	Albert Kingman	Methods of Analyzing Fluorosis Evaluations Made By Dean's Scoring System
Z01 DE00325-03	Horace Stiles	*Streptococcus mutans* Transmission in Rats
Z01 DE00343-02	Christopher W. Kemp	Methanogenesis in Dental Plaque
Z01 DE00344-02	Michael F. Cole	Mucosal Immunity to *Pseudomonas & Stapholococcus*
Z01 DE00345-02	Michael F. Cole	Natural Transmission of *Streptococcus mutans* in Rats
Z01 DE00346-02	Michael F. Cole	Rapid Purification of Rat SIgA & IgM
Z01 DE00348-02	Wayne A. Little	Purification of Cell Protein Antigens
Z01 DE00349-02	Joseph E. Ciardi	Effect of Sugar Substitutes on Bacterial Growth
Z01 DE00350-02	M. Curtis	Quantitation of Peptostreptococci in Dental Plaque
Z01 DE00351-02	M. Curtis	Protein Reduction in Dental Plaque
Z01 DE00352-02	M. Curtis	Metabolism of the Amino Acid Fermenting Organisms
Z01 DE00353-02	Albert Kingman	More Efficient Use of Initial DMFS
Z01 DE00354-02	S. Li	Statistical Methods of Analyzing Microbiological Changes in Plaque
Z01 DE00357-02	Joseph E. Ciardi	Hydrophobic Interactions
Z01 DE00358-01	Albert Kingman	Stratification Methods in Caries Clinical Trials

Z01 DE00364-01	S. Li	Small Behavior of Standard Deviation for Kaplin-Meier Estimator
Z01 DE00365-01	Horace Stiles	Effect of A Non-sucrose Sweetener on Rat Caries
Z01 DE00367-01	Michael F. Cole	Methods to Assay Glucosyltransferase (GTF) and Glucans
Z01 DE00368-01	M. Curtis	Kinetics of Acid Formation in Dental Plaque
Z01 DE00369-01	Stanley A. Robrish	Characteristics of Cervical and Coronal Dental Plaque
Z01 DE00370-01	Christopher W. Kemp	Amino Acid Metabolism in Binary Cultures of *S. mutans* and *S. sanguis*
Z01 DE00371-01	Wayne A. Little	Effect of Antisera on Glucose Uptake of *S. mutans* 6715

Extramural Program

EXTRAMURAL PROGRAMS

National Institute of Dental Research

October 1, 1982 - September 30, 1983

REPORT OF THE ASSOCIATE DIRECTOR

The Extramural Programs of the National Institute of Dental Research support a wide spectrum of research ranging from laboratory investigations on the basic causes of oral diseases to clinical trials of new methods of treatment and prevention. This broad array of scientific activity is divided into five categorical programs, each supporting a specific area of dental research, and a non-categorical program which consists of five university-based Dental Research Institutes and Centers conducting research in the entire field of oral biology. Continuing accomplishments in these programs have set the stage for an expansion in clinically oriented research and research training. The progress achieved during the past year is reflected in this report.

The goal of the Periodontal Diseases Program is to eradicate periodontal diseases, which pose a major threat to human health throughout the world. To achieve this goal, the Program supports research on the cause, nature, diagnosis, treatment and prevention of these diseases. Because the etiology of periodontal diseases is multifactorial, the research includes microbiologic studies to identify the suspected pathogens, immunologic studies on the host response, as well as studies to develop new therapeutic approaches. Emphasis is placed on anaerobic bacteria, on the cellular and biochemical mechanisms involved in inflammation and tissue destruction and on clinical pharmacology. Coordinated clinical and laboratory studies are carried out in three clinical periodontal research centers established since FY 1977.

The Craniofacial Anomalies Program Branch supports research and training in research related to the etiology, prevention, diagnosis, and treatment of craniofacial anomalies. Studies on basic mechanisms controlling craniofacial growth and development provide a foundation for understanding the cause and prevention of oral clefts and other congenital malformations. Clinical research is directed at improving the treatment of these conditions. Basic and clinical research on craniofacial defects and disfigurement resulting from injury is also a major concern of the Program. A third area of activity involves malocclusion and related functional problems.

The Soft Tissue Stomatology and Nutrition Program Branch supports research in oral soft tissue diseases, nutrition, salivary glands and their secretions, and mineralization. The main objectives are to obtain knowledge of a) the etiology, diagnosis, treatment, and prevention of oral soft tissue diseases and disorders, b) the role of nutrition in the growth, maintenance, function and health of hard and soft tissues of the craniofacial complex, c) the development and function of normal and abnormal salivary glands and their secretions, and d) the mechanisms of mineralization, with special emphasis on the cells and regulatory systems which affect the structure, function and repair of bones and teeth.

The Pain Control and Behavioral Studies Program supports research to increase our knowledge of dental and orofacial pain and of the behavioral and social factors involved in dental health and disease. Since the human pain response is a complex multifactorial experience, pain research in humans requires multidisciplinary approaches which consider individual, cultural and societal factors as well as physiologic parameters. The behavioral and social research includes studies of attitudes and behaviors related to the prevention and treatment of dental disorders and studies of the psychosocial impacts of certain orofacial diseases and therapies. In addition, the program supports research on oral-facial motor function and on taste and smell.

The Restorative Materials Program supports research and development in dental biomaterials and instrumentation. These research areas include: 1) restorative filling materials; 2) bonding agents, adhesive coatings and cements to prevent decay; 3) intraoral prostheses to replace teeth, maxillofacial prostheses to replace extraoral defects; 4) artificial tooth implants to replace teeth and to serve as anchors for bridges and dentures; 5) materials and techniques for improved root canal therapy; 6) transplants and replants of natural teeth; 7) diagnostic equipment and devices.

FY 1983 FUNDING (Based on year-end estimates)

Research

During FY 1983 the NIDR Extramural Programs awarded research funds of approximately $40.0 million, which included $32.1 million for research grants and

career awards, $349 thousand for contract research by two of the five categorical programs, and $7.6 million for the 5 university-based Dental Research Institutes and Centers. (These figures do not include grants and contracts by the National Caries Program.) Altogether, the Extramural Programs made 373 grant awards: 343 for research projects, 4 for scientific conferences, 5 for the university-based Dental Research Institutes and Centers, 3 for periodontal clinical research centers, 17 for research career development awards, and one for a research career award. Of the 343 project awards, 242 were made for regular research grants (RO1), 9 for program projects (PO1), 43 for new investigator awards (R23), 46 for small research grants (R03), and 3 for small business innovative research (SBIR) grants (R43).

In FY 1983 nearly 35% of the research grant funds was awarded for new grants and competing renewals and slightly more than 65% was awarded for noncompeting continuations and supplemental grants. The new awards included 48 regular grants, 15 new investigator awards, 38 small grants and 3 small business awards. The competing renewals included 34 regular grants, 5 program projects, one periodontal clinical research center and one noncategorical center.

Overall, there was a 9.6% increase in NIDR-EP research funding during FY 1983. Increases ranging from 12-16% were seen in the Periodontal Diseases, Soft Tissue Stomatology and Restorative Materials Programs and an increase of 28% was evident in the Pain and Behavioral Studies Program. On the other hand, the Craniofacial and Centers Programs sustained declines of 5.5% and 7.2%, respectively.

Training

In FY 1983, NIDR awarded research training funds estimated at $3.87 million, which represents an increase of 9.6% over the FY 1982 level of $3.5 million. A total of 87 awards were made, including 42 individual fellowships, 26 institutional fellowship grants, 2 senior fellowships and 16 short-term training grants to dental schools, and one minority award. The minority award supported one student under the Minorities Biomedical Support (MBS) Program through an agreement with the NIH Division of Research Resources. The 26 institutional grants provided 23 predoctoral and 122 postdoctoral trainee positions, and the 16 short-term grants supported 102 fellows. In addition, the National Institute on Aging awarded 8 Geriatric Dentistry Grants to dental schools.

RESEARCH HIGHLIGHTS

Clinical Periodontology

Evidence to confirm the possible cyclic nature of periodontal disease activity has recently refocused on the old question of whether bacteria and/or bacterial products invade the periodontal tissues. Clinical investigators at SUNY, Buffalo studied the effectiveness of subgingival scaling and root planing (excluding soft tissue curettage) in removing *Actinobacillus actinomycetemcomitans (Aa.)* from the deep pockets of patients with localized juvenile periodontitis (LJP). Even after two to six weeks following scaling and root planing procedures, these investigators found evidence for the presence of *Aa.* in surgically removed periodontal soft tissue specimens. The tissues were examined by immunofluoresence, light and electron microscopy. The use of crystal violet and silver staining techniques suggested the presence of bacteria in the connective tissues, especially near the pocket epithelium and between the numerous plasma cells. Immunofluorescent staining demonstrated granular and patchy tissue-bound IgG, and to a lesser extent IgA, in the connective tissue. Antisera specific for *Aa.* reacted with bacteria-like structures in the gingival tissue of all biopsies, and *Aa.* antigens were detected in the connective tissues and in cells resembling phagocytes deep in the gingival tissues. One of the serotypes of *Aa.* detected in the tissues corresponded to the serotype of *Aa.* cultured from the same patient's pocket.

These studies at SUNY, Buffalo suggest that *Aa.* is present in the gingiva of LJP lesions and may explain why soft tissue debridement and/or surgical excision of the gingival connective tissue, together with root planing may result in the elimination of cultivable *Aa.* from the subgingival pocket area, whereas root planing alone is less reliable. The new findings suggest that since root planing does not effectively remove connective tissue, this treatment regime may leave a reservoir of organisms in the gingival connective tissue. Furthermore, the success of systemic tetracycline administration in eliminating *Aa.* from periodontal lesions may also be explained by the concept of a gingival connective tissue reservoir of the organisms.

In studies on the clinical microbiology of different periodontal diseases, investigators at Forsyth Dental Center obtained plaque samples from patients with healthy gingiva, gingivitis, adult periodontitis, and localized juvenile periodontitis (LJP) as well as healthy control sites in individuals with LJP and adult periodontitis. *Eikenella corrodens* was elevated in number in all 3 disease conditions. *Capnocytophaga gingivalis* was strongly associated with gingivitis. *C. ochracea* and *Aa.* were strongly associated with LJP sites. Darkfield

microscopy indicated that motile forms including spirochetes were elevated in all periodontal disease states including gingivitis ($p < 0.01$) but only minimal differences in the microbiota could be observed by darkfield microscopy between samples taken from the different forms of destructive periodontal disease. The presence of spirochetes and motile rods showed a very high positive correlation with the depth of pockets. This result suggests that these organisms may become inhabitants of the pockets only after the pockets have formed and favorable conditions characteristic of deep pockets have developed. According to this interpretation, the motile forms would play no role in pocket formation itself.

In one study the Forsyth investigators focused on adult periodontitis with suppurative lesions. In this clinical condition, *Bacteriodes intermedius* was significantly elevated in diseased sites of all 10 patients studied. None of the other black pigmented *Bacteroides* species were significantly different in their proportions between healthy and diseased sites.

In a clinical study at the University of Connecticut, additional evidence for the role of complement in periodontitis was obtained from a recently completed longitudinal study. Thirty-seven total sites in 9 patients were assessed prior to and after treatment of periodontitis by root planing and plaque control. Following treatment, a significant reduction of clinical parameters of disease was observed. Paralleling this reduction in clinical inflammation was a significant reduction in the presence of complement activation products. These studies suggest that activation of complement may be an important pathogenic mechanism in periodontal disease.

Studies by investigators at SUNY, Stony Brook have led to the development of a new therapeutic concept that tetracyclines are effective against periodontal disease, not only because they suppress pathogenic bacteria but also because they directly inhibit tissue collagenase activity. The results show that tetracycline suppresses the excessive collagen degradation which occurs during periodontal disease, and also suppresses collagen breakdown in other diseases such as parathyroid hormone induced bone resorption as well. The investigators have preliminary data from studies in which tetracycline (minocycline) was administered orally, on a daily basis, to diabetic and control rats for 3 weeks. The rat gingiva was incubated in tissue culture on ^{14}C glycine-labeled collagen fibrils. After a 3-day incubation, collagenolytic enzyme activity of the gingival tissue was assessed by measuring the release of radiolabeled collagen degradation products. The gingiva from the untreated diabetic rats produced significantly elevated collagenolytic activity compared to gingival tissues from the control rats. However, treating the diabetic rats with minocycline reduced gingival collagenolytic activity by 62%. When this experiment was repeated under germfree conditions, similar results were obtained. Gingival collagenolytic activity was abnormally elevated in the germfree diabetics, and minocycline treatment inhibited it. Since this treatment effect occurred in the absence of bacteria, the tetracyclines apparently decreased tissue collagenase activity by a mechanism independent of the drug's antibacterial efficacy.

These findings on diabetic rats caused the Stony Brook investigators to reinterpret a previously described case report on a 13 year old diabetic girl with severe periodontal disease. This diabetic patient had exhibited severe periodontal destruction around the anterior teeth and unusually high collagenase activity in her gingival crevicular fluid (GCF). After 10 days of only tetracycline (doxycycline) therapy, GCF collagenase activity was reduced about 50% and stayed relatively low for at least 4 months during which time she was treated by repeated scalings but not with additional tetracycline. At that time the success of this treatment was attributed to the suppression of the Gram-negative flora in the gingival crevice by tetracycline and the repeated scalings. However, the investigators now believe that the successful result was due in part to direct collagenase inhibition by the tetracycline as well as to bacterial suppression. These promising preliminary data on rats and humans suggest future clinical uses for this new therapeutic rationale of inhibiting gingival collagenase activity.

Periodontal Microbiology

One of the more important research contributions in periodontal microbiology has been the evidence for microbial specificity in the etiology of the periodontal diseases. Over the past year, investigators at the University of Pennsylvania have focused on one of these bacteria, *Actinobacillus actinomycetemcomitans (Aa.)*, which is now considered to be the causative agent of localized juvenile periodontitis (LJP). *Aa.* is consistently found in LJP lesions but usually absent or in reduced numbers in normal patients or in patients with other periodontal diseases. Previous studies have shown that this organism produces a protein (a leukotoxin) capable of killing human white blood cells. During the past year work at Pennsylvania resulted in the isolation and characterization of this substance. This isolated leukotoxin is antigenic and monospecific antibodies have been produced to it; the leukotoxin reacts with antibodies in antisera from virtually all LJP patients and *Aa.*-infected monkeys. In addition to producing the leukotoxin, *Aa.* also produces another substance that is toxic to human fibroblasts, the cells that form the connective tissue attachment of teeth to the surrounding bone. Unlike the leukotoxin, the fibroblast toxin (called

PIF or Proliferation Inhibitory Factor) does not kill the fibroblasts but prevents their reproduction. This effect of the toxin appears to be irreversible; once exposed to the substance, the fibroblasts cannot recover to continue reproduction even after the toxic material is removed. The results suggest that the reproduction inhibition is due to a direct effect of PIF on the fibroblast's gene replication process (inhibition of DNA synthesis). PIF, also a protein, has just recently been isolated and is currently being characterized. Not only do *Aa.* products have the potential to adversely influence human cells, but also to suppress the growth of other plaque bacteria. For example, *Streptococcus sanguis, Actinomyces viscosus* and *Bacteroides gingivalis,* three organisms often found growing in plaque on tooth surfaces are killed in the presence of this product (tentatively termed Actinobacillus Inhibitory Factor). This observation may help to explain the fact that periodontal lesions harboring *Aa.* often have minimal plaque deposits and decreased numbers of many other commonly found oral bacteria.

An unexpected but potentially significant observation was made by University of Massachusetts investigators when they undertook growth studies of *Aa.* in an attempt to increase cell yield. Since acetate is an end-product of metabolism of many anaerobic microbiota which inhabit periodontal pockets, the investigators reasoned that acetate might supply needed carbon and some additional energy for growth. Accordingly, they increased the concentration of sodium acetate in the *Actinobacillus* synthetic medium. As a result, the organisms produced a lipopolysaccharide (SA-LPS) which was toxic to macrophages. At 50 ug SA-LPS/10^6 macrophages, essentially all of the macrophages were killed within 9 hours. Even at 5 ug SA-LPS/10^6 macrophages, 40% were killed within 9h and the remaining macrophages were damaged. In contrast, 80-100% of control macrophages were viable at 24 hours, and at least 70-85% were viable at 72 hours. The reason for this unprecedented increase in LPS cytotoxicty in the presence of such small amounts of acetate is unknown; however, preliminary analysis by gas-liquid chromatography of the Lipid A from the *Aa.* revealed both qualitative and quantitative alterations. As a result of these studies, another potentially pathogenic mechanism can be linked to *Aa.*

A team of investigators at SUNY, Buffalo are studying *Bacteroides gingivalis (Bg),* an organism implicated as a pathogen in adult periodontitis. As a first step, this team had previously established the colonization potential of *Bg.* Next, they wished to determine whether the bacteria or its products had invasive properties. All test strains of *Bg.* studied at SUNY, Buffalo have demonstrated strong positive fibrinolysis, whereas strains of black-pigmented *Bacteroides* including *intermedius, melaninogenicus, denticola, loescheii, asaccharolyticus,* and *levii* demonstrated negative to weak fibrinolytic activity as did closely related non-pigmented *Bacteroides* strains from the oral cavity. Hence, *Bg.* is unique among the oral black-pigmented *Bacteroides* in elaborating one or more enzymes that strongly catalyze fibrin. By effectively dissolving or lysing a fibrin barrier, this fibrinolytic activity may enable *Bg.* or its products to invade the gingival connective tissue.

Periodontal Immunology

In their studies on the role of lymphocytes, investigators at the University of Pennsylvania have identified still another potential periodontal disease mechanism related to the organism *Aa.* The Pennsylvania investigators have been interested in the interactions of lymphocytes with bacteria believed to cause periodontal tissue destruction. Specifically, they have studied the ability of these microorganisms to alter the response of the lymphocytes to antigenic and mitogenic stimuli. Recently they demonstrated that several bacteria including *Aa.* (associated with juvenile periodontitis), *F. nucleatum,* and the small spirochete *Treponema denticola* (the latter two associated with adult periodontal disease) can inhibit several lymphocyte functions in vitro. The Pennsylvania studies have concentrated on the characterization and purification of the inhibitory substance present in *Aa.* The investigators expect to complete purification and biochemical characterization during the coming year. Meanwhile, they have utilized the partially purified preparations to initiate studies on its mode of action. Preliminary findings in vitro suggest that *Aa.* is able to inhibit lymphocyte responses in a unique way by activating a subpopulation of regulatory lymphocytes, the T-suppressor cells. When these cells are activated they suppress other lymphocyte responses involved in immunologic protection, including cell mediated and humoral immunity. If these bacterial products initiate the same events in vivo as they do in vitro, an infected individual might develop a state of immunologic incompetence at least in the local area, and local defenses against infection by both pathogenic and opportunistic periodontal organisms might be severely compromised. These findings are particularly relevant since impaired host defenses are known to be present in some patients with periodontal disease.

University of Florida investigators are developing vaccines to control colonization onto tooth surfaces by bacteria associated with periodontal disease. They had determined earlier that the adherence (adsorption) of *Actinomyces viscosus* strain T14V is mediated by cell surface fibrils designated as Type 1. After isolating and purifying these Type 1 fibrils, they produced specific salivary antibody to the Type 1 fibrils for in vitro experiments. Subsequently, they showed that not only

did anti-type 1 salivary immunoglobulin (IgG) inhibit the attachment of *A. viscosus* to tooth surface crystals, but it also detached bacterial cells that were already adhering to the crystals.

In *in vivo* studies, the Florida investigators vaccinated mice to protect them against subsequent infections with *A. viscosus* cells. Preliminary results showed that mice immunized with a mixture of fibrillar antigen became resistant to infection by T14V strains of *A. viscosus*. Repeated testing showed that fewer immunized animals became infected than controls. These experiments suggest that it may be possible to prevent periodontal infections by immunizing specifically against antigens which mediate adsorption of bacteria to teeth.

Bone Metabolism in Periodontal Disease

To gain a better understanding of bone loss associated with chronic periodontitis, researchers at the University of Southern California have been investigating mechanisms of macrophage-mediated bone resorption. Their observations, as well as those of investigators at Washington University and the Jewish Hospital at St. Louis have suggested that the macrophage participates in the process of bone resorption either as a precursor to or as an auxiliary for the osteoclastic cell.

The USC scientists have focused on what mechanism regulates the recruitment of macrophages from the circulation to the bone prior to resorption. Recently, they have discovered a new factor present in extracts of bone as well as macrophage culture media that elicits chemotaxis (directed locomotion) in the macrophages. This factor, termed BDCF (bone-derived chemotactic factor) is found in more abundance in resorbing bones than in non-resorbing bones, and does not appear to be collagenous. The migration response of macrophages to BDCF is modulated by prostaglandin E_2 (PGE_2) such that low levels of PGE_2 augment chemotaxis, and higher levels of this mediator inhibit chemotaxis. Since it is known that bone synthesizes prostaglandins when it is stimulated to resorb by certain agents associated with inflammation, such as complement components, these investigators have advanced the following hypothesis. There exists in bone a local regulatory mechanism in which locally produced BDCF and prostaglandins regulate the recruitment of macrophages from the circulation and control their participation in the destruction of bone. This mechanism becomes particularly involved in chronic inflammation which stimulates bone cells to produce increased amounts of these factors.

The USC group is actively pursuing the identity of the BDCF. Chemotaxis is assayed using a modification of the Boyden chamber method with mouse peritoneal macrophages as the test cell. The BDCF activity appears as a low molecular weight molecule (10 - 20K daltons) in EDTA extracts of bone and does not appear to be related to either collagen or to the gammacarboxyglutamic acid-containing protein of bone. BDCF is also extractable from bone using lipid solvents. It is not clear at this time whether the BDCF activity from bone extract and that from culture medium represent identical molecules. The next stage of this project is to purify and identify this potentially important mediator of macrophage-mediated bone resorption.

Investigators at the University of Washington have studied the relationship between the severity of osteoporosis and alveolar bone density and height in edentulous and dentulous post-menopausal women. Osteoporosis was assessed by 125 I photon absorption of wrist and spinal bones and by total body calcium; mandibular bone density was determined by microdensitometry. In a pilot study of 30 patients, it was found that the microdensitometry technique has a high degree of precision. The data indicate that there is a significant correlation between skeletal osteoporosis and mandibular bone density. There appeared to be no relationship, however, between the severity of skeletal osteoporosis and the amount of mandibular bone loss in height in edentulous subjects. Likewise, there was no correlation between skeletal osteoporosis and periodontal bone loss.

Investigators at the University of Minnesota have examined the effect of various dietary Ca:P ratios ranging from 1:3 to 3:1 on the alveolar bone and general skeletal bone of male STR/N mice during a twelve-month experimental period. Alveolar bone loss was unaffected by either the calcium content of the diet or the dietary calcium to phosphorus ratio. In contrast, femur mass was reduced in all groups except the one receiving the diet with a Ca:P ratio of 3:1. Up to 20% net loss was experienced by the group receiving the diet with the calcium to phosphorus ratio of 1:3. The results indicate that under these conditions alveolar bone loss was unrelated to generalized skeletal disturbances relating to calcium and phosphate nutrition.

Previous work indicated that purified bone morphogenetic protein (BMP) is effective in inducing bony healing of trephined defects in the skulls of adult rats. These defects do not spontaneously heal. It appears that in an adult animal the obstacle to healing is lack of cell differentiation. The effect of the BMP was to stimulate mesenchymal cells which normally produce only fibrous tissues to differentiate into cartilage and bone instead. BMP has been isolated from demineralized dentin matrix of the rabbit, from rat, rabbit, bovine and human bone matrix and from mouse and human

osteosarcoma. Experimentally, BMP has been found to cause cartilage and bone formation in a muscle pouch by diffusion across as many as five millipore membranes (up to 450 micrometers) with pore sizes as small as 25 nanometers. Physiologically, it has been found that BMP activity is low in rickets, in lathyrism and in post-menopausal women with osteoporosis, but is unaffected by scurvy.

Herpes Labialis

Investigators at the Medical College of Georgia are testing two anti-viral drugs vidarabine phosphate (ARA-AMP) and Acyclovir (ACV) for the treatment of herpes labialis. Since ordinary topical applications of anti-viral drugs fail, presumably due to lack of skin penetration, the method being studied uses a weak electrical current to carry the drug into the tissues (iontophoresis). To date, all Phase I studies for allergic and systemic effects of the drug applied in this manner have been negative, and Phase II (open studies) feasibility studies to determine optimal electric current time and drug concentrations have been completed. In these preliminary investigations the results were impressive. Using ARA-AMP 5 out of 10 subjects were virus free after 24 hours and 8 were virus free after 48 hours. ACV was less effective in producing negative cultures by 48 hours (2 of 6) but healing time was considerably reduced for both drugs compared to healing time in control subjects receiving sodium chloride. There was also a decrease in lesion area and reduction in pain soon after ACV treatment but pain loss after 24 hours was best achieved with ARA-AMP. Phase III (double blind) studies of herpes oralabialis and cutaneous herpes treatment by iontophoresis have been started.

Smokeless Tobacco

Investigators at the University of Colorado examined over 1,000 teenagers in the Denver Public School System in grades 9 through 12 to determine the prevalence and frequency of the use of smokeless tobacco and to determine the oral tissue alterations associated with such use. Slightly over 10% of the sample admitted to using smokeless tobacco, and 97% of these were male. Nearly 50% of users examined had oral hard or soft tissue lesions directly in the area of quid placement. The vast majority of mucosal lesions were white, corrugated and raised. In addition, 7 individuals had specific gingival recession with apical migration of the gingiva to the CE junction or beyond without accompanying mucosal lesions. Twenty-three individuals had a combination of mucosal lesions and periodontal involvement. One case of severe cervical root erosion was also identified. On the average, smokeless tobacco users with oral sequelae had exposed their tissues for approximately 3 hours per day for slightly more than 3 years.

Developmental Biology

The neural crest consists of mesenchyme formed early in embryonic development from epithelium at the lateral margins of the neural plate. This important mesenchyme is then distributed throughout the head where it subsequently forms bones, cartilages and connective tissue of the face and neck, the dentine of the teeth, and major elements of the peripheral sensory ganglia.

A good deal is known about which adult tissues are derived from neural crest mesenchyme but a number of important questions remain to be investigated. These include: How the cells escape from the epithelium; what factors control their migration; what determines their distribution in the craniofacial region; and finally, what are the mechanisms controlling differentiation into their diverse derivatives.

Scientists at Creighton University have focused on the question of how neural crest cells escape from the epithelium in the region of the neural plate to begin their migration. The escape appears to be related to two processes — disruption of the organization of the epithelium and changes in the secretion of extracellular matrix constituents such as collagen, glycoproteins and glycosaminoglycans. A key element in reorganization of the epithelium has been shown to be a molecular interaction between cells of the epithelium and the subjacent layer of extracellular material, the basal lamina. Work by the investigators at Creighton has shown that disruption of the basal lamina is non-uniform and highly localized. They found that certain neural crest cells which penetrated the basal lamina precociously, disrupted it rapidly and completely, whereas adjacent cells slowly eroded patches of basal lamina. These latter cells only gradually assumed the characteristic morphology of crest mesenchyme.

Little is known of the timing of the switch which must occur to produce changes in the secretion of extracellular matrix constituents during neural crest formation; nor is there information on the significance of these constituents in crest formation. These problems are being addressed by promising studies in which specifically labeled antibodies are being used to identify matrix materials associated with the forming neural crest mesenchyme.

Once formed, crest mesenchyme must be distributed to appropriate sites. In recent literature, distribution is described as resulting from individual cell motility — cells crawling on a substrate. However, results from these investigations have shown two other mechanisms

to be factors. First, during crest formation, large amounts of the glycosaminoglycan hyaluronic acid are secreted into the spaces surrounding crest mesenchyme. Because of its charge properties, this hyaluronic acid absorbs water and swells dramatically. Crest mesenchyme cells embedded in this swelling matrix move with and are distributed by the matrix. In the case of the crest mesenchyme which forms the jaws, distribution is a largely passive process. The mesenchyme itself remains stationary, but its relative position changes because the surrounding structures move.

Researchers at the University of California, Davis, have been investigating some of these same questions in mice. They found that in *splotch*, a mutant with a central nervous system malformation, neural crest cells do not migrate from the regions where the spina bifida occurs. This model provides another approach to studying factors responsible for the initiation of crest cell migration. These investigators are determining the chemical composition of the basal lamina around the neural tube in both normal and mutant mice using antibody localization techniques.

During epithelial — mesenchymal interactions associated with tooth development, inner enamel organ epithelial cells (ameloblasts) are induced to synthesize and secrete a group of enamel extracellular matrix proteins. Two classes of enamel proteins have thus far been identified — enamelins and amelogenins. Amelogenins constitute about 90 percent of total enamel matrix proteins.

Recently, investigators at the University of Southern California cloned the complementary DNA (cDNA) for mouse amelogenin. In brief, the investigators first isolated and partially characterized the major mouse amelogenin proteins. Then, they were able to make polyclonal antibodies against these proteins. In turn, these antibodies were used to identify the messenger RNAs (mRNAs) for enamel proteins from the mouse enamel organ epithelia. Then, the investigators were able to produce cDNAs against these mRNAs, and finally, select the specific cDNA for a major amelogenin. This is the first time that a "dental" gene has been constructed and identified. The implications for future dental research, particularly in regard to problems of enamel gene expression, like amelogenesis imperfecta, are potentially great.

Previously reported work on the use of adenovirus type 2 as a probe for studying keratinocyte differentiation and maturation have been expanded. Under culture conditions keratinocytes do not undergo complete differentiation and thus the virus persists in the cell as a stable non-integrated epitome. This blockage of viral reproduction occurs at least at one and possibly two sites. First, viral DNA replication appears to be linked to cellular replication. During keratinocyte differentiation which follows cessation of replication, there is no increase in level of viral DNA relative to cellular DNA. Second, studies of viral mRNA indicate a relative absence of cytoplasmic viral RNA suggesting that during the differentiation stage a block in the transport of viral RNA from the nucleus may exist.

Trainees on the nutrition training grant at the University of Wisconsin have developed technology which make it possible to identify several hundred individual mRNA species from a typical eukaryotic tissue. Methods include new procedures for isolating RNA, in vitro translation technology, two-dimensional polyacrylamide gel electrophoresis (PAGE) and the use of quantitative fluorograms. Detection of an individual mRNA species accounting for as little as 0.01% of the total translatable mRNA is routinely achieved. This methodology will be applied to studies of hormonally regulated changes in mRNA in various tissues.

Cleft Lip and Palate

Knowledge of embryonic palate development is important in understanding the pathogenesis of cleft palate and the development of other embryonic facial structures derived through similar complex tissue interactions. The palate forms from two extensions of the maxillary processes which initially grow down on either side of the tongue. These shelves later remodel and reorient in a horizontal position above the tongue where they fuse with each other.

The palatal shelves are composed of a core of mesenchyme cells surrounded by collagen and other large molecules including glycosaminoglycans. They are covered with epithelium which rests on a basal lamina. Scientists at the University of Michigan are currently investigating how the interactions of these cells and molecules are responsible for shelf reorientation prior to fusion. They previously showed that some areas of the epithelial perimeter of the shelf increase in cell density during remodeling whereas other areas do not. These have been designated as morphogenetically active and inactive areas.

Current studies have shown that during shelf reorientation, the morphogenetically active areas of the posterior two-thirds of the palatal shelf contain an increased number of mesenchymal cell processes and cell bodies which closely approach the lower surface of the basal lamina. In contrast, neighboring inactive regions show no change in the number, distribution or location of mesenchymal cell processes. Studies of palatal shelves from drug-treated embryos with altered glycosaminoglycans indicate a significantly different

relationship between the mesenchymal cells and the basal lamina. These results suggest that local changes in the epithelial cell-basal lamina-mesenchymal cell relationships may underly changes in epithelial cell density and thus may play an important role in shelf elevation.

At the University of North Carolina, researchers have identified and quantitated the number of gap junctions in mesenchyme throughout the developing maxillary process and roof of the stomodeum of the chick embryo. Gap junctions are structures on the surface of adjacent cells thought to provide a means of intercellular communication. Gap junctions were found in all the regions examined but differences were found in their number. These quantitative differences were correlated with previously determined rates of cell proliferation of these regions, but the number of gap junctions were not simply the a result of cell density, nor was their distribution related to blood vessels. The number of gap junctions was markedly higher in regions adjacent to the epithelium compared to the interior of the maxillary process. These data suggest that the distribution of gap junctions is related to proliferative rate and that epithelial mesenchymal interaction may be significant in establishing this pattern.

The operation of a metabolic gradient has often been cited as a mechanism for mediating developmental events. Metabolic coupling of cells by means of gap junctions would be a direct means for establishing gradients. Differences in the number of gap junctions, their distribution, or functional state could provide the means for directing, amplifying, or regulating the steepness of a gradient.

Inbred mouse strains are known to vary in their susceptibility to teratogen-induced cleft palate. The A/J strain for example, is much more susceptible to cleft palate induction by glucocorticoid than is the C57BL/6J. Recent studies have shown that the genetically determined sensitivity of A/J and C57 strains of mice to glucocorticoid-induced cleft palate can be altered by diet. High fat content in the diet increases the susceptibility of both strains to induced cleft palate. During the past year, the investigators at the University of North Carolina have obtained results which suggest a mechanism to explain this effect. First, they showed that in mice on a normal diet, liver glucose 6 phosphate dehydrogenase (G6PD) activity is lower in the susceptible A/J mice than in the C57 mice. Second, they found that in both strains of mice, a high fat diet produced G6PD activity many times lower than levels found in animals on low fat diets.

The cellular activity of G6PD is known to regulate, in part, cellular NADPH (co-factor) levels. A decrease in G6PD activity lowers tissue levels of G6PD-generated NADPH which in turn plays a role in the activity of the cytochrome P-450 mediated monooxygenase enzyme system responsible for the metabolism of a variety of drugs. Thus, environmental factors which alter cofactor or monooxygenase activity could reduce the capacity of the liver to metabolize noxious substances and therefore increase susceptibility to drug-induced malformations such as cleft palate.

Malocclusion

Altered respiration accompanying nasal obstruction and mouth breathing may influence growth, affect facial morphology and produce malocclusion. Also, changes in size and shape of the oral and nasal cavities produced by orthognathic surgery may produce neuromuscular changes which can profoundly influence the stability and ultimate success of treatment.

Researchers at the University of California in San Francisco have been concerned with the relationship between alterations in neuromuscular patterns and changes in craniofacial morphology. In previous studies, the nasal airway of rhesus monkeys was mechanically blocked for two years. The resulting oral respiration produced changes in recruitment patterns of craniofacial muscles as well as marked skeletal changes and dental malocclusion.

Current studies involve the neuromuscular and skeletal recovery patterns after removal of nasal blockage. Within a week after removal of the nose plugs, a return toward normal activity in the oral and facial musculature was observed. After 18 months, however, certain altered muscle patterns remained and the skeletal changes and dental malocclusion induced by mouth breathing remained.

In other studies, acrylic wedges were placed over the palate of monkeys to reduce the size of the oral cavity and to alter tactile stimulation and sensory feedback from the tongue. Immediate effects were observed. The animals responded by lowering their mandibles and protruding their tongues. Increased tonicity and other changes were recorded in the oral and facial muscles. Since these studies have been active for only 6 months, no skeletal changes have yet been observed.

In future studies palatal wedges will be placed and the tongue will be denervated. This will provide information on whether the mandibular response to reduced oral space depends on tactile stimulation to the tongue or on proprioceptive feedback from the masticatory muscles and the temporomandibular joint.

Researchers at the University of Connecticut are investigating the potential role of prostaglandins as mediators of mechanical stresses applied to bone undergoing remodeling. Tensile forces were applied to osteoblastic cells cultured on collagen ribbons by stretching the ribbons in a controlled fashion. The initial studies showed that a 5-10 percent elongation of the collagen ribbon caused a 3-4-fold increase in the rate of prostaglandin synthesis in the attached osteoblasts. In this system the ribbon simulates the periosteum or periodontal ligament. The results so far support the hypothesis that prostaglandins mediate the mechanical effects on bone remodeling.

Investigators at Washington University in St. Louis have been trying to optimize the osteoinductive potential of composite bone grafts — grafts of allogenic bone plus autologous marrow. Usually, the osteogenic performance of the composite graft is equal to that of an autologous bone marrow graft, but it performs better than the autograft if the hosts are treated with the lectin phytohemaglutinin (PHA-P), a T-lymphocyte mitogen. Studies indicate that the mitogen PHA-P does not directly influence the proliferation of osteoprogenitor cells or influence the differentiation of these elements into osteogenic and chondrogenic cells. Rather, the efficacy of PHA-P appears related to its ability to promote resorption of the bony substrate of the graft and liberate matrix-bound osteoinductive glycoproteins.

Salivary Secretions

Investigators at the University of Missouri are studying the role of calmodulin, a protein known to regulate Ca^{++} in other systems, in regulating salivary gland intracellular Ca^{++} concentration and thus enzymatic activities which mediate secretory responses. In other tissues calmodulin has been shown to regulate ATPase activity and thus intracellular Ca^{++} concentration. In initial studies it was determined that the concentration of calmodulin was relatively high and exhibited similar distribution patterns in rat submandibular gland acini and exocrine pancreas. Compared to the richest sources of calmodulin known, rat submandibular gland contained approximately 1/3 of that found in rat brain and testes. Approximately 70% of the calmodulin in the two tissues studies was found in the intracellular fluid. The remainder was membrane bound in a Ca^{++} dependent manner. In related physiologic studies these investigators obtained evidence for the presence of a calcium ATPase which is stimulated by Ca^{++} in submicromolar concentrations. This specific ATPase is also affected by manipulation of membrane calmodulin; thus the tentative conclusion has been drawn that rat submandibular gland contains the enzymatic basis of a Ca^{++} "pump."

Dental Innervation

Neuroanatomical investigations at the University of Washington in Seattle continue to be productive. Recent studies of normal teeth show that more than 50% of the dentin tubules adjacent to the pulp horn in cat and monkey teeth are innervated by trigeminal sensory receptors, whereas greatly reduced innervation is found in mid-coronal and cervical regions. Since the number of receptors is greatest where the heaviest occlusal forces are expected, these findings suggest that sensory receptors in dentin may react to mechanical stimulation of dental cusps and protect teeth from excessive occlusal force. The investigators also identified sensory axons in root dentin, especially at the buccal and lingual poles where the predentin is wide. This finding may explain the painful sensitivity of exposed roots.

Finally, by electron microscopic autoradiography, it was demonstrated that no gap junctions exist between odontoblasts and sensory nerve endings in monkey as well as in rat teeth. This finding is opposed to the widely held view that odontoblasts are functionally coupled to sensory nerve endings.

Clinical Pain Studies

Investigators at the University of California at San Francisco continue to report progress in studies whose immediate goal is to develop an optimal approach to the management of pain in the dental postoperative patient. These studies have already developed a reliable human paradigm for studying clinical pain and generating improved quantitative methods for pain measurement.

An earlier series of experimental findings suggested that the surgical removal of impacted wisdom teeth is associated with the release of an endogenous morphine-like compound and that the analgesic effect of placebo administration is due to the action of these endogenously released opioids. More recent studies have shown that these earlier conclusions, while still basically valid, do not apply to every experimental situation. It is becoming increasingly clear that sorting out the complex psychological, neurophysiological and neurochemical factors involved in the human response to pain is an extremely demanding and difficult process.

Oral-Facial Motor Function

A research project at the University of California at Los Angeles is studying the central nervous system organization of motor activities involved in chewing movements. Two major findings have been reported during the past year. In guinea pigs, stimulation of special sites in the midline raphe nucleus of the brainstem blocked the rhythmic jaw movements evoked

by activation of the masticatory area of the cerebral cortex. Neurons in this nucleus are believed to be serotonergic. To demonstrate the involvement of serotonin more definitively, scientists first injected a known serotonin agonist, quizapine, which blocked the chewing movements produced by cerebral cortex stimulation just as the midline nucleus stimulation had. In a subsequent experiment, this blocking action of quizapine (due apparently to serotonin release) was reversed by pretreatment with mephysergide, a specific serotonin antagonist. Thus, it was clearly demonstrated that serotonin is involved in the control of coordinated, rhythmic jaw movements.

Myofascial Pain Dysfunction (MPD) Syndrome

Investigators at Columbia University are conducting longitudinal studies to determine how exacerbations and fluctuations in myofascial pain dysfunction (MPD) symptoms relate to a wide range of life-stress measures. The MPD syndrome is a rather widespread, often-disabling chronic facial pain disorder. The syndrome's etiology remains unclear, although many clinicians and researchers regard stress and associated chronic muscle tension as significant factors in its onset and progression. Approximately eighty percent of those seeking treatment for this disorder are female.

These researchers are currently using a prospective case/control design to compare 200 female MPD patients with healthy female control subjects matched for age and education. Initial baseline measures are taken of the subjects' personal characteristics, social supports, patterns of coping with stress, recent life events, physical health, and pain experiences. All the MPD and control subjects are then interviewed on a monthly basis to monitor stressful life events, patterns of coping with life events, social functioning, levels of pain, and treatment-seeking. Because menstrual problems are often reported by MPD patients and because anti-inflammatory drugs have been found somewhat effective in treating both MPD and menstrual and premenstrual pain, measures related to menstrual history and complaints are also included.

The procedures being used should permit a much clearer delineation of which aspects of life-stress are antecedents, and which are consequences, of the MPD syndrome. Thus, the study could make an important contribution to our understanding of this perplexing pain disorder.

Gag Reflexes in Dental Patients

Investigators studying factors influencing avoidance of dental treatment have identified a subgroup of patients troubled by gag responses when dental instruments are placed in their mouths. Exaggerated gag reflexes can interfere with many types of dental treatment including full denture therapy.

A preliminary study at Florida State University involved behavioral assessments of adult volunteers who sought help in controlling gagging during dental treatment. These volunteers had all avoided dental treatment for intervals ranging from 18 months to over 7 years. First, a dental mirror was placed in contact with the tip of the subject's tongue. The instrument was gradually moved back across the tongue in very small, measured increments until the subject signalled that he felt he was starting to gag. This measure provided a baseline against which later responses could be compared. Subjects were then taught to control their gagging response. They received brief instructions in relaxation procedures and practised specific controlled breathing and distraction techniques. Then each subject applied the new techniques while a dental instrument placed on his/her tongue was gradually moved back. When the subject signalled any distress, he/she was again "coached" in how to use the relaxation and distraction techniques.

Preliminary results of this behavioral therapy were encouraging. After an average of 8 30-minute training sessions, subjects had learned to tolerate dental instruments in their mouths, and were able to resume regular dental treatment.

Behavioral Reactions to Orthognathic Surgery

Investigators at the University of Washington are studying how patients' expectations relate to their decision to have orthognathic surgery. Patients who decide to undergo surgery are being compared with those who elect to have orthodontic treatment only, and those who decide to forego treatment completely. The aims of this research are to determine how best to aid patients in decision-making, to improve pre-surgical emotional preparation, and to determine in advance which patients are likely to be satisfied with the outcomes of orthognathic surgery.

All of the 237 patients studied completed a computerized questionnaire made up of items developed from pilot research on patient concerns and motivations. The computerized questionnaire provided a graphic representation of the patient's expectations and decision-making strategies.

Patients who decided to receive only orthodontic treatment placed less value on achieving a better occlusion and an improved profile than did those who decided to have orthognathic surgery. The no-treatment and orthodontic treatment-only subjects were more likely to report that their friends and families had

advised against treatment. Patient treatment decisions (no-treatment *vs.* orthodontic treatment *vs.* orthognathic surgery) could be predicted from responses to the computerized questionnaire more than 80% of the time.

The investigators are now attempting to enhance the predictive power of the computerized questionnaire and are utilizing it in developing more effective pre-treatment counseling and preparation approaches applicable to orthodontic and orthognathic surgery patients.

Radiology

The X-Ray Physics Group of the National Bureau of Standards is engaged in developing a digital radiographic imaging system for dental applications. The advantage of such a digital system is that sophisticated image processing techniques can be used to observe dental pathology which is not observable in conventional film radiography. This system has the ability to rapidly record and process multiple images of the same subject and display a composite image which is selectively enhanced for a particular dental task.

Experiments conducted so far have shown that the new digital system also has the ability to perform digital subtraction between members of successive sets of images. In these experiments as few as 8 images in each set was sufficient to provide this subtraction capability. This technique makes possible the detection of small changes in pathology. Artificially induced lesions as small as 1 mm in diameter introduced between sets of images were reliably detected using this technique. In addition, the exciting discovery was made that from this small set of images it is possible to make tomographic reconstructions with a lateral spatial resolution of approximately one millimeter. This technique will enable a dentist to trace pathologies through a plane of the teeth, a task that is impossible with conventional procedures.

An active intraoral detector (or film) consisting of an x-ray intensifying screen coupled to a fibre optic bundle is being constructed. Tests have shown that resolutions more than adequate for dental imaging tasks can be achieved with this combination. To provide the necessary sensitivity, a microchannel plate light amplifier has been interfaced to the prototype intraoral detector described above.

Also under development is a miniature x-ray tube that will provide multiple target positions (location of x-ray source). By means of computer control this tube will provide the many target positions and multiple angulations required by this system.

Parallel studies are underway to develop an imaging system with an intraoral x-ray source and an extraoral x-ray detector, an arrangement which greatly simplifies the requirements for an x-ray imaging device. Recently, a small high power x-ray source with an extremely small focal spot has been developed that can be accommodated in the mouth. Use of this source not only produced superb images of a dental phantom, but also demonstrated that the system can magnify the image up to 25 times without losing significant resolution. In addition, this new x-ray source can direct the electron beam to any point on the x-ray target. With minor modification, this positioning can be accomplished under computer control to produce the source-to-detector geometry required by this system. Calculations indicate that tomo-synthetic reconstructions can be achieved with 0.5 mm lateral resolution. The source can be made to produce 15 separate independent source locations on an anode (film) less than 10 mm in diameter. It is estimated that for a given probe location in the mouth, these source positions would depict the optimum "open contact" geometry for 40% of the teeth. These developments represent major advances in diagnostic dental radiography.

Dental Adhesives

In last year's annual report, we reported a novel method of obtaining improved adhesion between composite resins and the dentin as well as the enamel of human teeth. This research is being done by American Dental Association investigators working at the National Bureau of Standards. The new procedure uses an acidic mordanting solution, aqueous ferric oxalate, and then two coupling agents (surface-active compounds) to prepare the dentin and enamel for bonding with composite resins. One of the variables recently studied was the concentrations of these compounds in their respective solvents. Increasing or decreasing the concentrations of the ferric oxalate did not improve the adhesive bond strengths, but increasing the first surface-activity comonomer, NTG-GMA, from a 5% to about a 10% acetone solution increased the average tensile bond strength from 1,900 psi (pounds per square inch) to 2,140 psi for dentin and from 1,960 psi to 2,400 psi for enamel not previously etched. Changing the concentration of the second surface-active comonomer, PMDM did not give further increases in tensile bond strengths. However, prior etching of enamel with 30% phosphoric acid solution raised the adhesion obtained by these three materials to 3,600 psi.

Compared to the commercially available materials offered for bonding to dentin or enamel, the experimental material gave bond strengths 8 to 13 times greater on dentin, but slightly less on acid etched enamel. The experimental material also performed

significantly better than two polycarboxylate cements and two glass ionomer cements. In preliminary *in vivo* observations with Class V restorations in primates, the adhesive bond with the new formulation withstood stresses occurring during the hardening of the composite and also the stresses and strains due to mastication.

Restorative Filling Materials

For the past five years investigators at the U.S. Army Institute of Dental Research in San Francisco have been conducting a study of the clinical performance of an experimental composite resin based upon a strontium glass filler particle. The control restorative material was the amalgam alloy *Ease*.

The investigators found that there was a systematic decrease in the percentage of composite restorations which remained functional at each recall period. After five years, only 58.1 percent of the composite restorations remained functional as compared to 86.1 percent for the amalgam. Approximately one-half of the failures were due to caries and the remainder due to fractures, open margins, excessive marginal leakage and hyperemia. Although there was a difference between the wear rate of amalgam and composites, only one composite was replaced because of excessive wear during the five year period.

Unlike amalgam, composite resins do not produce corrosion products which seal minor marginal defects. Such defects seem to be unavoidable. They occur as a result of bubbles or voids which localize at the margins, or inadvertent operator variables. Other factors responsible for the decreased longevity include: a. the low mechanical properties of composites; b. differences in thermal expansion between composites and tooth structure; and c. the limited availability of enamel at the gingival area of multiple surface restorations for acid-etch bonding.

Maxillofacial Prostheses

In developing maxillofacial materials to match the tissues they are replacing, researchers strive to make prostheses more "life-like" in color, form, function and tactile properties. At the University of Michigan investigators have developed methods and materials for improving the capacity for matching color and tactile properties in silicones. Several types of silicones are used for facial prostheses. In general silicones are durable and have stable physical and mechanical properties, but often they are not sufficiently pliable. To produce more pliable maxillofacial prostheses, the Michigan investigators combined Type A adhesive, a dimethyl siloxane-triacetoxy terminated silane, with the base component of a siloxane elastomer called MDX. They are now determining the force/displacement characteristics of mixtures of Type A adhesive and an uncured MDX and comparing these values to force/displacement in facial tissues. Test samples were prepared using various ratios of Type A adhesive to MDX. Half of the samples were placed in a WeatherOmeter and aged. The force/displacement ratios or slopes for the weathered and non-weathered silicone specimens were obtained with a modified linear voltage displacement transducer (LVDT) which was connected to an x-y recorder. Force/displacement plots were also obtained for tissues of four human subjects at three different locations: the ear lobe, the cheek at a position in line with the lateral corner of the orbit, and the mid-forehead just above the glabella.

As more MDX base was added to the Type A silicone, higher displacements were obtained for a specific force. For the weathered samples the trends were similar except that for a constant force a smaller resultant displacement was obtained.

The major advantage of blending Type A and MDX is that one is able to obtain a variety of force/displacement plots with these silicones that can simulate the behavior of different facial tissues. By laminating mixes with the various ratios, a prosthesis could be tailor made to duplicate the force displacement characteristics of a specific tissue.

This study has also provided a computer based color mixing system which is used to determine the percent by weight of pigments to achieve a color match. A computer program working in conjunction with an analytical mixing model enables clinicians to quickly and accurately match the color of a patient's skin with maxillofacial prostheses. A reflectance spectrophotometer with a reflectance sphere is used to obtain the input data for this program. A computer program in FORTRAN connects the system equations to the data base and to the graphics display terminal. The program allows the operator to select a pigment mix that will match the color of a patient's skin or any other object placed in front of the sample port of the spectrophotometer. The computer program displays the reflectance curve of the object being matched and overlays it on the predicted reflectance curve. By changing the percent-by-weight of pigments the operator can match the color of the test object.

This computer program and associated hardware for color matching has already been used to produce good matches. As the program and experimental variables are refined, it will be possible to determine the pigment ratios with a high degree of reliability and accuracy.

Endodontics

The mixture of eugenol (oil of cloves) and zinc oxide has been used by dentists for many years in the treatment of toothache and dental decay. This mixture is accepted as an effective "sedative" agent for inflamed dental pulp, but it can also damage soft tissues on contact.

Working on the assumption that it is the eugenol component which has these therapeutic and toxic actions, scientists at the University of California have studied the release of eugenol from a zinc oxide-eugenol mixture, its diffusion through dentin to the pulp, and some of its effects on soft tissues. Surprisingly, eugenol does not move out of the mixture by simple diffusion; instead, it is released slowly from the surface layer only. This release depends on a reaction with water, and it becomes slower as the water has to penetrate deeper into the mixture. In teeth the released eugenol diffuses slowly through dentin, where it accumulates in concentrations high enough to kill bacteria and to inhibit nerve action, but not high enough to cause damage to the cells. In the soft tissue of the pulp, eugenol increases blood flow, and thus helps to achieve its own removal from the tissue. Higher concentrations of eugenol do kill cells. There is strong evidence that the dual effects of dentin in limiting the availability of water, and therefore the rate of release, and in slowing diffusion of eugenol to the pulp, prevent the eugenol from reaching toxic concentrations, so that it remains both safe and sedative.

SUMMARY OF RESEARCH HIGHLIGHTS

Studies of tissues from patients with localized juvenile periodontitis (LJP) indicate that infected connective tissue may harbor a reservoir of bacteria for as long as six weeks after root planing. Immunoflorescence and electromicroscopy were used to show the presence of *A. actinomycetemcomitans* (*Aa.*). The results indicate that removal of the organisms required either surgical excision or systemic tetracycline in addition to root planing. Related studies implicated *Eikenella corrodens*, *Capnocytophaga gingivalis, C. ochracea* and *Aa.* in one or more clinical diseases as well as spirochetes and motile rods, which showed high positive correlation with pocket depth. *Bacteroides intermedius* was elevated in adult periodontitis with suppuration. In a treatment study, the reduction in clinical inflammation was accompanied by a reduction of complement activation products in the crevicular fluid.

An animal study produced evidence to explain the efficacy of tetracyclines in combatting periodontal diseases. Tetracyclines not only suppressed pathogenic bacteria, but also inhibited tissue collagenase activity directly. Having first shown that gingiva from untreated diabetic rats produced elevated collagenolytic activity, the investigators showed that minocycline reduced the collagenase by 62%. Repeating the experiment under germ-free conditions gave similar results. Earlier studies had shown that *Aa.*, implicated as a pathogen for LJP, produced a protein toxic to human neutrophils, the white cells which protect the body against infection by engulfing bacteria. Recently, additional toxins against human cells were discovered. One is a protein toxic to human fibroblasts, the cells that synthesize the connective tissues which attach teeth to bone. This toxin does not kill fibroblasts but irreversibly prevents them from reproducing. Another *Aa.* product suppresses the growth of other common oral bacteria. This observation may explain why *Aa.* lesions often have minimal plaque. Still another *Aa.* toxin was discovered when *Aa.* organisms were grown in high concentrations of sodium acetate. This toxin is a unique lipopolysaccharide which kills macrophages with great efficiency.

B. gingivalis, implicated in adult periodontitis, was shown to produce a fibrinolysin which is believed to enable the organism to invade tissues. A fourth toxin from *Aa.* was shown to inhibit lymphocyte functions related to host defense. This *Aa.* factor activates T-suppressor cells which suppress both cell mediated and humoral immunity. Such effects might lead to a local state of immunologic incompetence.

In studies to develop vaccines against periodontal bacteria, investigators showed that mice immunized with a specific fibrillar antigen from T14V strains of *A. viscosus* became resistant to that organism. The fibrillar antigen is believed to be necessary for the organisms to attach to teeth.

To understand chronic periodontitis, investigators have studied how bone macrophages are recruited from the circulation to the bone prior to resorption. They have discovered in extracts of bone and in macrophage culture media a lower molecular weight protein which elicits chemotaxis, or directed locomotion, in the macrophages. Studies of post-menopausal women found no relationship between osteoporosis and alveolar bone height. A nutritional study showed that alveolar bone loss was not affected either by the calcium content or the calcium to phosphorous ratio in the diet.

After preliminary success in treating herpes labialis with either of two anti-viral agents applied by iontophoresis, investigators completed studies to determine optimal electric current time and drug concentrations. With both drugs, vidarabine phosphate and Acyclovir, a significant number of lesions were virus-free after 48 hours and healing time was reduced.

To study oral tissue alterations associated with smokeless tobacco, investigators examined more than one thousand teenagers and found that 10% were users and 50% of these had oral lesions. Those with oral sequelae had used smokeless tobacco for more than 3 years.

In developmental research on how the embryonic neural crest cells escape from the neural plate epithelium to begin migration, scientists focused on disruption of the epithelium and secretion of extracellular matrix. So far, they have found that epithelial disruption is non-uniform and highly localized. Although crest migration appear to reflect cell motility, new evidence indicates that the cells may not undergo actual movement; instead, their relative position changes because of large shifts in newly formed matrix surrounding them.

In a mouse mutant with spina bifida, it was found that neural crest cells did not migrate from the region of this defect. This system may serve as a useful study model. In studies of tooth development, investigators performed experiments to construct and identify the dental gene responsible for the major mouse amelogenin.

In studies on hormonally regulated changes in mRNA in various tissues, the investigators have developed methods to detect an individual mRNA species accounting for as little as 0.01% of the total translatable mRNA.

Studies on cleft lip and palate have focused on the cellular events during fusion of the maxillary processes. During reorientation of the palatal shelves, certain areas show increased mesenchymal cell processes and cell bodies which closely approach the basal lamina. In drug-treated embryos, these processes are altered. In another study, the researchers have quantitated the number of gap junctions in mesenchyme throughout the developing maxillary process. Gap junctions are thought to provide intercellular communication. Their data suggest that the distribution of gap junctions is related to proliferative rates and may provide a mechanism for metabolic gradients to mediate reactions.

Studies showed that the genetically determined susceptibility of A/J and C57 strains of mice to glucocorticoid-induced cleft palate can be altered by diet. High fat diets increased susceptibility in both strains of mice. Scientists then showed that the high fat diet reduced glucose 6 phosphate dehydrogenase (G6PD), an effect which would set in motion a chain of reactions which could result in a lessened capacity to metabolize noxious drugs including those that cause cleft palate.

Malocclusion studies have focused on residual effects following blockage of the nasal airway in Rhesus monkeys for two years. Eighteen months after removal of the nose plugs, certain altered muscle patterns, skeletal changes and the dental malocclusion induced by the mouth breathing were still present.

In studies of bone undergoing remodelling, elongating the collagen ribbon on which osteoblastic cells were being cultured caused an increase in prostaglandin synthesis. Improved results with composite grafts of allogenic bone plus autologous marrow were obtained when the host was treated with a T-lymphocyte mitogen.

Studies on the role of calmodulin in regulating salivary gland intracellular calcium indicate that salivary glands may contain a calcium "pump."

Studies of normal teeth showed that more than 50% of the dentinal tubules adjacent to the pulp horn in cats and monkeys are innervated by trigeminal receptors, whereas greatly reduced innervation is found elsewhere. The findings suggest that sensory receptors in the dentin may protect teeth from excessive occlusal forces. Sensory axons were also identified in root dentin, a finding which may explain the painful sensitivity of exposed roots.

In studies on pain, investigators had shown that analgesic effect of placebo is due to endogenously released morphine-like compounds, but more recent studies indicate that these conclusions do not always apply.

In studies of orofacial motor function in guinea pigs, investigators have shown that serotonin is involved in the control of coordinated rhythmic, chewing movements. First, they discovered that stimulating the midline raphe nucleus of the brain stem would block rhythmic jaw movements. Subsequently, by specific activation and inhibition experiments, they proved that the midline stimulation blocked jaw movement by releasing serotonin.

In longitudinal studies to determine how exacerbations and remissions of myofascial pain dysfunction (MPD) syndrome are related to life stress, a prospective case/control design will be used to compare 200 female MPD patients with controls. This design should permit the investigators to differentiate between contributing causes and consequences of the MPD syndrome.

Patients troubled by exaggerated gag reflexes when undergoing dental treatment were taught to use behavioral therapy techniques to control their gagging response. After an average of 8 30-minute training sessions, they had learned to tolerate instrumentation and were able to resume regular dental treatment.

In a study of behavioral reactions to orthognathic surgery, investigators developed a computerized questionnaire to determine patients' expectations and decision-making strategies in regard to the surgery. The questionnaire enabled the scientists to predict more than 80% of the time whether a patient would choose orthodontic treatment or surgery.

A newly developed X-ray system has the ability to record multiple images, display a composite image with selective enhancement, and perform digital subtraction between images. Artificially induced lesions as small as 1 mm can be detected. Also under development is a system in which a miniature X-ray tube positioned intraorally and controlled by computer can magnify X-ray images and provide optimal views of interproximal tooth surfaces.

Compared to commercial material offered for bonding to dentin or enamel, an improved experimental material gave bond strengths 8 to 13 times greater on dentin and only slightly less on acid-etched enamel. In a five-year clinical study, more than 40% of composite restorations with strontium filler particles failed, whereas less than 15% of control amalgams had failed. Half of the failures were due to caries and the remainder were due to other factors. Only one composite failed because of excessive wear.

To make a maxillofacial prosthetic material more life-like, scientists combined two silane adhesives in various ratios. By this approach, coupled with a computer program to aid in color matching, the investigators developed prosthetic material closely resembling skin even in tactile properties.

In studies of endodontic materials, investigators obtained evidence to explain the efficacy of zinc oxide and eugenol. They found that the eugenol diffuses slowly into the dentin where it accumulates in concentrations high enough to kill bacteria and to inhibit nerve action, but not high enough to cause damage to pulp cells.

Intramural Research Program

INTRAMURAL RESEARCH

National Institute of Dental Research

October 1, 1982 - September 30, 1983

SCIENTIFIC SYSTEMS SECTION

The Scientific Systems Section has continued this year to provide data processing support and consulting services to the NIDR research community by operating a central computing facility, by providing support for the dedicated laboratory systems in our Institute, and by developing a plan in conjunction with the NIDR Computer Services Committee for computer hardware upgrade. In addition, our scope has expanded to now include support for the NIDR Intramural Research Program's administrative office. Much of this support was made possible by the addition of a full-time programmer to our staff in January.

The scope of the Section's work this year has been rather diverse. Tasks undertaken can generally be divided into the following categories, and will be described in more detail below: the addition of "user friendly" software to the system and advocacy of "workstations" to be conveniently located in the laboratory, generation (under the auspices of the NIDR Computer Services Committee) of a plan to upgrade existing computer equipment over the next five years, addition of new hardware to the central system, addition of a common command language to all systems, the interface of laboratory instruments, new user-specific applications software written, and a highly successful series of computer classes taught to our user community.

A major emphasis of our work this year included making our central system more "user friendly." This was accomplished in two ways. Two software packages were added to the central system, SATURN word processing software and C-CALC electronic spreadsheet software, both of which are easy to use, and contain on-line help facilities which enable users to receive help text while on the system without consulting a reference manual. The word processing software has proven to be quite successful, with several users now creating their own letters, memos, and various other documents. This system will also enable users to prepare first drafts of scientific manuscripts which may then be transmitted over a communications link to the office word processing equipment for final polishing and printing. The other software package, C-CALC, has been used primarily for the automatic maintenance and projection of personnel work hours and laboratory personnel budgets. Discussions with laboratory personnel concerning ease of computer usage at NIDR resulted in the advocacy of the workstation concept, which has as its goal enabling the researcher to perform his data entry, analysis, and printing of results in the workplace, that is, in his laboratory. The recommended workstation might consist of either a personal computer which could be used to collect data from laboratory instruments, perform some local analysis, and transmit the data to the central system, or could simply be a terminal and personal printer. A few users have proceeded to develop such workstations at locations convenient to them.

Another major undertaking this year was the study, in conjunction with the NIDR Computer Services Committee, of all computer facilities within the Intramural Research Program. A five year replacement plan was adopted, the overall thrust of which is twofold. It was decided that the Diagnostic Systems Branch, because of its specialized image processing hardware requirements, should continue to operate independently of the central system. The top priority for the first year of the plan included replacement of DSB's computer system; an order has in fact been placed now and the equipment, a Digital Equipment Corporation VAX/750 computer, will arrive in late fall. The plan contained recommendations for the continued operation of the Neurobiology and Anesthesiology Branch's laboratory computers as satellites of the central system. In fact, these systems will be replaced by microcomputers, one per laboratory. Two such microcomputers for the NIDR Pain Clinic have been purchased. Note that the result of modernizing this equipment will be physically smaller systems, in some cases movable, that are easier, and thus less costly, to maintain. Two new pieces of hardware were added to the central system this year: a terminal multiplexor board to allow interfacing of 16 more terminals, and 256K words of memory, which results in more efficient system operation when several tasks are running. Where possible, the operating systems of our computers were upgraded to the latest versions. This resulted in a common "user friendly" command language interface across all NIDR computers.

Interfacing of several laboratory instruments was initiated this year and will be completed in early FY84. One, a Guilford 300N spectrophotometer in the Laboratory of Microbiology and Immunology has been interfaced to the central system using a microprocessor interface which shares a common line with a hardwired terminal. Data is thus collected, and growth curves are computed after experiment completion. Upon request, a

study was done at the Caries Prevention and Research Branch's intramural research laboratory at the Park Building to determine the most appropriate way to collect data from four pieces of equipment, a Biochrom 4400 amino acid analyzer, a Hewlett Packard 5840A gas chromatograph, a Titertek multiskan spectrophotometer, and a Packard scintillation counter. A plan was developed whereby a notebook sized microcomputer would be used to collect data from the equipment via an RS232 port. The data would then be transmitted to the central system over a communications line for archival storage and analysis.

Enhancement has continued this year on the Neurobiology and Anesthesiology Branch's neurophysiology laboratories' automation software. For these laboratories, a small minicomputer performs the experiment control, and then collects and displays on a terminal screen in real-time, the resultant neural, EMG, and behavior data collected from the research animal. The minicomputer communicates with the laboratory equipment using a series of codes which are sent to and received from a microprocessor which is hardwired to the equipment. Data thus collected is moved to the central system at the completion of the experiment, where it is analyzed behaviorally, and where the neural and EMG data may once again be analyzed graphically. The software for this entire system was reviewed thoroughly early this year to determine what difficulties would be encountered when two laboratories, both acquiring data at high transfer rates, and graphing, were running on the system simultaneously rather than one at a time as had previously been the case. Initial tests showed that modifications were indeed necessary to make this feasible. Modifications were then made to both the data acquisition and the on-line graphics software to ensure properly timed event intervals, prompt program response to monkey actions, and prompt graph display at the conclusion of a requested time window. Final testing on the system showed that it would be possible to perform experimental control and acquire data from two laboratories at once, however, it would only be possible to run the on-line graphics system in one laboratory at a time. A scheme has been developed whereby a microprocessor is used to collect the laboratory, neural, EMG, and environment data, reduce it into 100 millisecond bins and transmit it using a low speed terminal line to the central system, where the data is then displayed back in the neurophysiology laboratory on a graphics terminal. Thus, the original goal of two simultaneously running laboratories has been accomplished, although the on-line graphics of the second lab has somewhat limited capabilities, because of the prior data reduction. Enhancements were made to the experiment control software as well this year, specifically, several new paradigms were added to the extensive collection already available, and behavior analysis software was modified to include the new paradigms. It should be noted here that a second computer will soon be made available to the group, using components gleaned from two other systems which will be replaced in the very near future. The result will be two separate but equal computer facilities, one for each neurophysiology laboratory.

A series of programs has been written for the new DEC PRO/350 computer system, recently acquired by NAB for its Pain Clinic in the Clinical Center. The programs are designed to test patients' subjective measurement of pain, and thus present subjects with a variety of textual and graphical representations of pain intensity and unpleasantness, to which they must compare and evaluate their own pain experience. The experimental tests and data collection programs are run on the PRO/350, thereby allowing flexibility in the choice of a location for an experiment session. Data is transferred over a communications line to the central system where it may be analyzed and stored.

A real-time data acquisition project has been undertaken for the Diagnostic Systems Branch. The objective is to collect data at video rates from a prototype dental x-ray system currently being built at the National Bureau of Standards. The acquisition of initially eight, and potentially sixteen, pictures will take place in less than one second. Because of the speed constraints, data must be interleaved into the four memories of the DeAnza image processing system before being stored on the disk. Subtraction techniques will then be applied to the resulting images. The joystick box connected to the DeAnza system has been modified to pass a synchronizing signal from the new device to the software in DSB's minicomputer so as to coordinate camera positioning and picture digitization.

Numerous other projects have been completed and include the following: analysis of immunoprecipitation data for the Laboratory of Microbiology and Immunology, and conversion to run on the central system of a set of programs which were written at Harvard University to search for and display symmetries and homologies in nucleotide sequences of DNA. These programs have been used by researchers in both the Laboratory of Microbiology and Immunology and the Laboratory of Oral Medicine. The Dental Clinic Workload Reporting System has been modified to accept data from the Commissioned Officers Dental Clinic and then to produce the requested reports. The Workload Reporting System has also been enhanced so that error checking may be performed on the entered provider number, and an additional report of Total Patient Encounters by Institute has been developed. A cassette reader program which reads data and then transmits it to the central system from a Microelisa Data

Recorder has been developed as well. In addition, a large data base formerly stored at DCRT has been relocated to the central system for the Laboratory of Developmental Biology and Anomalies, and data analysis is being performed using our in-house statistical software, BMDP. A program to allow digitizer input and analysis of electrophoresis data was modified for the Laboratory of Microbiology and Immunology to run on the central system. Finally, radioimmunoassay software running on a Hewlett Packard 1000 computer at USUHS was modified to allow entry of variable format data for a scientist in the Neurobiology and Anesthesiology Branch.

Several classes were taught this year in a continuing effort to improve the computer literacy of our users, and also to introduce them to some of the software available both here and at DCRT. Specifically, classes were offered on such topics as the central system and its new command language, DCL, the RS1 interactive graphics and statistics package, and the SATURN word processing software. Also offered was a seminar on the programs that are available at NIDR for the use of molecular biologists, and finally a class in the computer production of publication quality graphs, and production of posters suitable for use at scientific meetings.

Plans for next year include further study of the workstation concept, primarily as it applies to the scientist in his/her laboratory. It is becoming increasingly apparent that our investigators would greatly benefit from the bringing of computer power directly to the workplace. This would enable a scientist to possibly collect data from laboratory instruments (if his workstation consisted of a personal computer), analyze data and print results locally, perform word processing, and perform library searches. If a personal computer is used for this purpose, files may then be transferred to the central system for archival storage and more sophisticated analysis. Another area requiring consideration is the usage of the office word processing equipment as a terminal for the central system. This would allow the secretarial staff who so choose to use the C-CALC software on the central system to maintain laboratory budgets, and also to receive from other word processors, such as SATURN, first drafts of manuscripts which need additional editing or printing. In general, I view next year as one in which an integrated approach to computing at NIDR will be formulated and initiated.

MICROBIAL SYSTEMATICS SECTION

The Microbial Systematics Section is charged with establishing a data bank for information describing diverse strains of microorganisms. Special emphasis is placed on the human oral microbiota. For this purpose, collaborative projects are on-going with microbiologists distributed throughout the world.

At present there are tens of thousands of scientists, physicians, public health personnel, and others involved in some aspect of microbiology. The number of microbial strains isolated, characterized, and (in many cases) preserved, by individuals runs into the millions. Hundreds of millions of bits of information have been developed on these strains. However, these data are not resident in a single, centrally located system, permitting rapid and efficient utilization. Because of the large volume of information involved and because, in several applications such as classification and identification, mathematical manipulations of the data are required, electronic processing of these data is necessary.

In collaboration with personnel of the American Type Culture Collection, the Food and Drug Administration (FDA); the Centers for Disease Control (CDC), the Veteran's Administration and numerous academic microbiologists, strain data are being entered into the data bank which provides such services as: data on specific organisms and/or groups of organisms, location of strains with special characteristics, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids for research purposes, aids in quality control of tests, methods, and laboratories, and communication of data via common format.

Data files of primary data on a large number of microorganisms found in the oral cavity and related types are established. These files provide a resource for asking both ecological and epidemiological questions of interest in dental research.

Programs have been developed and tested to enter, retrieve, and analyze the data in a variety of ways for epidemiological, diagnostic, taxonomic, ecological, etc., uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. As experience grows, improved programs are being designed and implemented to form a computer utility for microbiological data management and analysis. The utility is termed the Microbial Information System (MICRO-IS).

The system of coding descriptors for computer input of information on bacteria, protozoa, fungi, algae, and its current extension to hybridomas has gained international attention and is of consequence to various components of NIH as well as other agencies in the Public Health Service (specifically FDA and CDC) and the National Science Foundation. The coding system has been adopted as the standard coding method for microbial data by FDA, the World Data Center of the World Federation for Culture Collections, and the Japanese Federation of Culture Collections. The Committee on Data for Science and Technology (CODATA) of the International Council of Scientific Unions has agreed to sponsor the publication of the coding system for world-wide distribution.

CODATA has established a Task Group on a Hybridoma Data Bank for the purpose of establishing an international data bank on hybridoma and monoclonal antibody information. The Task Group is collaborating with the MSS in extending the microbial coding system to hybridomas using the Micro-IS programs. Two staff members of NIH (Dr. M.I. Krichevsky, MSS, NIDR and Dr. B. Janicki, National Institute of Allergy and Infectious Diseases) are members of the Task Group. The World Health Organization Immunology Program has volunteered staff for abstracting literature. The staff establishing the hybridoma data base is located at the American Type Culture Collection.

Extensive files of descriptions of filamentous and pleomorphic organisms are being assembled. The files cover all the described types of *Mycobacteria*, blend into the *Nocardia*, then through the *Actinomycetes* (especially a unique set on oral isolates), and finally, *Bacterionema*. An extensive cooperative study, covers the oral pleomorphic bacteria (many of which are associated with disease). The study will provide a standard set of well characterized bacteria for the Dental Research community. The data from this study has been incorporated into the files on pleomorphic organisms. These files are being actively analyzed in collaboration with the submitters of the data as well as numerical taxonomists to revise the badly confused taxonomic relationships of these bacteria. Such revision is necessary to avoid the misidentification (leading to erroneous epidemiological conclusions) which are found in some recent dental research literature.

Other files on non-filamentous oral organisms (streptococci, lactobacilli, veillonella, etc.) are being constructed to study correlations among caries activity, phenetic span of characters, serology, source of isolation, and host descriptions.

One of the long term goals in establishing all these files is the establishment of probability tables to allow computer-aided probabilistic identification of oral isolates. Probability matrices, for on-line identification of bacteria (including Gram negative rods, lactobacilli,

streptococci, bacilli, etc.) have been constructed. They are available to research workers for use.

MICROBIAL SYSTEMATICS SECTION

Intramural Projects

Z01 DE00044-13	**Micah I. Krichevsky**	Handling of Microbial Strain Information By Computers
Z01 DE00250-06	**Cynthia Walczak**	Algorithms for Microbial Systematics

MINERALIZED TISSUE RESEARCH BRANCH

The establishment this year of the Mineralized Tissue Research Branch brought together the major NIDR groups investigating the structure, chemistry, development and pathology of the skeletal tissues. The Bone Cell Biology Section, the Skeletal Matrix Biochemistry Section, and the Mineral Chemistry and Structure Section of the former Laboratory of Biological Structure, and the Proteoglycan Chemistry Section and the NMR group of the old Laboratory of Biochemistry were combined to form the new Branch. Each of these groups led by an active, highly respected scientist, and the creation of the Branch emphasizes the Institute's commitment to these programs and places basic research on mineralizing tissues at the forefront of the NIDR Intramural Program.

As during any period of organizational change, the staff of the Branch has experienced a degree of anxiety, particularly regarding space and leadership of the Branch. It is anticipated that both of these issues will soon be resolved. Completion of the second and fifth floor renovations has already created new space for the Bone Cell Biology and Skeletal Matrix Biochemistry Section, and some temporary space has been provided for the Skeletal Biophysics Section. Continuing sources of concern are the constriction in personnel ceilings and the need for adequate budgetary resources to allow the MTRB to move into promising new areas of investigation, especially those related to the inherited and acquired diseases of the skeletal system.

Despite the difficulties encountered during this transitional year, MTRB scientists have made substantial strides in a number of areas, providing important new information on the basic mechanisms of skeletal growth and development, mineralization and disease processes. The introduction of new methodology, such as molecular biology and monoclonal antibody techniques, the formation of new collaborative relationships, and the development and uti lization of new research models are beginning to have a major impact on the types of studies undertaken in the MTRB laboratories. The acquisition of new equipment, such as HPLC instrumentation and a superconducting magnet, will further expand the Branch's investigative capabilities. The progress made during the past year is summarized below.

SKELETAL MATRIX BIOCHEMISTRY SECTION

The activities of the Skeletal Matrix Biochemistry Section focused on three main areas during the past year: development and characterization of bone cell cultures; characterization of new animal models for osteogenesis imperfecta; and molecular biology studies of enamel matrix proteins. Fetal calf bone cell cultures, rich in osteoblasts as determined by alkaline phosphatase activity and cyclic AMP response to parathyroid hormone, were shown to synthesize type I collagen, osteonectin, the bone sialoprotein, and the bone proteoglycan. Similar results were obtained with fetal pig calvarial cell cultures. Additionally, post-secretion processing of osteonectin from 32,000 daltons to breakdown products of 20,000 and 10,000 daltons was demonstrated in the fetal pig cell cultures, confirming earlier whole bone biochemical studies. The fetal calf cell cultures, when grown in appropriately supplemented medium, were capable of producing a mineralizing extracellular matrix.

The methodology developed for culturing bone cells and the characterization of normal osteoblast populations *in vitro* are an important first step for future studies of the molecular basis of various bone diseases. Experiments were begun this year with a bovine model of the human genetic bone disease, osteogenesis imperfecta. Biochemical studies of the bone matrix and biosynthetic studies of skin biopsies from these animals showed minimal alterations in type I collagen structure and synthesis, but revealed a marked deficiency of osteonectin. Dentin osteonectin levels were also decreased, as was the content of dentin phosphoprotein. These studies suggest that factors in addition to collagen abnormalities may be operative in human osteogenesis imperfecta. Characterization of the defect in other affected tissues in this animal model, as well as studies of human tissues, are currently underway.

Advances in the area of enamel matrix proteins included development of polyclonal and monoclonal antibodies to the high molecular weight enamelin and amelogenin proteins, isolation and cell-free translation of ameloblast messen ger RNA, and cell culture of early enamel organ epithelia from rats and calves. The enamelin and amelogenin proteins were shown by immunological studies to be completely unrelated molecules, as previously suggested by chemical and biosynthetic data. Messenger RNA isolated from ameloblast-rich enamel organ epithelia directed the synthesis of 2 major and 1 minor amelogenin proteins of 25-35,000 daltons and an enamelin protein of about 55,000 daltons. About 50% of the total enamel cell mRNA was specific for amelogenin synthesis. Finally, ameloblasts grown *in vitro* were shown to contain immunoreactive amelogenins intracellularly, and to secrete enamelins into the culture medium.

SKELETAL BIOPHYSICS SECTION

Studies conducted during the past year by the Skeletal Biophysics Section fall into 2 main areas: the chemistry

and precipitation of calcium phosphates; and the molecular dynamics of connective tissue macromolecules. Within these general categories, however, a broad range of biologically relevant problems, and an equally broad range of investigative approaches can be found.

A major goal of the Section is elucidation of the physical and chemical processes controlling biological mineralization. In work reported last year, a model system for matrix vesicle-initiated mineralization, based on a 3 compartment Pressman cell, was established and characterized. This year a system utilizing multilamellar liposomes, more comparable in dimensions and properties to matrix vesicles was developed. Potassium-driven calcium transport from the medium into the liposomes was facilitated by the ionophore X-537A. In the absence of added phosphate, the transported calcium was largely bound to the liposomal membrane. When the liposomes contained encapsulated phosphate, over 3 times as much calcium was taken up by the liposomes, mostly in the enclosed aqueous phase. Sufficient calcium concen trations were achieved to precipitate calcium phosphate within the liposomes, first as amorphous calcium phosphate, which subsequently converted to apatite. These results support the concept of matrix vesicle initiated mineralization which proceeds by way of the now well established inorganic precipitation process leading to apatite formation, and suggest an important role for the vesicle membrane in crystal nucleation and proliferation.

A variety of substances can inhibit the precipitation or crystal growth of calcium phosphate. These inhibitors are important from the standpoint of regulating calcification in various sites where the extracellular fluids are metastable in regard to the calcium and phosphate concentrations, and as potential therapeutic agents to prevent calcification in the urinary tract or other tissues. The effects of the amelogenin and enamelin proteins on the growth of apatite crystals were assayed *in vitro*. Both classes of protein retard seeded crystal growth, without altering the basic crystal growth mechanism. The enamelins were much more potent than the amelogenins. Within the amelogenin class only those fragments of the 27,000 dalton amelogenin B protein which were 16,000 daltons or larger in size were effective as inhibitors. From the available data on the structure of the amelogenin protein the inhibitory domain appears to be located near the center of the molecule. *In vivo* the most rapid phase of enamel mineralization coincides with the loss of the larger inhibitory amelogenin proteins. Studies of inhibitory substances present in various body fluids have identified citrate complexes of the trace metals Fe(III), Al(III) and Cr(III) as significant inhibitors at normal physiological concentrations. At high citrate to Fe(III) ratios a small molecular weight complex is formed which effectively inhibits calcium phosphate precipitation. At low citrate to Fe(III) ratios, a high molecular weight complex is formed which inhibits calcium oxalate crystal growth. This citrate-Fe(III) system provides a potential biological regulatory system for urinary stone formation.

Treatments which alter the solubility of human tooth enamel are of interest because of their clinical implications. Laser irradiation previously has been shown to reduce subsurface enamel demineralization rates. A comprehensive study of laser irradiated enamel was undertaken to determine the mechanisms underlying this change in solubility. High energy density laser irradiation caused formation of new calcium phosphate phases; by comparison with enamel and synthetic analogs heated in a conventional furnace, the laser induced temperature changes and resulting solubilities could be predicted. Changes which render the enamel apatite more hydroxyapatite-like are considered to reduce solubility. In the temperature range 200-650°C, phases form which decrease the enamel solubility; from 650-1100°C, either a decrease or increase in solubility occurs, depending upon the overall Ca/P ratio; and above 1100°C, a marked increase in solubility is found. Surface melting and cratering by the laser occurs at temperatures above 1400°C. Thus, beneficial effects of laser irradiation on enamel solubility are limited mainly to regions where the induced temperatures are GE650°C.

Studies of the molecular dynamics and interactions of connective tissue macromolecules by nuclear magnetic resonance (NMR) have focused mainly on collagen. Studies of various collagens in which amino acids labeled with ^{13}C, ^{15} and ^{2}H have been incorporated demonstrate collagen backbone reorientation of 41°, 33°, and 14° at 10AG5-4 seconds, and 10°, 8°, and 5° at 10^{-8} seconds for reconstituted fibrils, tendon and calvarial collagen, respectively. Thus, collagen motion is restricted in mineralized tissues compared to soft tissue collagen. In contrast, preliminary observations indicate no differences between amino acid side chain mobility between hard and soft tissue collagen. Model peptides are also being studied, and a novel method for synthesizing deuterium-labeled proline was developed.

Other studies have examined cartilage proteoglycan chain mobility under various conditions. Loss of up to 50% of water caused by compressive stress has little effect on chain mobility. Calcium ions, in the absence of sodium ions, cause a reduction in mobility, possibly indicating calcium binding to proteoglycans.

BONE CELL BIOLOGY SECTION

The major thrust of the research conducted during the past year by the Bone Cell Biology Section has been the mechanism of bone induction by demineralized bone matrix. The previously reported method of dissociative extraction and reconstitution of demineralized matrix was utilized to study the effect of matrix particle size on bone induction, the species specificity of bone inductive proteins, and the influence of experimental vitamin D deficiency on bone inductive proteins.

Small-size (fine) matrix particles fail to induce bone when implanted subcutaneously. Dissociative extraction of the small particles followed by chromatography and gel electrophoresis revealed the same protein profiles as obtained with larger (coarse) particles. Reconstitution of column fractions of molecular weight 20-50,000 daltons from fine extracts with inactive coarse matrix residue resulted in bone induction following implantation. These results confirm the critical role that matrix geometry (size) has in trigger ing the biochemical cascade of bone induction. Similar studies compared the effectiveness of matrix proteins from human, monkey and bovine bone to induce endochondral bone formation in rats. Of the three, only bovine matrix had a weak activity, suggesting species specificity of the response. Extraction of the matrices and reconstitution with inactive rat residue also failed to induce bone. However, chromatography of the extracts and reconstitution of fractions less than 50,000 daltons in size resulted in bone induction by all three species. Thus, inhibitory or immunogenic components in the residue or extracted proteins $>50{,}000$ daltons probably account for the apparent species specificity, and the bone inductive proteins appear to be homologous in human, monkey, bovine and rat bone matrix. Finally, intact matrix derived from vitamin D-deficient rats was virtually devoid of inductive activity, but showed weak activity (20-30% of control) after extraction and reconstitution with inactive control matrix. These findings indicate that bone inductive proteins are vitamin D-dependent. Further, the experiments described above demonstrate the utility of this system for determining the basis for changes in bone inductive activity in various experimental and pathological con ditions.

Other studies conducted by the Section employed the matrix-induced bone forming system to investigate proteoglycan metabolism and the effects of various experimental procedures on endochondral bone development. Proteoglycans isolated from cartilage on day 7 showed two chromatographic peaks, one of which had glycosaminoglycan chains similar to femoral cartilage. The half-life of this proteoglycan was about 2.5 days, and its concentration began to decline by day 9. Bone proteoglycans extracted in 4.0 M guanidine from day 14 plaques had a half-life of 2.7 days, whereas those extracted after dissolution of the mineral turned over slower, with a half-life of 5.8 days. The influence of estradiol and progesterone on endochondral bone formation was assessed in ovariectomized female rats. Mesenchymal cell proliferation was enhanced by both hormones in combination, but not singly. Progesterone alone or in combination with estradiol increased bone formation and mineralization. Finally, irradiation of rats prior to implantation of bone resulted in inhibi tion of bone formation, primarily due to decreased mesenchymal cell prolif-eration.

PROTEOGLYCAN CHEMISTRY SECTION

Proteoglycans are ubiquitous components of connective tissues, where they play a major role in determining the physical properties of the tissue, and serve a number of important biological functions. These molecules have complex structures and differ markedly in size and in composition. The research efforts of the Proteoglycan Chemistry Section are directed toward elucidating the structure and biosynthesis of proteoglycans in various tissues and their alteration in pathological conditions.

Studies carried out this year on cartilage proteoglycans have examined biosynthetic and structural aspects of core protein and glycosaminoglycan chain formation. Two-dimensional peptide mapping revealed that the hyaluronic acid binding region is at the N-terminus of the core protein and that there are at least two disulfide bonds in this region of the molecule. Synthetic experiments using labeled glucose as a precursor to chondroitin sulfate suggest that addition of the glycosaminoglycan chains to the protein core may occur nearly simultaneously. Organ cultures of articular cartilage are employed to study proteoglycan metabolism in a steady state system or after perturbation by treatment with bacterial lipopolysaccharide or vitamin A. Lipopolysaccharide inhibits synthesis and increases degradation, resulting in a net loss of proteoglycans from the culture. Significant changes in intra cellular carbohydrate metabolism also occur. Vitamin A was found to affect the organ cultures in a manner identical to lipopolysaccharide.

The structure, synthesis and metabolism of proteoglycans have also been examined in other tissues. Ovarian granulosa cells synthesize three different proteoglycans which exhibit a variety of metabolic fates. A heparan sulfate proteoglycan migrates to the cell surface, where about one-half is released into the medium, and the other half is internalized and degraded in lysosomes. A small dermatan sulfate proteoglycan behaves similarly to the heparan sulfate proteoglycan, and a large dermatan sulfate proteoglycan is primarily

secreted into the medium. Mouse skin cultures produce a heparan sulfate proteoglycan with properties similar to that produced by the granulosa cells. Whether the skin cultures are maintained as stem cells or induced to differentiate in a high calcium medium, little difference is seen in proteoglycan synthesis. However, under low calcium conditions considerably greater amounts of hyaluronic acid are synthesized. Finally, keratan sulfate proteoglycans synthesized by human corneal tissues are being characterized. Although similar amounts of proteoglycan are synthesized in patients with corneal macular dystrophy, the glycosaminoglycan chains appear to have an abnormal structure and lack incorporated sulfate groups.

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MINERALIZED TISSUE RESEARCH BRANCH

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00012-21	Bruce O. Fowler	Infrared & Raman Spectroscopic Studies of Teeth, bones, compounds
Z01 DE00074-11	John D. Termine	Bone and Tooth Matrix Biochemistry and Metabolism
Z01 DE00088-10	Edward D. Eanes	Interaction of Apatitic Substrates with Calcium Phosphates
Z01 DE00134-09	Vincent Hascall	Structure and Biosynthesis of Proteoglycans
Z01 DE00157-08	Dennis A. Torchia	Biophysical Studies on the Structure of Connective Tissue
Z01 DE00162-07	John L. Meyer	Kinetic & Thermodynamic Portrait of Calcium Phosphate Precipitation
Z01 DE00204-06	Akepati H. Reddi	Collagenous Matrix and Bone Differentiation

LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY

During the course of the past year major changes have taken place in the Laboratory of Microbiology and Immunology. Most noteworthy has been the departure of a section chief and senior investigator as well as several support personnel from the section of cellular immunology. It should be pointed out that this section, under the leadership of Dr. Joost J. Oppenheim, has brought considerable recognition to the laboratory and the NIDR because of its sustained and outstanding achievements in immunology during the past decade. For this reason, it comes as no surprise that these scientists have been offered several attractive positions in recent years within and outside the NIH.

Dr. John J. Farrar moved to Hoffmann LaRoche in September, 1982 while Dr. Oppenheim took a position as a Branch Chief at the National Cancer Institute in January, 1983. At these institutions they have been provided considerable opportunity to broaden the scope of their research interests on lymphokines and their regulatory role in the immune response. Dr. Sharon Wahl will assume a leadership role in a section that will focus more directly on the immunobiology and biochemistry of mononuclear leukocytes and their cytokines which have an impact on connective tissue metabolism and are involved in the pathogenesis of chronic inflammatory diseases.

Considerable effort has gone into a modest expansion and solidification of certain of our other programs which have shown genuine progress during the past year particularly in the areas of microbial ecology and pathogenesis as well as molecular biology. With the reality of severe constraints on replacing personnel who have left the Laboratory our immediate goals have been to promote our research by enhancing collaborations within the Laboratory as well as on the outside. This has been a rewarding endeavor as indicated by the many visible signs of cooperation between immunologists and bacteriologists. Thus, the two basic science disciplines that are represented in LMI no longer exist in semi-isolation but are pooling their resources and expertise toward the solution of problems of first importance. These and other shifting fashions in our approaches to biomedical research have justified the principal premises on which programs of the Laboratory have contributed consistently and meaningfully during the past quarter of a century.

MICROBIOLOGY SECTION

The Microbiology Section continues its broadly based research program in microbial ecology, biochemistry and molecular biology. The program has, as its ultimate objective, to explain physiological and pathological traits of oral microorganisms in molecular terms. The underlying theme of this program is founded on the premise that new knowledge accruing from such inquiries will establish the basis for the rational formulation of future approaches to the control or eventual eradication of oral diseases having a microbial etiology.

The ecology program is investigating intergeneric coaggregation reactions that occur among various members of the oral microbial flora. Our previous work established that interactions between oral actinomyces and strains of *Streptococcus sanguis* are mediated by a complex array of cell surface molecules. A model has been proposed to account for all of the coaggregation patterns observed, which involves seven different complementary pairs of surface molecules on the actinomyces and streptococci. One aspect of our current work involves an attempt to characterize the surface structures involved in the lactose non-reversible types of coaggregation. To this end, bacteriophages have been used to probe the cell surface of the actinomyces. In collaboration with Dr. Alan Delisle at the University of Maryland School of Dentistry, four lytic phages have been isolated from *Actinomyces viscosus* MG1. One of the phages (AV3) was selected for further study. Mutants of *A. viscosus* MG1 that are resistant to AV3 were isolated and tested for their coaggregation patterns with *Streptococcus sanguis*. The phage resistant mutants had lost their ability to coaggregate with group 1 and 2 streptococci, but retained their ability to participate in all other previously described coaggregation patterns. This and other data indicate that the phage receptor site on the actinomycete cell carries the surface molecule that mediates coaggregation with *S. sanguis*. This significant finding has prompted the preparation of antibodies against AV3 and *A. viscosus* MG1 in an attempt to further analyze this particular type of coaggregation.

Another aspect of this study involves surveys of other oral bacteria that might participate in this form of cell-cell recognition. It has been found that *Cytophaga* species coaggregate with *Actinomyces israelii*, but not with *S. sanguis*. This suggests that certain of the actinomyces might serve as a "bridge" between the initial tooth colonizer, *S. sanguis*, and members of the gram-negative flora. The coaggregation reaction between *Cytophaga* strain PC 1000 and *A. israelii* was effectively inhibited by N-acetyl galactosamine, N-acetyl glucosamine, and N-acetyl neuraminic acid. Recent evidence indicates that there may be three distinct surface molecules involved in this type of coaggregation.

About 70 strains of *Bacteroides* species have also been analyzed for their coaggregation properties. An interesting result from this survey was that strains of *B. loescheii* participated in a lactose-inhibitable coaggregation with *S. sanguis*. Since one of the coaggregation reactions between *S. sanguis* and *A. viscosus* is also inhibited by lactose, it is likely that the same surface molecule on *S. sanguis* is involved in the coaggregation reaction with both *B. loescheii* and *A. viscosus*. This hypothesis was reinforced by the observation that a spontaneous mutant of *S. sanguis* that failed to coaggregate with *A. viscosus* also failed to coaggregate with strains of *B. loescheii*.

Our biochemical studies have focused on mechanisms of carbohydrate transport and metabolism by lactic acid bacteria. Last year we reported the discovery of a novel three-step futile cycle in *Streptococcus lactis* that explained the bacteriostatic effect of the glucose analogue, 2-deoxyglucose (2DG). The first step of the cycle involves translocation and phosphorylation of 2DG to 2DG-6 phosphate (2DGP) *via* the mannose-PTS. In the second step, intracellular 2DGP is dephosphorylated by a hexose-6P: phosphohydrolase and, the third step involves the export of 2DG from the cell. Operation of this cycle depletes the cell of energy (ATP) and accounts for the bacteriostatic effect of the 2-deoxy glucose. As part of our approach to understanding the molecular events in the cycle, the hexose-6P: phosphohydrolase responsible for step 2 has been purified and extensively characterized. The final and more complex step is now being analyzed. In order to study the export step, mutants have been isolated that are defective in the mannose-PTS. In addition, a novel strategy has been developed to introduce non-phosphorylated glucose analogues into the cell. [^{14}C] 2-deoxyglucosyl-lactose and [^{14}C] 2-fluoroglucosyl-lactose have been synthesized enzymatically and shown to be rapidly translocated into the cell T3via the lactose-PTS. It has also been shown that both analogues are hydrolyzed by phospho-β-galactosidase to generate high intracellular levels of 2DG or 2-fluroglucose (2FG). By means of this "Trojan Horse" technique, it has been possible to pre-load cells with 2DG or 2FG and to study their export in the mannose-PTS defective mutants. Results indicate that either Enzyme II^{B} or a non-PTS glucose permease mediates analogue efflux.

Significant progress has also been made in our efforts to characterize the structure and function of enzymes involved in the transport of xylitol by *Lactobacillus casei*. The soluble xylitol specific component of the phosphotransferase system (Enzyme III) has been purified to homogeneity and characterized as a highly hydrophobic protein. This unexpected property has now been found to be due to the fact that Enzyme III was isolated and purified in a phosphorylated state. Treatment of purified Enzyme III with alkaline phosphatase resulted in a dramatic alteration of its properties. First, its electrophoretic mobility in anionic gels increased, indicating conversion to a more basic molecule. Second, antiserum prepared against phosphorylated Enzyme III did not react with the dephosphorylated form. It appears that the hydrophobic nature of phospho-Enzyme III allows its association with the cell membrane to facilitate its interaction with other membrane associated components of the xylitol phosphotransferase system.

The section's molecular biology program continues to explore recombinant DNA technology for the study of physiological and pathologenic traits of the oral bacterial flora. Last year we reported cloning a phospho-β-galactosi-dase gene carried on the *L. casei* plasmid, pLZ 64, into *E. coli*. This important undertaking not only allowed the definitive location of the gene coding for phospho-β-galactosidase on the *L. casei* plasmid, but was instrumental in developing an approach for investigating potential pathogenic traits of other oral bacteria at the molecular level. Recombinant DNA technology has now been used to establish a cosmid library of *A. viscosus* DNA in *E. coli*. Over 500 *E. coli* clones containing overlapping fragments of *A. viscosus* DNA have been isolated. On a statistical basis, this accounts for the entire *A. viscosus* chromosome. This library can now be used to study all *A. viscosus* gene and its product that is expressed in *E. coli* and for which a suitable selection technique exist.

In collaboration with the Humoral Immunology Section, the library has been screened for the expression of a "subunit" of *A. viscosus* type 2 fimbria. The type II fimbria carry the molecule responsible for the lactose-inhibitable coaggregation between *A. viscosus* and *S. sanguis*. To date, one clone has been identified that produces a protein that reacts specifically with antiserum prepared against purified type II fimbriae. Subclones are now being prepared to locate and characterize the gene(s) coding for this important *A. viscosus* protein.

Although cloning genes from *L. casei* and *A. viscosus* into *E. coli* has clearly been successful, it is also important to develop direct genetic exchange systems for the lactic acid bacteria. To this end, a technique is being developed for DNA transformation of *L. casei* protoplasts. The first step in this difficult undertaking has been accomplished. A procedure has been developed for protoplast regeneration. Unfortunately, DNA transformation attempts have, to date, been unsuccessful. Recently, however, it was discovered that *L. casei* produces a DNase which rapidly degrades transforming DNA. DNase negative mutants have recently been isolated and they are now being tested for their transformability.

HUMORAL IMMUNITY SECTION

The focus of the Humoral Immunity Section has changed dramatically in the past year. With the removal from the section of several personnel and their transfer to the Cellular Immunology Section, the emphasis of this section has been placed on pathogenic mechanisms which are involved in microbial adherence, colonization and the subsequent initiation of inflammation. This sequence of events is dependent on initial basic interactions between surface structures present on bacteria with those on other bacteria, mammalian cells or components of host defense mediator systems. The activities of this Section are now concentrated in two major areas: 1.) immunochemical and physical characterization of bacterial surface structures involved in adherence and 2.) the interaction of a major host defense system, the complement system, with oral bacteria.

In extending previous findings that two antigenically distinct fimbriae are present on the surface of *Actinomyces viscosus* T14V, the functional properties of both of these fimbriae have now been precisely defined. Recent studies of the type 1 fimbriae clearly define their function in adherence to tooth surfaces as determined experimentally by attachment to saliva treated hydroxyapatite (SHA). Monospecific antisera against these fimbriae specifically inhibit bacterial attachment to SHA and previously attached bacteria are desorbed by antibodies or Fab fragments specific for the type 1 structures. Mutants of *A. viscosus* T14V which lack type 1 fimbriae have been selected by their lack of reactivity with anti-type 1 monospecific antibodies and these mutants are unable to attach to this experimental matrix. The type 2 fimbriae on *A. viscosus* T14V are associated with a lectin which mediates lactose reversible adherence to streptococci and neuraminidase treated erythrocytes. In examining other strains of actinomyces, only type 2 fimbriae have been found on typical strains of *A. naeslundii* by stringent immunochemical criteria. These findings clearly establish that the type 2 fimbriae on these strains of *A. naeslundii* are antigenically related to the type 2 on *A. viscosus* and, moreover, it has been shown that their functional properties are also similar. The fact that typical strains of *A. viscosus* and *A. naeslundii* differ in their abilities to colonize tooth and epithelial surfaces is apparently related to the distribution of the two fimbriae, each with a distinct functional property, on the two species. These findings provide a sound basis for the possible immunological intervention of attachment of oral bacteria to specific surfaces by immunization with the purified fimbriae. Such studies have been initiated in collaboration with Dr. William Clark at the University of Florida.

Investigations are also underway to identify the carbohydrate receptor on streptococci for the lectin associated with type 2 fimbriae on *A. viscosus*. An antigen with this property has been identified on *S. sanguis* 34; mutants lacking this receptor have been obtained and monospecific antibodies against the receptor have been prepared. These antibodies have identified a similar surface structure on several streptococci which exhibit lactose reversible coaggregation with *A. viscosus*.

A major breakthrough has been achieved through a collaborative effort with the Section of Microbiology. A protein detected by its reactivity with monospecific and monoclonal antibodies to *A. viscosus* type 2 fimbriae has been cloned in *E. coli*. This relatively low molecular weight unit may, in fact represent a subunit of the type 2 fimbriae. This finding has important implications for future studies of the biosynthesis and assembly of these fimbriae, an area which until now has not been possible to explore due to the inability to obtain subunits of these structures by conventional techniques.

A new area, the interaction of the complement system with oral bacteria, was initiated following restructuring of this Section. Studies of the activation of complement by monoclonal antibodies reactive with the *A. viscosus* fimbriae are providing information concerning the localization of epitopes on these fimbriae and, in addition, are generating valuable data concerning the ability of the different classes of monoclonal antibodies to activate this mediator system, an area in which only meager information exists. These investigations have demonstrated that different classes of monoclonal antibodies with different specificities can cooperate in the activation of complement. A basic requirement for the expression of this biological activity by antibodies of the IgG class is that two antibodies must interact with epitopes within a defined spatial distance to permit the Clq subcomponent of the first complement component to bind to the Fc portions of both antibodies. Studies utilizing the five monoclonal antibodies reactive with the type 1 fimbriae have revealed three distinct patterns of fixation: addition, synergy or inhibition. Additive complement fixation curves are obtained with two different antibodies, both of which fix complement alone and react with the same epitope. Antibodies which react with different epitopes and fix complement alone are synergistic in activating the complement sequence and exhibit this effect at concentrations of each antibody which do not activate the complement sequence alone. Inhibition of complement fixation is observed when two antibodies, both of which react with the same epitope or epitopes very close to each other but only one of which fixes complement are incubated with fimbriae and complement. The critical demonstration that the Fc fragments of both the participating antibodies are

involved in this activity has nearly been completed utilizing combinations of $F(ab')_2$ fragments (which bind to and aggregate antigen identically to native antibodies but do not activate complement in the assay system employed) and intact antibodies. In all cases examined thus far, the Fc fragments have been found to be required for additive and synergistic effects.

Studies have been completed on another aspect of complement initiated inflammation, namely, the ability of complement to induce the synthesis of prostaglandins by macrophages. Antibodies or their $F(ab')_2$fragments reactive with cell surface antigens initiate complement dependent synthesis of prostaglandins by macrophages. This effect requires an intact complement system in that serum from rabbits genetically lacking the sixth component of complement does not support prostaglandin synthesis. The addition of exogenous arachidonic acid, the prostaglandin precursor, markedly enhances prostaglandin synthesis and no prostaglandins are produced if the inhibitor of prostaglandin synthesis, indomethacin, is added to the cultures. Since many bacterial products are known to activate complement, this may be a mechanism by which prostaglandin mediated damage to oral connective tissue is initiated or potentiated.

CELLULAR IMMUNOLOGY SECTION

As indicated earlier, the Cellular Immunology Section has recently been reorganized and has experienced a significant loss of personnel. However, the Section will continue to pursue the basic mechanisms by which the host defenses to microbial and other antigens mobilize and modulate cellular and antibody-mediated inflammatory reactions. The biological and biochemical properties of the hormone-like immunoregulatory molecules produced by stimulated lymphocytes and monocytes in response to antigenic stimulation are being investigated. Current efforts focus on how these lymphokines and monokines interact with other inflammatory cells and noninflammatory cells, influence the integrity of connective tissue and bone in addition to effecting the killing of microorganisms.

Immune responses can lead to either excessive destruction or formation of connective tissue (collagen). The changes in the connective tissue may be mediated by the local production and release of lymphocyte products. One such product of human peripheral blood T lymphocytes activated by mitogens or specific antigen is a soluble mediator, fibroblast activating factor, which stimulates division of fibroblasts, the major cells involved in the synthesis of connective tissue elements. Characterization of this lymphokine may contribute to understanding the mechanisms which mediate fibrosis in normal tissue repair as well as in the pathophysiologic fibrotic response associated with certain chronic inflammatory diseases. A human T cell line and T lymphocyte hybridomas have been identified which produce these molecules in sufficient quantity to enable characterization and monoclonal antibody production. It is also possible to isolate cells from an inflammatory site and determine whether these cells generate similar molecules. Synovial fluid and synovial tissue obtained from rheumatoid arthritis patients at synovectomy provide a unique opportunity to study an *in vivo* inflammatory response. Cells obtained in this way have been found to be predominantly T lymphocytes and monocytes which are already activated and which generate fibroblast growth promoting activity without further stimulation. Thus, these cells activated in the joint produce molecules which can cause expansion of the fibroblast population. These products appear to be similar or identical to the factors produced by peripheral blood lymphocytes and monocytes stimulated *in vitro*. Furthermore, the synovial fluid also appears to contain these mediators, further documenting the role lymphocytes, monocytes, and their products may have in the overgrowth of the synovial membrane and the inflammatory synovitis which is characteristic of arthritis.

Since lymphocytes play a significant role in perpetuating the inflammatory synovitis of rheumatoid arthritis, lymphocyte depletion (leukapheresis) has been used to ameliorate synovitis. In collaborative studies with NIADDK, it has been shown that improvement does not correlate with lymphopenia, but rather with the apparent immunological status of the patient at the time of treatment. The clinical and immunological effects of a brief course of leukapheresis were studied in two clinically similar groups of patients with active refractory rheumatoid arthritis. On completion of a brief course of leukapheresis (6 runs in 2 weeks), 6 of 7 patients in Group A (subnormal lymphocyte proliferation) demonstrated a 40% decrease in their articular indices, whereas 0 of 7 patients in Group B (normal lymphocyte function) demonstrated improvement. Furthermore, the clinical responders but not the clinical nonresponders, showed significantly improved lymphoproliferation (200% increases in stimulation index) and an augmentation of originally depressed skin test responses. Thus the clinical improvement produced by leukapheresis in a subset of RA patients was not dependent upon induction of immunosuppression and appeared to reflect the depletion of functionally abnormal mononuclear cells. Therefore, immunosuppressed patients responded favorably to leukapheresis whereas patients with normal lymphoid cell function failed to improve clinically after treatment. Thus, inroads are being made into the mechanisms of chronic inflammatory joint disease and how the course

of the disease can be altered by manipulation of the immune system.

An experimental rat model of streptococcal cell wall-induced arthritis is being utilized in collaboration with NIADDK scientists to define the genetic mechanisms underlying arthritis susceptibility and resistance. Evidence suggests that arthritis susceptible rats have a defect in their inflammatory cells which allows the streptococcal cell walls to reach the joints. These animals are unable to contain the streptococcal cell walls locally and thus the cell walls are disseminated to other sites in the body, including the synovial tissue, causing inflammation and arthritis. In the arthritis-resistant rats, however, the inflammatory cells (macrophages) more effectively contain the bacterial cell walls, and therefore prevent them from reaching the synovial tissue. Thus, these studies suggest that the ability of inflammatory cells to contain streptococcal cell walls or other agents may play a central role in determining whether or not arthritis will be induced.

Another facet of the regulatory role of the immune system on connective tissue metabolism involves the immunologic modulation of bone resorption. Two model systems of bone resorption are currently being explored: The osteopetrotic (op) rat and the toothless (tl) rat. Although the two models have in common defective bone resorption, they do not share common immune defects. The op rats have excessive suppressor function which is similar to osteopetrosis in humans, whereas the tl rats have hyperactive immune responses. How these immunological variants translate into defective bone resorption is being investigated. Recent observations indicate that the tl rat spleen cell macrophages generate greater levels of 5-hydroxy-eicosatetraenoic acid (5-HETE) than normal littermates which may be involved in regulating bone resorption.

Additional involvement of monocytes in contributing to connective tissue metabolism is through the production of the neutral protease, collagenase. New steps in the activation pathway leading to the production of collagenase have recently been elucidated utilizing various inhibitors of lipomodulin, PGE_2, phospholipase A_2 and microtubules. In addition, ornithine decarboxylase and polyamines have been shown to participate in the production of collagenase at a stage subsequent to the elevation of intracellular cAMP. Through the recognition of the intracellular sequence leading to macrophage PGE_2 and collagenase synthesis, it becomes possible to pharmacologically intervene in the tissue destructive potential of an inflammatory response. In additional studies, human peripheral blood monocytes isolated in large quantities by counterflow centrifugal elutriation have been shown to be a productive source of collagenase, an observation that has been elusive because of the inability to obtain adequate cell numbers in earlier studies.

Important insights into the mechanisms of monocyte-macrophage killing of microorganisms have been obtained in the investigations with *Giardia lamblia*, an extracellular protozoan parasite. The mechanisms of monocyte chemoattraction for extracellular organisms, the role of oxygen metabolites in the extracellular killing of mucosal organisms and the role of prostaglandins in macrophage regulation of cytotoxicity are currently being defined. Little is known of the mechanisms by which monocytes deal with extracellular parasites and using giardia as a prototype, monocyte-parasite interactions can be analyzed.

Another important product of activated macrophages is the soluble mediator, interleukin 1 (IL1), which has a multiplicity of biological effects on inflammatory and noninflammatory cells. Investigations continue to focus on its mechanisms of action as well as on its biochemical purification and the generation of monoclonal antibodies towards the isolated molecule. To expedite this approach, a human monocyte cell line has been identified which produces significant amounts of IL1 and can be used as a continuous source of the mediator. Taking IL1 production a step further involves the use of recombinant DNA technology to clone the genes for IL1. Using monoclonal antibodies to IL1, the translation products of mRNA can be identified and positive cDNA clones can then be used for selected gene transfer to study the regulation of the expression of IL1. Similar techniques will be used for other mediators.

Significant advances have been made by members of the Cellular Immunology Section in the NIH investigation of the acquired immune deficiency syndrome (AIDS). By utilizing numerous assays to evaluate monocyte functions, multiple defective monocyte-macrophage functions have been identified in this syndrome. Prior to the contribution of this Section, the disease was considered to be the consequence of a defective T cell population. However, the defective monocyte functions may play a significant role in the development of this lethal disease.

The Clinical Immunology Section continues its fundamental studies on the mechanisms of cell secretion. A rat basophilic leukemia cell line was established which has surface immunoglobulin receptors and contains histamine and serotonin. These cells can be activated to secrete their cellular content of histamine. The crosslinking of the IgE receptor with either antigen or hybridoma anti-receptor antibody results in a number of biochemical events including increased phospholipid methylation, the influx of Ca^{++} into the cells, the release of arachidonic acid and of histamine. The release of

arachidonic acid is due to the activation of phospholipase enzymes, which is an essential step in the histamine release pathway. A series of experiments compared in parallel the effect of pharmacological agents on the Ca^{++} influx, phospholipase activation and histamine release. A very interesting finding was that microtubule depolymerizing agents had no effect on Ca^{++} influx but blocked the IgE-or ionophore induced arachidonic acid and histamine release. Therefore, the coupling of receptor bridging to Ca^{++} influx is independent of the microtubular system but the further activation of the phospholipase system requires the function of the microtubules. Another approach is to isolate mutants of the rat basophilic leukemia cell line which are defective for secretion and also have deficiences in enzyme systems. Variants have been isolated with inhibitors of the microtubular system and these should prove useful for analyzing the early steps in cell activation and secretion.

Further studies on cell secretion have concentrated on the variation which has been observed in cell lines. By fractionating the cells according to cell size it was found that there was heterogeneity in the capacity of cells for histamine release at different stages in the cell cycle with the least release in the cells in the G_1 phase and the optimal reaction with the more mature cells.

Monoclonal antibodies are also being used to dissect the immunoglobulin receptors on cells involved in triggering the release of mediators. A series of four monoclonal antibodies have been produced which block the binding of IgE with its cell surface receptor. Three of these monoclonal antibodies bind to the receptor activation site and can be used to precipitate the receptor from the cell surface. The fourth antibody binds to a receptor associated protein which has not been previously recognized. These antibodies will be useful for studies to define the structural components of the cell surface receptor and as tools in attempts to clone the receptor genes. A clearer definition of the structure of the receptor could allow manipulation to inhibit the secretion of mediators involved in acute inflammation.

A series of monoclonal antibodies have been produced which react with *Cytophaga* species. These are gram negative filamentous organisms which preferentially colonize the cementum of teeth and might play an important role in periodontal disease. In collaborations with microbiologists in the Laboratory, a series of four monoclonal antibodies have been produced and purified which react with *Cytophaga* (strain NS1001). Immunofluorescent and enzyme-linked assay procedures utilizing these antibodies are being developed to detect these organisms in dental plaque. In other studies, these antibodies are being used to inhibit the attachment of these organisms to solid surfaces. Such studies will hopefully shed light on the mechanisms by which these bacteria attach to the cementum of the tooth.

LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY

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LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00007-23	Charles L. Wittenberger	Carbohydrate Metabolism & Lactic Acid Production in Microorganisms
Z01 DE00022-17	Jack P. London	Phylogenetic Mapping of the Homofermentative Lactic Acid Bacteria
Z01 DE00034-15	Reuben Siraganian	Immunological Mechanisms Involved in Histamine Liberation
Z01 DE00042-13	Bruce M. Chassy	Regulation of Carbohydrate Metabolsim in Oral Streptococci
Z01 DE00043-13	Jacob A. Donkersloot	Physiological Studies on Pathogenic Oral Microorganisms
Z01 DE00046-12	Sharon M. Wahl	Chemotactic Factors of B & T Lymphocytes & Inflammatory Lesions
Z01 DE00061-10	Ann L. Sandberg	Complement Activation & Its Relationship to Inflammatory Response
Z01 DE00216-07	Larry M. Wahl	Immunological Control of Connective Tissue Metabolism
Z01 DE00254-06	John O. Cisar	Adherence of Oral Actinomycetes to Oral Streptococci
Z01 DE00273-05	Paul E. Kolenbrander	Studies on the Properties of Oral Actinomycetes
Z01 DE00290-04	Reuben Siraganian	Production of Hybridomas
Z01 DE00316-03	T. Krakauer	Biochemical Characterization of Biological Medicators
Z01 DE00341-02	J. Thompson	Regulation of Sugar Transport & Metabolism in Lactic Acid Bacteria

Z01 DE00374-01	D. Pluznik	Generation of GM-CSF By Short-Term Cultures of Murine Bone Marrow Cells
Z01 DE00375-01	P. Smith	Monocyte Responses to an Extracellular Protozoan, *Giardia lamblia*

LABORATORY OF ORAL BIOLOGY AND PHYSIOLOGY

The past year witnessed several changes which had a major impact on the former Laboratory of Biological Structure. The realignment of research groups from LBS and the Laboratory of Biochemistry to form the new Mineralized Tissue Research Branch considerably reduced the size of the resulting Laboratory of Oral Biology and Physiology. The incorporation of the Enzyme Chemistry Section into LOBP has redefined our research goals and opened potential areas for new collaborative interactions. At the same time, the proximity of MTRB research groups encourages continuation of our close relationships with them at all levels. The completion of the long-scheduled renovations provided a modest increase in laboratory space on the second floor for LOBP, and a new electron microscope and photography suite on the fifth floor. Relocation of the transmission electron microscopes and installation of a new scanning electron microscope was completed in February, and the instruments are in full operation. Delivery of a new JEOL 100-CX transmission electron microscope, anticipated this Fall, should ensure state-of-the-art instrumental capability for the next several years.

Despite these major changes and their consequent disruptions and demands on the time of our professional and support staff, considerable research progress has been achieved during the past year, and is summarized below.

EXPERIMENTAL MORPHOLOGY SECTION

The research efforts of the Experimental Morphology Section continue to focus on the mechanisms of exocrine secretion. Our approach to these problems has been to correlate structural analyses with biochemical, immunological and physiological methods. One area of investigation which continues to expand is that of the regulation of secretory cells, at the cellular, subcellular and molecular levels. The rat parotid gland has proved to be a useful model for these studies.

Beta-adrenergic receptor-ligand interactions are mediated through changes in intracellular cyclic AMP levels, which regulate the activity of cyclic AMP-dependent protein kinases. On a short term basis, the most prominent effect of beta-adrenergic stimulation is exocytosis. Our studies have shown that there are stimulation-dependent shifts in the distribution of cyclic AMP-dependent protein kinase, and that the regulatory subunits exhibit behavior similar to secretory proteins: they are associated with particulate cellular components (mainly secretory granules) and are released into the saliva. Another sequela of beta-adrenergic stimulation is an increase in the permeability of the tight junctions between adjacent cells. The physiological (or pharmacological) alteration of the paracellular pathway and potential regulation of the passage of macromolecules from the interstitial spaces to saliva (or vice versa) may have important implications. Despite the clear evidence for tracer penetration through the tight junctions, changes in junctional structure (as observed in thin sections or in freeze-fracture replicas) indicative of increased permeability have not been observed. No increase in junctional permeability occurs when secretion is stimulated with alpha-adrenergic or cholinergic agonists. Two recent developments have provided new tools to investigate the results of beta-receptor-ligand interactions. Tritiated and biotinylated derivatives of the irreversible beta-receptor antagonist, bromoacetylalprenololmenthane, synthesized by investigators at the National Institute on Aging, are being used to study receptor localization in the rat parotid gland. This work, carried out in collaboration with the Clinical Investigations Section, CIPCB, should yield valuable new information on the distribution of receptor sites on the surface of the acinar cells, the fate of the receptor following beta-adrenergic receptors. Other LOBP studies have demonstrated that many of the short term actions of beta-receptor-ligand interactions can be effectively mimicked by the drug forskolin. Forskolin directly activates adenylate cyclase, bypassing the beta-receptor. This provides an additional means to dissect and characterize the various steps in cellular signal processing.

Long-term effects of beta-adrenergic stimulation of the rat parotid gland are hypertrophy and hyperplasia of the acinar cells. The attendant increases in nucleic acid and protein synthesis and alteration of gene expression, provide a wealth of possibilities for investigating the regulation of various cellular processes. For example, our results demonstrate a marked reduction in cyclic AMP-dependent protein kinase regulatory subunits with chronic beta-adrenergic stimulation. Substantial changes also occur in cell structure, lysosomal activity and various other parameters. We are currently developing a number of methods to probe these alterations in depth: production of monoclonal and polyclonal antibodies to specific cellular and secretory proteins; in vitro translation of specific mRNA's; and immunocytochemical studies of specific protein localization.

Studies directed toward elucidation of endocytic processes and the fate of internalized membrane in secretory and other cell types constitute an important part of our efforts to understand the basic mechanisms underlying the process of secretion. A variety of probes have been utilized to label and identify various cellular

membranes. In addition to enzyme cytochemistry, colloidal gold, ferritin and horseradish peroxidase isozymes have been employed in these studies. Horseradish peroxidase, long used as a soluble-phase tracer for electron microscopic studies, appears to be endocytosed partly bound to the cell membrane. Horseradish peroxidase isozymes with different isoelectric points have different affinities for uptake from the luminal and basal-lateral cell surfaces, and various sugars inhibit or stimulate endocytosis of horseradish peroxidase. Once internalized, the fate of the horseradish peroxidase appears to depend upon its site of uptake, that from the luminal surface entering multivesicular and dense bodies, while that from the basal-lateral surfaces is first sequestered in the basal lysosome system described a few years ago. The basal lysosomes appear to be widely distributed among exocrine glands and between various species, and they exhibit changes in intracellular distribution following secretagogue administration, suggesting a close relationship between receptor activation and lysosomal functions. These studies soon will be extended with the development of monoclonal antibodies for the localization of cell-surface components, and the use of lectins to characterize the carbohydrate constituents of the cell surface and intracellular membranes.

ENZYME CHEMISTRY SECTION

The research interests of the Enzyme Chemistry Section focus primarily on the posttranslational modifications of proteins and the enzymes involved in these modifications. As in past years, the formation of gamma-glutamyl amide bonds by transglutaminases have been the principal subject of these investigations. Studies of substrate specificity of the transglutaminases have utilized model peptides synthesized with variations in the sequence of amino acids around the glutamine residue which is modified by the enzyme. While certain amino acid substitutions still allow recognition by the enzyme, it is evident that the enzyme-substrate interactions are dependent on the tertiary structure of the substrate molecule. A practical application of this molecular level knowledge of enzyme-substrate specificities can be seen in the development of a transglutaminase-mediated procedure for photolabeling of peptides and for production of cleavable crosslinks between molecules. Various photolabeled hormones have already been prepared for studies of cell surface receptors.

The products of transglutaminase activity are widespread, being found in fibrin clots, cell membranes, myofibrils, seminal plasma, wool keratin and hair proteins, among others. Thus, investigations of the physiological functions of transglutaminases and their products constitute an important part of the Section's work. An animal model to study transglutaminase deficiency was produced by injecting rabbits with antibodies to the catalytic subunit of Factor XIII (plasma transglutaminase). The ensuing rapid fall of plasma transglutaminase to less than 5% of control levels provides a means to study enzyme synthesis and turnover, as well as the role of transglutaminase in various physiological and pathological processes, such as wound healing and disseminated intravascular coagulation. In addition, it was shown that the incorporation of alpha 2 PI, a plasmin inhibitor, into fibrinogen and fibrin is catalyzed by red blood cell transglutaminase. This data suggests that transglutaminases may have an important function in the regulation of fibrinolysis. Significant new findings have also emerged from studies on the catabolism of transglutaminase-modified proteins, particularly those containing epsilon-(gamma-glutamyl)lysine crosslinks. These gamma-glutamylamines are not cleaved in intact proteins, nor during normal proteolytic degradation of the proteins. Instead, the gamma-glutamylamines are transported to the kidney, where they are broken down by the enzyme gamma-glutamylamine cyclotransferase, with release of the products back to the circulation.

A major discovery during the past year was the identification of eukaryotic initiation factor-4D (eIF-4D) as the single protein in all mammalian cells examined which contains the unusual amino acid hypusine. Hypusine is formed posttranslationally from the butylamine moiety of the polyamine spermidine and a protein bound lysine. Formation of hypusine occurs only after stimulation of cell growth. While such an unusual modification of a single protein would appear to be highly significant, the physiolgical role of eIF-4D is unknown. The production and utilization of antibodies to eIF-4D, studies currently underway, may provide important clues to its function.

LABORATORY OF ORAL BIOLOGY AND PHYSIOLOGY

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LABORATORY OF ORAL BIOLOGY AND PHYSIOLOGY

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00001-31	John E. Folk	Chemical, StereoChemical, Confirmational Aspects of Transglutaminase
Z01 DE00028-16	Arthur R. Hand	Ultrastructure & Cytochemistry of Salivary Glands
Z01 DE00049-12	Soo Il Chung	Physiological Function of Transglutaminases
Z01 DE00199-07	Constance Oliver	*In Vitro* Studies of Salivary Gland Structure and Function
Z01 DE00215-07	Sandra L. Lee	Connective Tissue: Formation and Structure
Z01 DE00217-05	Robert O. Wolf	Salivary Systems
Z01 DE00285-04	Maija Mednieks	Regulation of Protein Secretion in Salivary Glands
Z01 DE00311-03	John E. Folk	Unusual Amino Acid, Hypusine; Mechanism of Formation & Function

LABORATORY OF DEVELOPMENTAL BIOLOGY & ANOMALIES

Research in the Laboratory of Developmental Biology and Anomalies is concentrated in normal and abnormal development, on wound healing, and on various acquired and inherited disorders. In general, rather basic questions are chosen for study including the nature and structure of connective tissue proteins, their gene structure and cellular behavior. However, rapid application of research findings to important biological and pathological questions is encouraged. Diverse methodologies are utilized in LDBA including biochemistry, genetic studies, molecular biology and recombinant DNA technology, cell culture and animal experimentation.

Some changes have occurred in senior personnel in LDBA. Dr. Mark Sobel, our senior molecular biologist has returned to the National Cancer Institute to work on the genes involved in tumor metastasis. He has been replaced by Dr. Yoshihiko Yamada formerly of the Laboratory of Molecular Biology in the National Cancer Institute. Dr. Yamada is well known for his work defining the structure of the gene for type III collagen and the gene for fibronectin. Additional persons (Drs. K. Kohno and Y. Sakurai) have been recruited to the molecular biology group and work has been intensified on the structure of connective tissue genes and changes in gene structure and activity during development and in diseases (see below).

Further, due to his increasing interest in the biology of tumor cells, Dr. Elliott Schiffmann has undertaken a years training from July, 1983-July, 1984 in the Laboratory of Pathology, NCI. Dr. David Gershon, Professor of Biology at the Technion Institute in Haifa Israel will work in LDBA during the same period. Dr. Gershon is a world reknowned expert on cellular and molecular aspects of aging and will apply his expertise to the prominent aspects of aging of connective tissue. Dr. K. Brown of this Laboratory has received (his award) and Dr. Martin has been selected to receive a Senior Investigator Award by the Humboldt Foundation. Mrs. Frances Cannon was awarded the DHHS Handicapped Employee of the year in a ceremony at the Hubert H. Humphrey Building by Secretary Heckler.

RESEARCH ACCOMPLISHMENTS

Structure and Assembly of Extracellular Matrices

It is well established that the extracellular matrices in different histological sites such as tendon, cartilage and basement membranes contain different collagens, proteoglycans and glycoproteins. Recently, in studying the reconstitution of basement membrane in vitro from purified proteins, we noted that the individual components were soluble. However, when collagen type IV and laminin were mixed, they precipitated in defined proportions. Addition of the basement membrane proteoglycan increased the amounts of material precipitating, particularly the amounts of laminin. Components of other matrices such as type I collagen and fibronectin could not substitute in the reaction for the authentic components of basement membrane. These observations suggested that the basement membrane was assembled as a supramolecular complex of defined but regulatable composition.

Interestingly when an extract of basement membrane rather than purified components were incubating with type IV collagen, membranous structures were formed which contained the well recognized components (laminin, type IV collagen, heparan sulfate proteoglycan) plus one or two other proteins. Presumeably this other protein(s) was able to organize the components of basement membrane into membraneous structures. The appearance of these structures will be studied and the putative organizing protein will be purified for characterization. Similar studies are being carried out with other extracellular matrices to see if they contain proteins that organize their structural elements. To date these studies indicate that each matrix assembles as a defined supramolecular complex formed of the components specific to that tissue.

Isolation of the Core Protein of the Basement Membrane Proteoglycan

In previous studies we isolated a heparan sulfate proteoglycan from the basement membrane of the EHS tumor and showed that antibody to it reacted with authentic basement membranes. This proteoglycan has an important role in the basement membrane forming a charged shield over the surface which restricts the passage of protein. This proteoglycan is a large molecule (M_r=750,000) composed predominently of glycosaminoglycan chains linked to protein. Now we have used specific antibodies to the proteoglycan to identify its nascent protein core. These studies show that this proteoglycan arises from a unique protein (M_r=400,000) which is produced by various epithelial cells but not by fibroblasts. Subsequently heparan sulfate chains are added to the protein core. The completed proteoglycan undergoes modification with time with removal of part of the core protein and shortening of the heparan sulfate chains. Similar reactions were observed in the synthesis of the heparan sulfate proteoglycan by parietal yolk sac cells and by differentiating teratocarcinoma cells.

Previously, we and others have shown that diabetic basement membranes have reduced amounts of proteoglycan. We have proposed that the reduced content of proteoglycan accounts for the leakiness of basement membranes in diabetes and triggers a compensatory synthesis of matrix components causing the thickening of basement membranes observed in diabetics. These changes in the basement membrane presumeably cause the degenerative changes in a variety of tissues including blood vessels, kidneys, eyes, nerves, etc.

Now we have investigated whether the synthesis of the glycosaminoglycan chains, their sulfation or the synthesis of the core protein is limiting in diabetes. Xylosides, synthetic initiators of glycosaminoglycans biosynthesis, were added to EHS tumor tissue taken from normal and diabetic. Xyloside stimulated the synthesis of glycosaminoglycan and its sulfation equally in the diabetic and control tissue. The distribution of the xyloside stimulated material between media and tissue was similar, indicating normal processing and secretion in the diabetic. In contrast, the synthesis of the core protein appears to be reduced in the diabetic tissue and to be increased by insulin. It seems likely that the level of heparan sulfate proteoglycan is reduced in diabetes due to a decrease production of core protein.

Regulation of Metastatic Activity in Tumor Cells by Fibronectin and Laminin

We and others have identified some of the critical steps in the metastases of tumor. In brief, the metastatic tumor cells represent only a small proportion of the total cells in the tumor. Typically, these cells penetrate through basement membranes in metastasizing in the body. In vitro we have found that the metastatic tumor cells have a high affinity for type IV (basement membrane) collagen in comparison to stromal collagen (type I and III). The metastatic cells utilize laminin to bind to type IV collagen while the tumorigenic but nonmetastatic cells prefer fibronectin. The metastatic cells have a higher number of laminin receptors on their surface. Further, laminin and the laminin receptors are required for metastases and invasiveness of basement membrane, since antibody to laminin and fragments of laminin that bind to the receptor block invasivenss and metastases when administered along with the tumor cells. Finally metastatic but not nonmetastatic tumor cells secrete a special collagenase able to degrade basement membrane collagen. These studies indicate that the critical step in metastases is the ability of the tumor cells to bind to basement membranes and to degrade them. Laminin has an essential role in the process.

Most recently we have found that fibronectin and laminin can regulate the metastatic activity and the invasiveness of tumor cells. Exposure to fibronectin suppress metastatic activity and invasiveness while exposure of the cells to laminin increases these processes. Similarly after exposure to fibronectin, the number of laminin receptors on the cell surface decreases. Removal of fibronectin or the addition of laminin increases the number of receptors.

Taken together these results indicate that the metastatic cell has a defined phenotype which is subject to regulation and perhaps control by modification of laminin receptors on the surface of the cells.

Studies on Wound Healing

It is well known that different types of cells enter a wound in sequence with phagocytic cells arriving first followed by fibroblasts and smooth muscle cells and finally endothelial cells. We have been studying factors which might be involved in regulating the entry as well as the numbers of cells in the wound. During the course of this work, we discovered that platelets released a potent chemoattractant for fibroblasts and smooth muscle cells. This factor was isolated and shown to be the platelet derived growth factor (PDGF). PDGF has been studied for some time, since it is a very potent mitogen for these same cells. However, in our studies it was shown that neither DNA synthesis nor cell division was required for chemotactic activity. Nor do other growth factors have this activity. Further studies indicate that the phagocytic cells and endothelial cells do not respond to PDGF but utilize other chemotactic factors and mitogens produced in the wound by clotting and by cells entering the wound. Presumeably this cascade of chemotactic factors regulates the penetration of cells into the wound.

These observations have been applied to healing wounds. Here stainless steel chambers implanted subcutaneously in rats which elicits the standard wound healing response. These chambers can be removed at various times and their contents assay for cells, collagen and histology. In our experiments some of the chambers with PDGF, collagens, attachment proteins and other factors. These studies showed that the presence of additional PDGF in the wound chamber brought more fibroblasts into the wound and increased their proliferation. The PDGF supplimented wounds accumulated collagen at twice the rate of controls in the first 10 days but controls and supplimented reached the same level by 20 days. Similar studies have now been carried out in diabetic animal. As expected, based on human experience, the diabetic rats showed impaired wound healing. Supplimenting the wound chamber with PDGF or insulin greatly accelerated the entrance of fibroblasts. Affects of the suppliments on matrix deposition and the penetration of endothelial cells are

currently under study. In general, the results indicate that wound healing can be stimulated in the early stages with mitogens and chemotactic facts. Possibly such suppliments may aid individuals with defects in wound healing due to age or other disability.

Structure of Connective Tissue Genes

Studies have been underway for some time on the structure and regulation of collagen genes. Considerable attention has been directed toward the study of the type II collagen gene, since it represents the major structural component in cartilage. To date, we have isolated cDNA clones and genomic DNA clones comprising about 40% of the chick type II collagen gene. Under Dr. Yamada's direction recombinant DNA clones are being isolated from libraries of rat and human DNA. In addition to basic information on gene structure, these probes will be used to study the mechanisms used to coordinate the expression of cartilage genes, to look for defects in DNA in human chondrodystrophies and to study the state of these genes during differentiation and dedifferentiation. In the latter area, we are examining the state of the type II collagen gene in chondrocytes, in fibroblasts and in dedifferntiated chondrocytes using restriction enzymes which distinguish between methylated and unmethylated guanosines. These studies show that the type II collagen gene is more highly methylated in fibroblasts than in chondrocytes. These results are in accord with previous studies which show a lower methylation in active genes. However, this does not seem to be a sufficient feature for transcription. Chondrocytes treated with bromodeoxyuridine also show a low degree of methylation in their type II collagen gene but do not produce type II collagen.

Work continues on the isolation of genes for basement membrane proteins. Two approaches are being utilized. Synthetic oliognucleotides have been synthesized which correspond to known amino acid sequences in type IV collagen. These have been extended with reverse transcriptase using mRNA from tissues that produce basement membrane proteins. The extended primers have been cloned into plasmids and used to infect bacteria. The resultant colonies will be screened for the presence of type IV collagen DNA by hybridization using labeled mRNA from cells making high levels of the protein as well as screened with the origional primer.

Second, cDNA has been prepared from mRNA of cells making basement membrane proteins has been cloned and bacterial colonies with recombinant clones will be screened with labeled mRNA as above as well as with the various primers.

Chemotaxis

Since Schiffmann initiated studies on chemotaxis in this Laboratory, significant advances have been made including the identification of new chemotactic substances i.e. the formylmethionine peptides and the platelet derived growth factor, and the mechanisms involved in the activation of directed cell movement. Studies in this Laboratory have implicated chemotactic factors in several normal and certain pathological processes. Gleiber and Schiffmann have found that human carcinoma cells secrete a protein that is a potent attractant for fibroblasts and smooth muscle cells. When infused in vitro this protein induces the formation of a fibrous mass. Possibly the tumor capsule formed around such carcinomas is produced by host fibroblasts brought to the tumor by this factor.

After transformation by viruses or chemical fibroblasts lose the ability to responde chemotacticly to PDGF. Examination of SV40 3T3 cells and RAS transformed 3T3 cells indicate that they produce a mitogenic and chemotactic factor (s) that competes with authentic PDGF for its receptor. Possibly this factor maintains the uncontrolled growth of the tumor cells and are related to PDGF as part of a gene family.

Teratocarcinoma cells induced to differentiated with retinoic acid and cyclic AMP begin to produce a factor with potent mitogenic and chemotactic activity which also resembles PDGF. Since the differentiation of these cells mimics early stages in the differentiation of the embryo, it is possible that a similar factor is produced in the early embryo and is involved in the recruitment and proliferat;ion of mesenchymal cells.

Assays for Tissue Specific Factors in Blood-Chondronectin and Osteonectin

Previously we isolated chondronectin from chick serum and showed that it was produced by chondrocytes and concentrated in cartilage. Presumeably the chondronectin in serum represents material released from cartilage. Now we have purified human chondronectin and are preparing antibodies both monoclonar and polyclonal to the protein. Additionally in collaboration with Termine in the Mineralized Tissue Research Branch we have isolated human osteonectin, a bone specific protein, and are preparing antibodies to it. Preliminary observations indicate that osteonectin or immunologically related fragments exist in serum and their levels can be measured by immunoassays. It is possible that the physiological states of cartilage and bone can be inferred from measurements of these proteins and that characteristic alterations in their levels will be associated with specific human diseases.

Developmental Biology

A number of projects referred to above include studies on developing tissues and factors that influence differentiation. Recently we have isolated a protein (M_r=50-60,000) from calf serum that induces pluripotential neural crest cells to differentiate into melanocytes. It also stimulates melanogenesis in mouse melanoma cells which are dependent on the presence of serum to exhibit this activity. Antibodies against the isolated pigmentation factor prevent melanogenesis in both neural crest cell and melanoma cells. Preliminary assays show immunologically cross reacting material in pituitary and adrenal glands and extracts of these tissues stimulate melanogenesis. These tissues may be the source of the factor in serum.

A factor that suppresses the differentiation of limb bud cells, melanocytes, preadipocytes and teratocarcinoma cells has been found in fluid collected from the rete of Haller from rams testes. This factor was sought, since extracts of testes suppressed differentiation. Preliminary information suggests that it is a large protein which could be involved in maintaining stem cells in the testes and in various other organs in an undiffernetiated state. Materials with this type of activity have been detected previously by others in serum and extracts of various tissues. The rete testes fluid represents a particularly rich source for isolation.

Finally, studies continue on the influence of various components of the extracellular matrix on the differentiation of cells. Laminin has been found in collaborative studies with NINCDS scientists to induce an enhanced outgrowth of neurites from fragments of spinal ganglia and of Schwann cells. At the same time fibroblastic outgrowths are suppressed by laminin. It is possible that laminin can be added to damaged nerve tracts to enhance nerve regeneration.

LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

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LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00006-23	Elliott Schiffmann	Studies on Chemotaxis
Z01 DE00009-22	George R. Martin	The Chemistry and Biosynthesis of Connective Tissue
Z01 DE00024-17	Kenneth S. Brown	Developmental Processes in Genetically Controlled Malformations
Z01 DE00025-17	Mark Sobel	*In Vitro* Synthesis of Collagen & Characterization of Messenger Rna
Z01 DE00149-09	J. Hassell	Extracellular Matrix & Cellular Proliferation in Oral Facial Growth
Z01 DE00230-07	Hynda K. Kleinman	Attachment of Cells to Collagen
Z01 DE00253-06	Hugh Varner	Development of Cartilage
Z01 DE00275-05	George R. Martin	Biological Testing of Fluoride
Z01 DE00330-02	V. Terranova	Role of Attachment Proteins in Tumor Cell Metastasis
Z01 DE00331-02	G. Grotendorst	Connective Tissue Cell Chemoattractants

LABORATORY OF ORAL MEDICINE

The Laboratory of Oral Medicine studies the etiology and pathogenesis of both systemic diseases and diseases of the soft tissue of the oral cavity. Emphasis is on: (1) viral infections such as herpes simplex virus; (2) endocrine diseases, specifically virus-induced diabetes mellitus; (3) autoimmune disorders; and (4) ulcerative and proliferative lesions of the oral cavity. The program is disease oriented and highly interdisciplinary. The Laboratory is made up of investigators who are trained in a variety of disciplines including virology, immunology, pathology, molecular biology, and clinical medicine and dentistry.

Over the last year, in-depth studies have continued on the projects discussed in previous annual reports. Good progress has been made in our studies on the molecular biology of herpes simplex virus and on monoclonal autoantibodies.

Since last year, three members of the Laboratory have left: Dr. Martin Haspel, who was in charge of our hybridoma laboratory; Dr. Gurmit Aulakh, who developed *in situ* hybridization methods for studying herpes simplex virus; and Dr. Fumio Shimizu, who was working on hybrid cells that produce hormones. Progress has been made by the existing staff and by new members of the Laboratory to fill the void created by these departures. The hybridoma laboratory has been taken over by Dr. Bellur Prabhakar and in situ hybridization studies are being continued by Dr. Kenneth Cremer. The work on hybrid cells that make hormones, however, has been slowed down.

Three people have joined the Laboratory. Dr. Daniel Eskinazi is setting up a clinical project on the pathogenesis and treatment of leukoplakia and is beginning to use hybridoma technology to identify unique antigens associated with oral tumors. Dr. Floyd Taub was recruited to head up the pathology unit. This unit has been reorganized and its staff is now processing, for many members of the Laboratory, a large number of tissues on a regular and very reliable basis. Recently, Dr. Peter Brayton, from UCLA, joined the Laboratory. He is setting up a transfection system to look for oncogenes in oral tumors and to study the transforming potential of HSV DNA sequences.

In May of 1983, the entire Laboratory was reviewed scientifically by an 11 member outside Board that spent two and a half days at the Institute. In its written report, the Board was very complimentary, and the program of the Laboratory of Oral Medicine was commended for "excellence and its success in carrying out the missions of NIDR specifically, and NIH generally."

Since last year, a number of new techniques were introduced into the Laboratory and existing ones modified. Specific techniques include: (1) DNA sequencing using Sanger's dideoxynucleotide synthesis in M13 phage; (2) shotgun cloning of foreign DNA sequences using open reading frame (ORF) vectors to obtain the expression of HSV DNA sequences which code for herpes antigens; (3) *in situ* hybridization of DNA to metaphase chromosomes, with the ultimate goal of attempting to determine whether HSV is integrated into a particular host cell chromosome; (4) affinity chromatography for the purification of monoclonal antibodies and their subunits; (5) development and modification of methods for preparing human hybridomas to study monoclonal autoantibodies; (6) transformation of cells (pancreatic beta cells) with SV40 in an attempt to obtain continuous endocrine-producing cell lines; (7) detection of T cell lymphocyte subsets using monoclonal antibodies to evaluate the immunoregulatory changes in viral infections; (8) polyclonal B cell activation tests in mice to study the effect of viral infections; (9) avidin-biotin immunoperoxidase techniques to screen hybridomas for monoclonal antibodies and to detect islet cell antibodies in patients with diabetes mellitus; (10) measurement of soluble mediators from lymphocytes such as IL2; and (11) refinements in affinity column chromatography and Western blots to isolate and identify autoantigens.

Collaborations continue or have been initiated with investigators from other laboratories within NIDR and with investigators from other institutes at NIH and with colleagues from various universities. Active collaborative projects include: (1) screening of human tissues (ganglia and brain for herpes simplex virus DNA sequences (University of California, San Diego); (2) oncogenicity of herpes simplex virus DNA fragments and comparison with known oncogenes (NCI); (3) cloning and expression of HSV sequences (City of Hope Research Center, California, and the University of Tennessee); (4) *in situ* hybridization of HSV DNA to metaphase chromosomes (NCI); (5) studies to identify the polypeptides of Coxsackievirus that are involved in neutralization using monoclonal antibodies to Coxsackievirus B4 (Department of Microbiology, Hahnemann Medical College); (6) studies on the epidemiology of Coxsackievirus B4 variants in different geographic locations and at different times with monoclonal antibodies (Department of Microbiology, University of Rochester); (7) reactivity of human monoclonal autoantibodies with DNA, RNA, etc. (NIADDK); (8) cross reactivity of anti-Dengue virus monoclonal antibodies with normal tissues (Walter Reed Medical Research Center); (9) long-term prospective study on newly diagnosed insulin dependent diabetics (Mt. Sinai Medical Center, New York City); (10) characterization of monoclonal antibodies that react with

nervous tissue (NAB, NIDR); (ll) virus-induced diabetes and autoimmunity in non-human primates (NINCDS); (12) virus-induced diabetes in hamsters (Mt. Sinai Medical Center, New York City); (13) evaluation of islet cell cytoplasmic antibody and islet cell surface antibody in diabetics, in patients with rubella, and in patients receiving pancreatic transplants (Mt. Sinai Medical Center, New York City, and the University of Minnesota); (14) monoclonal antibodies to detect actively proliferating mesenchymal tumors (Georgetown University); (15) virologic and immunologic studies on diseases such as AIDS and autoimmune disorders (Johns Hopkins Hospital and several of the institutes at NIH); (16) evaluation of monoclonal antibodies that react with tumor antigens (UCLA and University of Loma Linda); (17) therapeutic treatment of leukoplakia with cis-retinoic acid (NCI); and (18) expression of herpes simplex virus DNA using a vaccinia vector (NIAID).

Some of our more important findings since last year's Annual Report are summarized below:

1. Over the last year we have identified and characterized several of the regions of homology between the herpesvirus genome and those of mice and humans. We have begun to obtain DNA sequence data for these regions and have confirmed the presence therein of repeated DNA sequence elements. A comparison of these sequences with those in the Los Alamos DNA sequences databank has revealed their presence within several known genes, including transplantation antigens of mice, immunoglobulin genes and switch-over regions in murine sarcoma viruses.

We have also discovered the presence of some of these regions of homology within the regulatory regions of at least one human oncogene, the v-Ha-ras homologue present in the T24 cell line derived from a human bladder carcinoma. The homologous regions are found in at least eight discrete sites of the herpes genome, several of which have enhancer functions, i.e., they enhance by several hundred fold the expression of nearby genes.

These two findings suggest that the herpes genome may recombine with the genome on the host cell and perhaps insert some of its regulatory genetic information into normal homologues of oncogenes, thus leading to the transformation (perhaps neoplastic) of the host. This possibility is at present being investigated by constructing recombinant DNA molecules that contain the viral enhancers replacing the indigenous regulatory regions of the T24 oncogene and its normal homologue.

2. Work on a subunit vaccine against HSV using recombinant DNA techniques has continued. Efforts have been made to achieve expression of the HSV-l B2 gene in E. coli and to this end we have instituted the use of the ORF cloning vectors. These are very powerful vectors which allow one to clone and select for those clones which are expressing the inserted DNA sequence in one step. These vectors are particularly suited to this project since detailed knowledge, such as DNA sequence data of the gene (fragment), is not essential to achieve expression of a portion of the gene, as a tribrid protein with the foreign polypeptide fused to two E. coli polypeptides.

A random series of DNA fragments was produced by partially digesting the HSV-l Bam G fragments with Sau3A and following size fractionation these fragments were cloned in the ORF-2 vector. Clones, identified as expressing the inserted DNA sequence by virtue of the indicator function carried by the vector, were isolated. To date about 25 such clones have been obtained and shown to express a new polypeptide (tribrid protein) not seen in extracts prepared from cells carrying the parental vector lacking an inserted piece of HSV-l DNA. Preliminary studies indicate that the tribrid proteins are insoluble and that they may be localized in the bacterial membrane. Studies are now underway to see whether any of these clones express the HSV B2 glycoprotein.

3. Latency of HSV in the mouse model was studied by *in situ* hybridization with 3H-labeled viral DNA probes. We detected the presence of HSV-specific DNA in the sensory neurons of the trigeminal ganglia, brain stem and hemispheres of latently infected animals and demonstrated the presence of HSV-specific mRNA in neurons of the trigeminal ganglia of the same animals.

The site of the latent infection in the brain stem, determined from anatomic and histologic examination, was found to be the trigeminal complex, predominantly in the nucleus caudalis. The nucleus caudalis receives direct afferents from the ganglia and has been implicated in herpes-mediated trigeminal neuralgia. In some brains, DNA-positive neurons were seen in the midline reticular formation and in the trigeminal motor nucleus. These results establish the site of HSV latent infection in the central nervous system of the mouse and suggest that in the ganglia at least some mRNA is being made during latency.

4. Good progress has been made in our studies on virus-induced diabetes. Last year we reported that encephalomyocarditis (EMC) virus not only produces the early metabolic changes, but also some of the long-term complications of diabetes, including kidney diseases, ocular changes and reduced lifespan. We now find that endochondral bone formation and mineralization are greatly impaired in these diabetic animals. The decreased bone formation and mineralization could be due to the persistent metabolic

alterations. These findings are similar to those seen in humans with diabetes mellitus.

5. The genetics of susceptibility to EMC virus-induced diabetes was studied in further detail. First, we found that New Zealand mice were susceptible to virus-induced diabetes. Second, males and females of parental NZB and NZW strains were equally affected, whereas in other strains males are usually much more susceptible than females. Third, the equal susceptibility of the two parental strains was not inherited by their F1 offspring. In the (NZB x NZW)F1 mice, males, but not females, were susceptible to virus-induced diabetes. Finally, the sex differences were found to be related to androgens in the F1 mice in that castration of the males rendered them insusceptible.

6. Over the last year, we have found another virus that can produce diabetes. Infection of mice with Mengovirus (i.e., the 2T variant) produces abnormal glucose tolerance tests, decreased levels of immunoreactive insulin and necrosis of beta cells. The spectrum of host susceptibility to Mengo-virus-induced diabetes is strikingly different from that produced by the D variant of EMC virus. Both viruses produce diabetes in certain strains of mice. However, Mengovirus can produce glucose abnormalities in the strains of mice that are resistant to EMC virus-induced diabetes. Studies reported last year showed that Mengovirus and EMC virus are distinct variants of the cardiovirus group that bind to different cell receptors. In this context, receptors specific for Mengovirus may be broadly expressed on the beta cells of many strains of mice, whereas the receptors for EMC virus may be restricted to a few mouse strains. This and other possibilities are now under investigation.

7. Previously we reported that mice infected with reovirus developed a polyendocrine disease with autoantibodies to the pancreas, anterior pituitary, thymus and gastric mucosa. Treatment with immunosuppressive agents decreased autoantibody production and prevented development of the polyendocrine disease. This year studies were initiated to determine the mechanism by which the virus triggers autoantibody production. Preliminary experiments indicate that the infection causes a decrease in the number of suppressor cells in the peripheral circulation and in the spleen. Detailed studies are now in progress to look for further evidence of virus-induced immunoregulatory abnormalities. In addition, a number of other viruses are being studied for their capacity to trigger the production of autoantibodies.

8. In the area of virus receptor, we are attempting to produce new receptor specific probes through the use of anti-idiotypic antibodies. These antibodies are prepared by immunizing animals with the antigen-binding fragment (Fab) of monoclonal antiviral (Coxsackie B4) antibodies. We have shown that the sera of some of the immunized animals contain antibodies that react with the antigen binding site of the antiviral immunoglobulin. After affinity purification of the sera on columns containing the Fab fragment, the immunoglobulin (presumably, anti-idiotypic antibody) is able to inhibit the monoclonal antiviral antibody from neutralizing Coxsackie B4. In addition, the "anti-idiotypic" antibody reacts with cell surface components on a variety of cell lines that are susceptible to Coxsackievirus B4 infection. Tests are now underway to see whether the "anti-idiotypic" antibody reacts specifically with the viral receptor on the cell surface.

9. Last year we made a number of monoclonal neutralizing antibodies to Coxsackievirus B4. We now have used these antibodies as a selecting agent to isolate antigenic variants of Coxsackievirus B4. These variants have been analyzed antigenically by screening with a large panel of monoclonal antibodies. Our studies show that variants selected in the presence of a specific monoclonal antibody not only lack the epitope with which the selecting antibody reacts, but also other epitopes. Moreover, some of these variants express epitopes that were not originally detected on the parental virus.

In other experiments we have analyzed multiple isolates of Coxsackie B4 virus, from the same patient, obtained sequentially during the course of a single infection. These studies showed that there are antigenic differences among the various isolates from the same individual. This suggests that antigenic variation may be a mechanism by which the virus persists in nature.

10. Lymphokines can help to facilitate some of the complex cellular interactions required for efficient immune function. Interferon-gamma (IFN), as part of the cascade of lymphokines, promotes a variety of host functions including antiviral and immunoregulatory responses. A defect in the production of IFN or other lymphokines can be indicative of an underlying alteration in regulatory signals. Retinitis pigmentosa is a disease of unknown etiology. We found that peripheral leukocytes from patients with retinitis pigmentosa when stimulated with mitogens produce subnormal amounts of IFN-gamma. These findings suggest that altered immune reactivity may be one of the abnormalities associated with this disease.

Patients with AIDS have multiple dysfunctions in immune reactivity. By measuring the ability of peripheral mononuclear cells to produce IFN-gamma, we have identified a defect in the lymphokine cascade. Leukocytes from AIDS patients produce subnormal

amounts of IFN-gamma in response to T-cell mitogens. This defect in the lymphokine cascade was frequently associated with a persistent cytomegalovirus (CMV) infection of leukocytes. In contrast, homosexuals with lymphadenopathy, who did not have AIDS, were free of infectious CMV in their leukocytes and produced normal amounts of IFN-gamma. An understanding of this defect in AIDS may be important in modulating the impaired T lymphocyte-monocyte interactions.

11. Over the last 12 months we have characterized some of the monoclonal autoantibodies that we described in last year's Annual Report. We have found that some of these monoclonal antoantibodies react with antigens in more than one organ. This suggests that multiple-organ reactive (MOR) monoclonal autoantibodies react either with the same molecule present in several organs or with common antigenic determinants on different molecules in multiple organs.

The autoantigens with which these antibodies react are now being characterized by affinity columns and Western blots. An affinity column was made with one of these antibodies designated MOR-4. Extracts from the stomach and pituitary were passed through this column. Thus far, three polypeptides from the stomach (25K, 43K and 53K) and one from the pituitary (25K) have been isolated.

Recently, we also succeeded in preparing a number of human monoclonal autoantibodies. Many of these autoantibodies are of the MOR type. This work suggests that MOR antibodies may be a partial explanation for the multiple organ autoimmunity found in a number of human autoimmune diseases, such as diabetes mellitus.

LABORATORY OF ORAL MEDICINE

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LABORATORY OF ORAL MEDICINE

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00080-10	Ji-won Yoon	Virus-induced Diabetes of the Endocrine and Exocrine Glands
Z01 DE00123-10	A. Puga-carrasco	Herpes Simplex Virus: Latency
Z01 DE00219-07	John J. Hooks	Interferon, Autoimmunity and Viral Diseases
Z01 DE00255-05	Patrick R. Mcclintock	Receptors, Membranes and Diseases
Z01 DE00309-03	Bellur Prabhakar	Hybridomas: A Probe to Study Viral and Other Diseases

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

The Clinical Investigations and Patient Care Branch functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (l) to conduct high quality, clinically oriented dental research programs; (2) to encourage and to provide support, consultation and facilities for clinical research activities of other Branches and Laboratories within the Institute; (3) to offer consultation on oral and dental problems to other Institutes; (4) to render clinical care to specified patients of the Clinical Center; and (5) to sponsor a training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research oriented dental clinicians.

The past year has generally been one of considerable success for this Branch. Organizationally fiscal 1983 was the first full year that the Branch operated under Bruce Baum as Clinical Director and Chief of the Branch and of the Clinical Investigations Section and Michael Roberts as Chief of the Patient Care Section. Substantial efforts have been made by Branch personnel to enable us to begin to attain our stated goals. All of our personnel have recognized that we are involved in a particularly challenging endeavor. Cooperation, assistance and understanding have been practiced to a high degree and we are most enthusiastic about our future activities.

PATIENT CARE SECTION

The Patient Care Section conducts the daily operation of the NIDR clinic and as such is the focus of clinical oral and dental health concerns at NIH. Two new Dental Staff Fellows joined the staff during the past year. The Section provides a wide range of diagnostic consultative services to NIH clinical care programs. The Section has continued to become more involved with the medical staff of the various Institutes and of the Clinical Center. Staff dentists and dental hygienists routinely participate in medical rounds and patient care discussions thus integrating oral health care concerns to total patient management. This Section is also responsible for providing clinical training and development for the Dental Staff Fellows.

The first complete year of epidemiology and patient services data was compiled using the Dental Data System computer program which was initiated in May, 1982. The data provided has been valuable in the evaluation of program direction and individual progress.

The Patient Care Section continued an affiliation with the Baltimore College of Dental Surgery, University of Maryland. This collaboration provided senior dental hygiene and dental students an opportunity to participate in alternative practice settings beyond those offered in the dental school core curriculum. The arrangement provided our staff with certain academic clinical dental teaching responsibilities and offered a professional development opportunity. Section staff members served as preceptors. Unfortunately, the University of Maryland has terminated this program, except for the dental hygiene students, at the end of this school year due to fiscal restraints.

The Section's staff have been deeply involved in the continued development of the Dental Staff Fellow program. Regularly scheduled rounds have been presented and a Fellowship lecture series initiated. The lecture series has brought in speakers from the various Laboratories and Branches of the Intramural Research Program, NIDR. A Journal Review Seminar was also begun which gives the staff an opportunity to present and discuss interesting articles from the dental literature. The monthly Brown Bag Seminar sponsored by the Patient Care Section has continued to bring in outstanding speakers to present information that is topical and clinically relevant.

Many of the Patient Care Section staff members have been actively involved in both clinical and laboratory research projects. They include the following studies: (l) the mechanism of adherence of the periodontal ligament to the root surface; (2) herpes zoster and the development of extensive facial and alveolar bone pathology associated with the disease; (3) oral developmental defects in Albright's Hereditary Osteodystrophy; (4) oral anomalies associated with Reiger's syndrome; (5) the effects of steroids on post-operative inflammatory response following extraction of third molars; (6) a protocol for the treatment of patients with acquired immune deficiency syndrome (AIDS); (7) oral and facial complications associated with Job's syndrome and mucopolysaccharidoses; (8) the use of occlusal fissure sealants as a possible vehicle for slow release of fluoride; (9) assay of salivary gland proteins after radiation; (10) the effects of radiation on the development of the oral tissues; (ll) epidemiological and clinical parameters of jaw lesions in Burkitt's Lymphoma in the American population; (12) oral and facial development in patients with precocious puberty; (13) laboratory and clinical studies of patients with osteogenesis imperfecta; (14) herpes simplex stomatitis; (15) small-cell osteosarcoma; (16) human papilloma virus DNA in oral lesions and (17) microflora associated with dental implants.

CLINICAL INVESTIGATIONS SECTION

This section was created 1 year ago to establish a high quality clinical-problem oriented, dental research program. At present there are two major general subject areas of interest: the primary focus is towards understanding the regulation of salivary gland function, and salivary secretion. A second area involves studying specific factors influencing the development and progression of periodontal diseases.

Salivary studies focus on understanding biochemical mechanisms of saliva formation and alterations in these processes occurring during normal aging and certain disease states. Saliva is of critical importance to the maintenance of normal oral and dental health. Many of the oral health problems of older adults likely are the result of either specific age-related alterations in salivary gland function or alterations in function secondary to diseases and therapeutic treatments common to the elderly situation. Using *in vitro* cell models considerable progress has been made in understanding neurotransmitter (α-, β-adrenergic; muscarinic cholinergic) regulation of saliva formation. The aged rat parotid gland has previously been shown by us to display an altered α-adrenergic physiologic response (K^+ efflux) in the presence of normally functioning α-adrenergic receptors. We continue to use this paradigm as a probe of α-adrenergic receptor signal transduction mechanisms. Thus far we have been able to demonstrate for the first time a dissociation of α-adrenergic and muscarinic-cholinergic transduction mechanisms in the same cell. Also we have shown that multiple transduction mechanisms exist in rat parotid cells for Ca^{++} mobilizing secretory events. Further work by the Clinical Investigations Section has demonstrated separate influx and efflux channels for Ca^{++} in these cells.

Other *in vitro* studies of α-adrenergic regulation of salivary glands have utilized rat submandibular cells. We have studied the influence of the α-adrenergic receptor on protein synthesis in this cell. We have shown that activation of the α-adrenoreceptor results in a profound inhibition of protein synthesis. This response is dose and time dependent and appears to require mobilization of intracellular Ca^{++}. Further studies have examined the influence of increased age on submandibular cell protein synthesis. Aged rat cells demonstrated approximately 25% less synthesis of new protein compared to cells from younger animals. More striking, however, was our observation of marked differences in the processing of the major mucous glycoprotein (mucin) synthesized by cells from older rats.

An *in vivo* model of studying rat parotid and submandibular gland saliva secretion has also been utilized. We have evaluated the effects of aging upon saliva produced by these glands. Our studies demonstrated the stability of submandibular secretions with age (Flow rate, [protein], $[K^+]$, $[Na^+]$). Conversely parotid secretions showed markedly depressed flow rate with age. Also some perturbation in Na^+ handling and in levels of exocrine protein secretion, were noted.

We have begun a series of studies examining the mechanisms of radiation damage to salivary glands. Importantly, the Clinical Investigations Section has shown that 3 days following irradiation *in vivo*, rat parotid cells are capable of normal secretory and metabolic functions *in vitro*. This is true despite the substantial (55%) diminution in salivary output by irradiated rats *in vivo* at this same 3 day time point.

Also during this year, we have initiated studies on α-adrenoreceptor coupled functions. We have shown that an irreversible β-adrenoreceptor antagonist, bromoacetylalprenololmenthane (BrAlpM), is a potent inhibitor of β-adrenergic agonist induced secretory responses in rat parotid cells. This result allows the use of BrAlpM as a probe of both β-adrenoreceptor turnover in these cells and determination of the absolute numerical requirements for β-adrenoreceptor occupation to achieve maximum physiologic response. The Clinical Investigations Section has, in addition, shown that stimulation of rat parotid cells by β-adrenergic agonists results in profound enhancement of N-linked protein glycosylation. This effect is fully blocked by propranolol and tunicamycin, is dose and time dependent, and occurs concommittantly with β-agonist induced secretion.

The other general group of laboratory studies by this Section have focused on phenomena and events related to periodontal diseases. One series of investigations have continued to examine the role of free radicals (superoxide, hydroxyl radicals) in immune response cells. Particular attention has been paid to defining the role of thiols in protecting cells against oxidative damage. Splenocytes develop disulfide cleaving capacity proportional to their antibody forming activity. Both antibody formation and cell proliferation were shown diminished by oxidative reagents. The Clinical Investigations Section demonstrated the presence of cysteinyl glycine in culture medium of splenocytes. This is a unique product of the amino acid transport enzyme γ-glutamyltranspeptidase (γ-GTP). We have shown that γ-GTP activity increases with splenocyte proliferation. Inhibiting the activity of γ-GTP decreases disulfide cleavage and cell proliferation. Cysteinyl glycine was shown to be the most rapidly oxidized of the thiols suggesting it has a primary role in thiol antioxidant protection. These studies have suggested an important role for disulfide cleavage and γ-GTP in chronic inflammation.

Another area of investigation has been the study of mechanisms regulation osteogenic processes. A bone specific protein, which displays chemotactic activity for osteoblasts, has been partially purified from extracts of human and rat bone. The protein was not observed in extracts of enamel or dentin or in adult bone. In addition, we have begun studies on glycosylation of non-collagenous glycoproteins in osteoblast-like cells *in vitro* . Incorporation of fucose and leucine were linear for over 5 hours while incorporation of mannose was linear for only 2 hours, suggesting extensive processing of poly-mannose oligosaccharides by these cells. Since the initial events in fracture healing and repair of alveolar bone destruction include the migration of cells to the healing site, non-collagenous proteins and glycoproteins such as studied here, are likely of considerable import.

Studies are continuing to evaluate the role of the gram negative oral microorganisms *Cytophaga* in the initiation and progression of periodontal diseases. Our approach has been to develop a series of monoclonal antibodies with varying specificity toward human oral isolates of *Cytophaga* and the related species *Caphocytophaga*. We have begun studies examining human plaque, crevicular fluid and serum samples, by indirect immunofluoresence with the antibodies, for the presence of these bacteria.

Human research studies have thus far focused on problems related to oral health in the aged. Salivary gland functional studies have evaluated Na^+ handling by stimulated parotid glands. With increased age, changes in Na^+ secretion were observed. Evaluating the extent of Na^+ reabsorption on Na^+ levels in saliva (using the accepted methods and model of Thaysen) have resulted in the important finding that the two stage model for salivary secretion is inadequate to describe this process. Results suggest that either Na^+ reabsorption occurs by more than the one mechanism proposed by Thaysen or that Na^+ reabsorption is modulated in concert with secretory stimuli.

We have initiated studies on salivary gland dysfunctions induced by radiation and chemotherapy. These studies evaluated both parotid and submandibular/sublingual secretions. X-irradiation caused virtually complete loss of unstimulated parotid secretions and 75% depression of stimulated parotid saliva output. Lesser, but still significant effects, on submandibular/sublingual salivas were also observed. Following chemotherapy, patients showed no change in salivary flow, but rather specific dimunitions in Na^+ and protein secretions were seen.

Studies on gustatory function across the human life span have continued. Considerable effort has been focused on the refinement of psychophysical methods for evaluating suprathreshold measures of taste intensity and the development of new methods of data analysis. These new approaches have been applied to studies of gustatory function during aging. Generally older persons perform slightly less well than younger persons. Changes are quality specific and modest in extent, though particularly striking negative changes were observed in ability to taste quinine sulfate (bitter). Also tests of olfactory function in patients with cystic fibrosis were performed. Data show no general deficit in olfactory performance in these individuals, rather specific patients (3 of 20) were severely impaired.

Using ultrasonic imaging techniques, we have begun evaluating the oropharyngeal phase of swallowing in different aged persons. A standard for normal swallow was developed and patients with clinically demonstrable dysphagia studied.

We have also established a new clinical service, the Dry Mouth Evaluation Clinic. Our purpose is to characterize the nature of dry mouth complaints and begin development of appropriate therapies for patients suffering from oral dryness with a broad spectrum of etiologies.

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

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CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00212-07	James M. Weiffenbach	Taste and Its Disorders
Z01 DE00320-03	Agnes H. Donahue	Survey of Jaw Lesions in Burkitt's Lymphoma in the NIH Population
Z01 DE00332-02	M. Roberts	Clinical Investigations & Case Studies
Z01 DE00336-02	B. Baum	Salivary Gland Secretory Mechanisms
Z01 DE00337-02	B. Baum	Oral Physiological Processes
Z01 DE00338-02	J. Terrell Hoffeld	Role of Oxygen Radicals in Inflammation
Z01 DE00339-02	M. Somerman	Regulation of Osteogenic Processes
Z01 DE00340-02	P. Fox	Role of *Cytophaga* Species in Periodontal Diseases
Z01 DE00359-01	M. Roberts	Evaluation of A Fissure Sealant as a Vehicle for Slow Release of Fluoride
Z01 DE00361-01	S. Martin	Application of Immunocytochemistry to the Diagnosis of Malignancy
Z01 DE00362-01	M. Roberts	Dental Development in Patients With Precocious Puberty
Z01 DE00372-01	E. Kousvelari	N-linked Protein Glycosylation and B-adrenoreceptors

DIAGNOSTIC SYSTEMS BRANCH

The Diagnostic Systems Branch (DSB) was consolidated into a single functional unit by the elimination of both the Diagnostic Methodology Section (DMS) and the Oral and Pharyngeal Development Section (OPD) subsequent to the retirement of the OPD Chief, Dr. James F. Bosma, and the permanent relocation of remaining OPD personnel. The thrust of the entire Branch is now unified by the systems-based research commitment previously associated with DMS. This action complements the change in research emphasis cited last year and simplifies operations in accord with a general effort on the part of management to streamline administrative practices.

In keeping with a more focused administrative perspective, DSB has concentrated existing resources on activities which facilitate research collaboration both within and outside the Branch. Most of this work involves theoretical analysis of method-specific diagnostic processes with particular emphasis on clinically promising x-ray systems. A significant experimental design constraint has been identification and analysis of specific factors limiting diagnostic performance obtainable from a microfocused x-ray spot source which can be moved electronically under computer control. Such a source when appropriately coupled to a suitable high-gain detector has the potential for practical implementation of 3-D image reconstruction via filtered tomosynthesis, and digital subtraction of sequentially-obtained radiographic images without the need for mechanical coupling of system components.

That such a system is technologically feasible has been determined through organized collaboration of scientists at DSB and the Radiation Physics Group, National Bureau of Standards. This cooperative interagency approach to clinical applications research has been augmented significantly by support from the Restorative Materials Program Branch, Extramural Programs.

This work is an extension of that reported previously which involved design of a prototype x-ray system which permitted exposure geometry to be reproduced accurately from one examination to the next. It also complements research being done in collaboration with the US Army Institute of Dental Research to develop a practical and portable dental fluoroscope.

Recent work has focused on the design constraints imposed by tomosynthetic reconstructions. Of particular interest are sampling strategies which maximize angular disparity within an x-ray examination while minimizing disparity between examinations. Such strategies have been shown to be optimal for both 3-D reconstruction and digital image subtraction. Specifically, the effects of such constraints have been modeled with the aid of an image-processing computer to determine the effective slice thickness of reconstructed images as well as the effects of noise, both quantum and electronic. The statistical influence of uncontrolled fluctuations attributable to variations in projective geometry as well as anatomical differences between patients also has been modeled.

Of particular interest in this context is the investigation of constraints underlying the general theory of radiologic image registration. Recent findings show that registration errors can be resolved into two components, depending on the spatial relationship shared by the x-ray source and detector relative to the tissues of interest. This research also shows that one component is amenable to computer correction via affine transformation, and the other can be handled by systematic manipulation of the position of the x-ray focal spot. This is important because it assures that there is no theoretical requirement for mechanical coupling of system components in order to achieve clinically useful registration of x-ray images.

Other efforts likewise have built systematically on previous work. They include the development and testing of automated lesion detection algorithms, systematic analysis of image filters designed to reduce method-specific artifacts asociated with various image-reconstruction methods, and the use of feedback during x-ray exposure to optimize the information carrying capacity per unit dose produced by the x-ray system.

The theory underlying some of the work also is applicable to more general diagnostic tasks involving a broad range of clinical measurements. These include statistical analyses of factors underlying objective determination of disease severity in cases where the end-point can be determined unequivocally. Work being done in collaboration with investigators at Childrens Hospital, National Medical Center, provides a specific example wherein a method has been develped to rigorously analyze factors associated with survival in a structured intensive care environment.

Other activities include consultation in long-term periodontal studies being done at the State University of New York at Buffalo with Dr. Robert Genco and colleagues, and in Europe with Dr. Bengt Rosling at the University of Lund in Sweden. All these investigations make clinical use of the digital radiographic subtraction method developed by DSB in recent years.

Particularly noteworthy is a related study being done in cooperation with Dr. Michael Rethman at the US Army Institute of Dental Research where bony lesions induced in the jaws of beagle dogs have been traced

radiographically using automated digital methods throughout months of healing. Preliminary results indicate that it is possible using these methods to unequivocally map the dynamics of bone remodelling over extended intervals.

This and other work has resulted in several ideas having potential commercial interest which are currently undergoing patent review by government authorities at the National Bureau of Standards. In this context it is of interest to note that previous collaborative research with NASA and the US Army Night Vision Laboratory directed toward the development of a hand-held isotopic fluoroscope has resulted in a commercial device licensed under a government patent which now is finding use in a variety of biomedical applications.

Efforts to recruit Dr.s Hans and Kerstin Grondahl have been circumvented by their recent decision to accept positions at the University of Washington. Accordingly, Dr. David Roberts, an anatomist from England, has been recruited as a Visiting Scientist. Dr. Roberts has considerable experience with new computer-based methods for displaying raiographic images in 3-D. His research while at NIDR will involve quantitative assessment of anatomical changes secondary to myofacial pain dysfunction (MPD) using occlusal photoplastic interferometry and computerized tomography of the temporomandibular joint.

In keeping with the priorities described above, research involving the application of symmetric-axis geometry to the description of biological shape has been uspended. This decision reflects the fact that our principle associate, Mr. Harry Blum, who originally conceived the symmetric-axis geometry, has retired recently from Government service and is no longer available to continue this work. This decision in no way compromises existing commitments to provide consultative support to others using our software orotherwise interested in applying this unique geometric tool.

Plans for the future will focus on continued collaboration with NBS in order to demonstrate a prototype dental x-ray system capable of high-quality electronically-generated radiogrphic images produced in real time which are tailored to a variety of diagnostic tasks including 3-D spatial analysis and digital subtraction of homologous projections exposed on different occasions. Efforts to implement algorithms designed to facilitate lesion detection and to quantify changes in shape and size likewise will continue.

The Branch also plans to commit a significant effort toward upgrading our image-processing computer facility. To this end, a larger and more efficient mainframe computer has been ordered which will ultimately simplify data management and algorighm design while simultaneously decreasing the time required to run existing software.

DIAGNOSTIC SYSTEMS BRANCH

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DIAGNOSTIC SYSTEMS BRANCH

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00065-12	Richard L. Webber	Development and Evaluation of Improved Diagnostic Systems
Z01 DE00211-07	Richard L. Webber	Enhancement of Diagnostic Images
Z01 DE00373-01	D. Roberts	Quantitative Assessment of Changes Associated with Pain Dysfunction Syndrome

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

The Neurobiology and Anesthesiology Branch is concerned with the study of oral-facial sensation, with particular emphasis on mechanisms of pain and the development of new methods for controlling pain in humans. The Branch is composed of three sections that utilize anatomical, physiological, behavioral, pharmacological and psychophysical techniques to study neural function as it relates to the processing of sensory signals about the threat of tissue-damaging stimulation. The Neural Mechanisms Section includes the following research activities: 1) correlative morphological, physiological and neurochemical studies of the organization of the medullary and spinal dorsal horns and the identification of putative neurotransmitters involved in sensory transmission; 2) correlative behavioral and physiological studies to determine the role of different peripheral and central neural populations in pain and temperature discrimination. The Neurocytology and Experimental Anatomy Section is concerned primarily with the study of synaptic connections in the medullary and spinal dorsal horns in normal tissue and following peripheral nerve injury. The Clinical Pain Section develops new methods for measuring and assessing experimental and clinical pain and applies these methods to the study of mechanisms of acute and chronic pain in humans as well as to the evaluation of new techniques for the treatment of acute and chronic pain.

This year we have continued our detailed analyses of the organization of the medullary and spinal dorsal horns and their role in pain transmission. By combining techniques from different disciplines we have been able to elucidate functional circuits within the dorsal horn that play a role in information transfer related to pain, temperature and touch sensation. The importance of two major ascending projection systems in the dorsal horn, the spinothalamic system and the dorsal column postsynaptic system, has been elucidated utilizing combined anatomical, physiological and cytochemical techniques. Combined behavioral and physiological studies also have examined the functional properties of the trigeminothalamic projection system in awake animals. Recent studies of the dorsal horn following peripheral nerve injury are beginning to shed light on the morphological basis of some chronic pain states precipitated by the loss of sensory input. Our animal research studies also provide the conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions. Human studies also have focused on improved methods for assessing acute and chronic pain, the evaluation of non-steroidal anti-inflammatory agents useful in controlling postsurgical pain, and the development of new methods for the treatment of pain associated with neuropathies, myofascial pain, and cancer pain.

Our clinical pain program was relocated to the Clinical Center Ambulatory Care Research Facility in March, 1983. The Clinical Pain Section coordinates this multi-Institute collaborative program on pain research. Present studies are evaluating pain associated with diabetic neuropathies, postherpetic neuralgia, myofascial disorders of the head, neck and lower back, and cancer.

Investigators in the Branch received recognition for their achievements and were chosen to chair and present their research at symposia and workshops at the annual meetings of the Society for Neuroscience, the International Association for Dental Research and the American Pain Society. In addition, Dr. Dubner was honored by being chosen chairperson of the Scientific Program Committee of the American Pain Society for 1983 and by being elected Chairperson of the Commission on Oral Physiology of the International Union of Physiological Sciences.

Research accomplishments this year are presented in more detail below.

The Neural Circuitry of the Medullary and Spinal Dorsal Horns

The lower end of the spinal trigeminal nucleus in the brain stem, called trigeminal nucleus caudalis, is directly continuous with the spinal dorsal horn and is homologous to it in terms of structure, chemistry and physiological function. For these reasons, it is more properly referred to as the medullary dorsal horn. This year we have continued our in-depth studies of the functional organization of the medullary and spinal dorsal horns and their role in pain mechanisms.

We have continued to examine the properties of local circuit neurons in the superficial layers and, in addition, have studied major projection neuron systems in the dorsal horn. The dorsal horn contains identified neuronal cell types that respond exclusively to noxious stimuli (nociceptive-specific), respond to both innocuous and noxious stimuli (wide-dynamic-range) or respond only to innocuous stimuli. The dorsal horn also contains a wealth of chemical mediators that are released by primary afferent or descending neurons projecting to this region as well as by intrinsic neurons. Using immunocytochemical techniques alone or in combination with the retrograde and intracellular horseradish peroxidase (HRP) methods, at both light and electron microscopic levels, we are examining the role of these putative neurotransmitters in the neural circuitry of the dorsal horn.

Enkephalin (ENK), an opiate peptide which may represent one of the natural ligands of opiate receptors, can be localized to several different laminae in the dorsal horn, some of which contain the neuronal somata and dendritic arbors of the thalamic projection neurons. Several studies have shown that ENK mediates inhibition of the response of neurons to noxious stimulation. An understanding of the neural circuitry accessed by ENK is thus critical to the elucidation of the mechanisms of pain and analgesia. By combining the techniques of immunocytochemistry and retrograde transport of HRP we have achieved the first direct anatomical demonstration of a synaptic relationship between axonal endings containing an opioid peptide and an identified postsynaptic neural element in the dorsal horn. Enkephalin immunoreactive axonal endings were shown to make direct synaptic contact with the soma and proximal dendrites of laminae I and V dorsal horn thalamic projection neurons in the cat and monkey. This observation demonstrates that one major site of opioid modulation of the transfer of nociceptive information in the dorsal horn is a direct synaptic event on the projection neurons themselves. In lamina I, approximately 30% of the projection neurons received ENK innervation proximally. In lamina V more than 50% of the projection neurons had ENK contacts. The presence of ENK contacts could not be attributed to a single morphological type of neuron. These observations indicate that ENK modulation of noxious input occurs to a substantial degree on the proximal portions of a subset of projection neurons. Such input is strategically located to suppress the effects of excitatory inputs originating on more distal portions of the dendritic tree.

Serotonin (5-HT) is a monoaminergic neurotransmitter which originates from brain stem nuclear groups. It occurs in all laminae of the dorsal horn and as such is situated to modulate a variety of intrinsic dorsal horn neurons. In double-label experiments, 5-HT contacts were identified on to thalamic projection neurons in the medullary, cervical and lumbar dorsal horn of the monkey. The pattern of innervation viewed at the light microscope level was somewhat different from that encountered in the ENK study, suggesting that ENK and 5-HT arise from different sources. At the ultrastructural level, 5-HT axonal endings formed synapses on the projection neurons. These observations suggest that descending monoaminergic modulation of the transfer of nociceptive information can occur directly on the projection neurons. Serotonin thus acts, in part, on postsynaptic receptors located on projection neurons.

In similar double-label studies, we showed at the light microscope level that dorsal horn neurons sending axon projections in the dorsal columns (the dorsal column postsynaptic system) received direct contacts from serotonin immunoreactive axonal varicosities on their somata and proximal dendrites. By modifying the immunocytochemical procedure for electron microscopy, we found that 5-HT axons did indeed synapse upon retrogradely-labeled dorsal column postsynaptic (DCPS) neurons. Such findings imply that descending serotonin systems modulate physiologically diverse types of neurons since some DCPS neurons respond exclusively to tactile input while others are nociceptive neurons.

By combining immunocytochemical techniques with the intracellular HRP method, we have been able to examine, at the light microscope level, the distribution of serotonin contacts on morphologically and functionally identified neurons in the superficial dorsal horn. Serotonin immunoreactive contacts were found on marginal neurons in lamina I and stalked and islet cells in lamina II. For all three neuron types, both nociceptive-specific and wide-dynamic-range neurons were represented. For all three cell types, serotonin immunoreactive axonal contacts occurred preferentially on dendritic shafts rather than on spines. The number of serotonin contacts on marginal and stalked cells was much greater than on islet cells. Axonal contacts were concentrated in the proximal 250 μm of the dendritic tree of marginal and stalked cells, but were more evenly distributed in the dendritic trees of islet cells. Stimulation of nucleus raphe magnus in the brain stem, a major site of origin of serotonin input, consistently resulted in inhibition of the nociceptive responses of marginal and stalked cells. Similar stimulation failed to influence the activity of islet cells. These findings confirm our previous interpretation that morphologically distinct cell types subserve different roles in dorsal horn function and that serotonin descending modulation is exerted via postsynaptic mechanisms mainly on neurons concerned with the rostral transfer of nociceptive information (marginal and stalked cells).

The experiments described above have identified several important sites of action of neurotransmitters in the dorsal horn. The analysis of monoaminergic axonal endings is of particular significance since the activation of descending aminergic pathways are implicated in mechanisms of analgesia. The study of enkephalinergic neural circuitry furthers our understanding of the role of opiates in pain and other somatosensory pathways.

Another important question relating to neural circuitry in the dorsal horn concerns the identification of the termination sites of different types of primary neurons activated by innocuous and noxious stimuli. Using immunocytochemistry combined with the intracellular HRP method, we have examined the distribution of substance P contacts on identified superficial dorsal horn neurons. Substance P, a polypeptide, is a candidate

neurotransmitter for small primary afferents activated by nociceptive input. Although substance P also is found in intrinsic dorsal horn neurons (and possibly descending axons), these studies do give some insight as to the possible termination sites of substance P primary axons. In contrast to serotonin contacts, substance P contacts preferentially occurred on spine heads of spiny neurons rather than on dendritic shafts, although aspiny neurons had substance P contacts on dendritic shafts.

Vasoactive intestinal polypeptide (VIP) is another candidate transmitter in primary afferent neurons and exhibits a unique distribution in the spinal cord. In the monkey, it is present as a dense band of axons in laminae I and II which enter the spinal cord through the dorsal root entry zone. VIP is concentrated in the sacral segments of the spinal cord with only an isolated fiber entering the lumbar level. Ultrastructurally, VIP axons are unmyelinated and are found in bundles of other unmyelinated axons in Lissauer's tract. Their axonal endings are quite large, the majority measuring more than 1.5 μm in diameter. They contain oval agranular vesicles and a large number of dense core vesicles. The VIP varicosities form small synaptic specializations, most often with dendritic shafts. Based on these observations, VIP may be a neurotransmitter in unmyelinated primary afferent axons involved in nociception.

In the superficial layers we have used the intracellular HRP method to examine whether distinct physiological types of marginal neurons — nociceptive-specific (NS) or wide dynamic range (WDR) — are associated with distinct morphological classes of neurons. We subdivided marginal neurons based on the shape of their somata and dendritic trees, number of primary dendrites, and presence or absence of spines. None of these classes was associated exclusively with a distinct physiological type. These findings indicate that NS and WDR lamina I neurons are not exclusively associated with separate morphological classes, although there is a tendency for NS neurons to be more numerous among the aspiny pyramids, and WDR cells to appear more frequently in the spiny pyramidal and multipolar-compact neuronal classes.

Studies of neurons in the deeper layers of the dorsal horn revealed the presence of a major system reaching the brain via the dorsal columns. This dorsal column postsynaptic (DCPS) system contains about 1000 neurons in the lumbosacral enlargement of cats and monkeys and appears to be one of the major sources of somatosensory input from the spinal cord to higher brain centers. Work from other laboratories has shown that DCPS neurons innervate the dorsal column nuclei (DCN) in the medulla. It was originally believed that the DCN's only spinal input came from primary afferent axons. However, the DCPS input to the DCN is surprisingly large since a reanalysis has shown that the cat's lumbosacral segments send approximately 3,000 primary afferent axons to the DCN.

Our retrograde labeling studies showed that DCPS neurons were concentrated in laminae III-IV. Previous work, in other laboratories, has established that another major ascending system, the spinocervical tract (SCT), also has cell bodies concentrated in this region. Our intracellular staining of DCPS neurons showed that their dendritic arbors and local axon collaterals looked very much like those of SCT neurons. Moreover, our electrophysiological analysis of the responses of DCPS neurons to cutaneous stimulation revealed many similarities to the responses of SCT neurons. These observations suggested that DCPS and SCT projections might not be independent and separate systems. In order to test this idea carefully, we separately stimulated the projection of these two systems in the rostral cervical spinal cord. We found 56 antidromically activated neurons in the lumbosacral enlargement; 23 of these were unequivocally activated antidromically from the axon projection sites of both SCT and DCPS neurons. This result confirms our suspicion that the DCPS and SCT systems share many cells of origin.

Pain is ameliorated by activation of low-threshold mechanoreceptors and this is the hypothetical basis for the analgesia produced by dorsal column stimulation, transcutaneous nerve stimulation, and perhaps acupuncture. The neural circuitry that mediates this touch-induced inhibition of pain is not known. Since low-threshold mechanoreceptors synapse primarily in laminae III-IV, we have begun to investigate the pool of interneurons that reside in this region. To date, we have found a population of small, lamina III interneurons that respond in a distinctive way to electrical stimulation. When characterized with natural stimulation, these laminae III cells were found to respond to activation of only one of three types of A-beta low-threshold mechanoreceptors. The cells had receptive fields that were quite small, suggesting that they were innervated by only one of a few afferents. Intracellular HRP staining showed that these laminae III cells are very small. They issue 2-3 primary dendrites which generally travel dorsally and rostrocaudally, branch infrequently, and generate dendritic arbors shaped like the letters L or U. We have also examined ENK-containing cells in lamina III. These cells also have small perikarya and only a few primary dendrites. The proximal portion of their dendritic arbors is oriented dorsally and rostrocaudally. They appear to be morphologically identical to the lamina III interneurons described above.

What happens to primary afferent neurons and central neurons after peripheral nerve injury or destruction of

their receptor endings? This has been examined in two different systems, in the trigeminal system following tooth pulp extirpation and in spinal nerves after peripheral nerve transection. At thirty and sixty days following tooth pulp extirpations, membrane-lined cavities formed inside many of the small caliber dendrites of second order neurons in laminae I and II. The process of cavitation ultimately resulted in the destruction of the affected dendrites. Many cavities became patent to the intercellular space with the cavity membrane establishing continuity with the dendritic membrane. The presence of synaptic connections from a number of different kinds of axonal endings were not sufficient to prevent cavitation. Evidence that dendrites were being lost from the neuropil was most readily apparent in many of the disrupted glomeruli in lamina II in which many of the scalloped depressions in the central axonal endings that normally contained small dendrites were empty. This study demonstrates that injury to the distal branches of primary trigeminal neurons which innervate tooth pulps resulted in transsynaptic degenerative changes in the dendritic arbors of second order neurons.

The central processes of the primary neurons which comprise the superficial radial nerve have been shown to survive peripheral nerve injury up to three months postoperatively and maintain their normal topographic position across laminae I-VI in the spinal dorsal horn. However despite the presence of these primary axons, many of the fine caliber dendrites in laminae I-IV less than 2 μm in diameter show transsynaptic degenerative changes in the form of small cavities at one month and three months postoperatively. These membrane-lined cavities are similar to those seen following tooth pulp extirpations. They appear to follow the same sequence of enlargement and hollowing out of the afflicted dendrites until their membranes fuse with the cell membrane of the dendrite and they become patent to the intercellular space.

A comparison of the dorsal horn following injury to either the peripheral or central processes of spinal nerves at one month postoperatively reveals two major differences. The cavitation of small caliber dendrites seen after peripheral injury does not occur after central process injury. Macrophages containing numerous electron-lucent vacuoles, some dark granules and myelin debris, are found in Lissauer's tract and in the spinal dorsal horn following central process injury but not after peripheral process injury.

Six months after pressure injury to the trigeminal root, there is extensive destruction of axons in the spinal tract and of second order neurons in subnucleus interpolaris. Virtually all of the axons including primary trigeminal axons as well as axons of neurons intrinsic to the trigeminal brain stem nuclei disappeared from the tract. Most of the neurons and neural processes have also disappeared from the subnucleus interpolaris.

These studies in the spinal cord and trigeminal brain stem nuclear complex show that extensive transsynaptic degeneration of second order neurons takes place after injury to the peripheral or central processes of primary neurons. It will be important to determine whether such changes in dorsal horn circuitry provide the morphological substrate for pathophysiological mechanisms associated with some chronic pain states.

Behavioral Correlates of Neural Function in the Medullary Dorsal Horn

We have extended our analysis of the neuronal properties of the medullary dorsal horn by correlating response characteristics with behavior in awake monkeys trained in sensory discrimination tasks. As mentioned above, two general classes of dorsal horn neurons (wide-dynamic-range and nociceptive-specific) convey information related to pain. Our major objectives this year were 1) to continue studies of medullary dorsal horn neurons that send axonal projections to the thalamus, 2) to determine discrimination levels to thermal stimuli in humans and monkeys utilizing newly developed behavioral tasks, and 3) to study the effects of drugs applied locally in the medullary dorsal horn on the activity of thermally-sensitive neurons and on the ability of monkeys to discriminate noxious and innocuous thermal stimuli.

In the behavioral tasks, human and monkey subjects are required to detect small changes in warm and heat pulses applied to the face. The tasks evaluate the effects of attention on the detection of thermal changes. In this task the monkey or human detects which of two thermodes placed bilaterally on the upper lip increases temperature above a fixed level applied to both thermodes. The subject presses an illuminated button and is presented simultaneous, identical heat pulses, one on each probe. After a variable time (3-10 sec) one of the thermodes rapidly increases temperature. The subject has two seconds in which to detect this second temperature change and report which probe produced the temperature increase by pressing a left or right response button. On approximately half of the trials the subject is aided in making this detection by the illumination of a red light above the left or right response button. Ninety percent of the time this light correctly signals the side on which the temperature shift will occur. However, on 10% of the trials, the temperature shift occurs on the unsignalled thermode. The signal light provides a means of guiding the subject's attention to one thermode or the other. Both reaction times for detecting the temperature change and

accuracy in reporting the thermode on which it occurred can be analyzed to determine the effects of correct and incorrect warning signals on performance.

Human subjects detected temperature changes of less than 1.0°C from bases of 39°C and 45°C while performing the attention task. From both innocuous (39°C) and noxious (45°C) baselines, detection latencies were shortest when the subject attended to the relevant thermode (correct signal condition) and longest when he attended to the irrelevant thermode (incorrect signal condition). When there was no location signaled, latencies were intermediate. In addition, the percentage of temperature changes not detected was less for the correct than for the incorrect signal condition. One monkey detected more of the temperature changes from a 45°C baseline and produced shorter detection latencies when the stimulus modality was signaled than when it was not. These data show that detectability of thermal stimuli is influenced by attentional factors, both within and between stimulus modalities.

Previously we have described responses of thermosensitive and mechanosensitive medullary dorsal horn neurons that are independent of stimulus modality or stimulus parameters. In the present project we identified several types of these task-related responses in medullary dorsal horn cells that project to the thalamus. Some cells discharge as the monkey depresses the button to initiate the trial. Some cells exhibit a sustained discharge during the trial while others show a burst of activity at the signal for button release, whether that signal is a temperature change or light onset. Interestingly, a significant proportion of neurons show a dependable decrease in discharge correlated with a specific aspect of the task. These inhibitory (as well as excitatory) task-related responses occur only during performance of a task and are related to sensory events that lead to successful completion of the task. Some neurons with each pattern of task-related activity project to the thalamus.

Neurons with task-related activity may be providing a gain control mechanism for somatosensory information that the animal must use for successful completion of the task. Additionally, these responses may be involved in the transmission of behavioral information to motor cortex to facilitate appropriate goal-directed behavior. The presence of inhibitory task-related responses may serve as an enhancing mechanism for these signals.

The effects of microinjection of morphine into the medullary dorsal horn are studied while a monkey performs a thermal discrimination task. The results we obtained are preliminary, but consistent and encouraging. Local applications of morphine in the medullary dorsal horn of up to 15 μg do not alter general performance of the monkey in the task, but do produce profound analgesia. The monkey is able to depress the lighted button and to initiate more than 500 trials. Moreover, morphine does not affect the accuracy or latency to detect innocuous temperature pulses. Nevertheless, morphine triggers powerful analgesic effects at all doses tested, since reaction times to discriminate noxious thermal stimuli are increased substantially for 47°C and slightly for 49°C. Finally, the monkey's behavior returns towards control levels within two hours after morphine administration.

These studies are important in determining the neurons critical for signalling the intensity of painful thermal stimuli and transmitting this information to levels of conscious sensation. By studying the monkey's behavioral responses within a task we also can assess the influence upon pain perception of such variables as behavioral significance, attention and predictability of noxious thermal stimuli. Our data show that the neural representation of oral-facial nociception can be influenced by environmental and behavioral factors at the earliest stage of central integration and that this modulatory information is relayed to a thalamic nucleus that receives thermal sensory information. In addition, the microinjection data demonstrate local pharmacological modulation of nociception directly within the medullary dorsal horn, in the absence of generalized perceptual or motoric effects. The findings further suggest a direct role of this area in pain discrimination and its modulation and can ultimately lead to new and important methods of pain control.

The Assessment of Experimental and Acute Clinical Pain

The purpose of these human studies is 1) to develop psychophysical and behavioral models of pain perception that assess the intensity and unpleasantness of experimental and clinical pain sensation and also assess the ability of subjects to judge their perceptual experience, and 2) to use these models to assess physiological and psychological mechanisms of pain and analgesia, and the efficacy of pharmacological and non-pharmacological methods of pain control.

One study assessed the relationship between the reduction in the magnitude of verbal pain reports following analgesic administration and the reduction in actual sensory experience. Patients used either a numerical category or verbal descriptor scale to rate the intensity of painful thermal stimuli applied to their forearm. After a placebo medication, the intensity of these stimuli were then reduced by a fixed amount on one-half of the occasions to simulate pain reduction after an analgesic. Results showed that the verbal descriptor scales were more stable and accurate than the category scales, although both were sensitive to the sham

analgesic. The within-session decrease in response magnitude found in the preliminary analysis was documented in the final analysis. This decrease may represent a response artifact or accurately reflect a temporal response suppression observed in previous studies. The results of this study provide strong evidence for the validity of these measures in analgesic assessment.

An additional study assessed the repeat reliability of the Descriptor Differential Scale (DDS). This scale was presented to 93 subjects at one and two hours after oral surgery. Results show that this instrument is reliable over time and that performance consistency is uncorrelated with pain intensity and improves significantly with practice. Data from this study is being used to develop alternative forms of the DDS that will be useful for repetitive assessments. Preliminary analyses have resulted in two 6-word sensory intensity forms with a split-half reliability of .96. In contrast to experimental pain scaling, clinical pain measures often collect only one response. The DDS scale presents multiple measures, thereby decreasing variability and providing a measure of internal consistency to assess scaling performance. This scale may improve the reliability of clinical pain assessments and also identify poor observers who produce unreliable results. The development of alternative forms will increase the validity of repeat assessments by assuring that a second response is based on experimental pain and not on the previous response.

Recent studies on pain measurement assessed the contributions of word meanings and category sequences to the perceptual values assigned by subjects in reporting the magnitude of skin stimuli. The results showed that category judgments were made on the basis of word meaning rather than position on a list and that the assumption of equal spacing between verbal categories on a list may be incorrect. These findings provide additional evidence that verbal descriptor scaling procedures produce more information about the perception of stimuli than do simple numerical estimation procedures and should be employed in the assessment of new analgesic agents.

Assessment and Treatment of Chronic Pain

We are continuing to evaluate the effects of narcotic analgesics and electrical brain stimulation on clinical and experimental pain in a group of chronic pain patients, some of whom received chronic brain electrode implants for pain relief. These electrodes are placed in brain pathways where they are presumed to activate descending, opiate-related, pain-suppressing systems. Thirteen patients have participated in this study this year. Two received postoperative evaluations, seven were admitted for a screening visit, and four completed the presurgical evaluation. One is a candidate for electrode implantation. Four patients participated in the study assessing the effects of morphine, naloxone and placebo on thermal sensations mediated by A-delta and C-fiber primary afferents. The design of this study has been improved by including an auditory stimulus to control for confounding motoric or confounding effects. Patients press a button to indicate when each of a series of 51°C stimuli became painful. Responses to the first two stimuli in a series reflect activation of A-delta fibers and responses to later stimuli reflect activation of C-fibers. Morphine, in comparison to either placebo or naloxone, increased the latency of A-delta responses and also decreased the number of C-fiber stimuli described as painful. These results suggest that morphine exerts differential effects on A-delta and C-fiber mediated pain. This method may be one of the first procedures capable of assessing the effect of pharmacological and non-pharmacological analgesic manipulations on separate primary afferent systems.

One patient was reevaluated following sympathectomy for causalgic burning and dysesthetic pain in the sacral and lateral peroneal distributions of her right foot. The burning had returned after one year. Four series of sympathetic, sacral and peroneal anesthetic blocks showed that the dysesthetic pain was sacral nerve dependent while the causalgic pain was dependent on both sural and peroneal innervation. The sacral distribution received a peroneal collateral innervation of light touch that persisted after sacral nerve regeneration. This study of causalgia suggests that such pain may involve both sensory nerve and sympathetic nerve pathways and that reinnervation can persist after regeneration of part of a damaged nerve.

The use of deep brain stimulation to control human pain evolved from the findings in animals that electrical stimulation of peri-aqueductal sites activated a descending analgesic system mediated by endogenous opioid-like compounds. The human brain stimulation procedure is assumed also to activate a descending opioid system. Our previous findings that the stimulation-produced analgesia does not show an opioid time course and is not reversed by a narcotic antagonist suggest that the analgesia is not produced by an opioid mechanism. The reduced analgesia found in many patients after one year questions the efficacy and ultimate clinical utility of this procedure.

We have initiated a number of studies this year to evaluate mechanisms of pain and new treatment plans for deafferentiation pain and pain associated with cancer. Diabetic neuropathy pain and shingles pain are chronic pain syndromes associated with sensory loss, hyperalgesia and dysesthetic sensations. They are poorly

treated with conventional analgesics. We are evaluating the efficacy of tricyclic antidepressant drugs in the treatment of these painful syndromes. These agents are thought to alter serotonin levels in the brain and may act by influencing descending pain-suppressing pathways in the brain. Cancer pain studies are evaluating new agents in patients tolerant to morphine. These agents act on opiate receptors not sensitive to morphine administration and thus not made tolerant to morphine. Such studies should provide alternative treatment possibilities for cancer patients with severe pain even after exposure to very high dosages of morphine.

In another study we are evaluating various methods for the treatment of myofascial pain of the oral facial region. Although a wide variety of pharmacological agents have been employed in the therapy of myofascial pain, most reports of success have generally been loosely controlled clinical observations. The purpose of this project is to characterize the presenting clinical symptoms, psychological state and neurohumoral profile of patients with chronic orofacial pain and to evaluate in a controlled clinical trial the efficacy of a drug combination (anti-anxiety agent and non-steroidal anti-inflammatory agents) in comparison to conservative non-drug treatment, placebo drug and no treatment. If successful, the results of this study will provide insight into the role of anxiety, depression and inflammation in the clinical symptoms of myofascial pain in the oral-facial region.

Control of Pain and Anxiety in Ambulatory Dental Patients

These investigations are evaluating novel drugs for controlling postoperative pain in an attempt to identify agents which possess greater analgesic efficacy or less side effect liability than standard agents. Standard therapy with postoperative analgesics usually involves the administration of a narcotic analgesic in combination with a mild analgesic, such as aspirin or acetaminophen. The use of narcotics in ambulatory patients is associated with nausea, vomiting and dizziness. The drugs under investigation in our studies are selected on the basis of having greater efficacy or lower side effect potential.

A within-subject, double-blind crossover design is being employed in these investigations. Patients in need of bilateral extraction of impacted third molars serve as subjects. Subjects receive one of the two treatments on a random basis at the first appointment, and the alternative treatment is administered at a second appointment, approximately two weeks later. Flurbiprofen, a non-steroidal anti-inflammatory agent, in combination with etidocaine, a long-lasting local anesthetic, was compared to standard treatment. Following the extractions, subjects remained at the clinic to rate their postoperative pain. The combination of flurbiprofen and etidocaine resulted in less postoperative pain then standard treatment with oxycodone plus acetaminophen and lidocaine. Approximately forty percent of the patients in the sample reported no postoperative pain during the seven hour observation period at the clinic following the experimental combination. Significantly fewer patients reported side effects following the flurbiprofen plus etidocaine treatment, indicating that the enhanced clinical efficacy of the combination was not at the expense of an increased side effect liability. These findings indicate that the combination of a non-steroidal anti-inflammatory agent and a long-acting local anesthetic provides superior postoperative pain relief than analgesic methods presently employed. The increased efficacy of these agents results in no postoperative pain or reports of only mild pain.

The relationship between the analgesic effects of non-steroidal anti-inflammatory drugs and their other anti-inflammatory effects has been evaluated in a study comparing flurbiprofen to a steroid, methylprednisolone. The results of the study indicate that flurbiprofen suppressed pain initially to a greater extent than standard treatment or methylprednisolone. Conversely, methylprednisolone suppressed swelling and loss of function to a greater extent than the other treatments. This dissociation suggests that the analgesic effect of flurbiprofen may be, at least in part, independent of its other anti-inflammatory effects.

The objective of other studies is to evaluate the modification by drugs of the neurohumoral, psychological and physiological responses to acute pain and apprehension in patients undergoing a stressful surgical procedure, the removal of impacted third molars. Prototype drugs employed include placebo, anti-anxiety agents, narcotic analgesics and barbiturates. The results of these investigations will clarify the role of these agents in the control of pain and apprehension as well as provide information on the interaction of these drugs with endogenous pain control systems.

Present studies are evaluating the effects of exogenous epinephrine administered with local anesthesia on cardiovascular and catecholamine responses to oral surgery. The results of our most recent study confirm our previous finding that epinephrine-containing local anesthetics result in a marked increase in circulating epinephrine levels which is associated with an increase in cardiac output. Surgical stress in unsedated patients results in a further increase in circulating epinephrine levels and a further slight increase in cardiac output. The catecholamine and cardiovascular response is also seen in diazepam sedated patients following an epinephrine containing local anesthetic, but not the response to surgical stress. Diazepam premedication results in a direct decrease in plasma norepinephrine

levels followed by a return to preoperative levels during surgery. In contrast, unsedated patients show a marked increase in circulating norepinephrine levels during surgery. These findings indicate that administration of epinephrine-containing local anesthetics does result in appreciable circulatory changes and that diazepam premedication attenuates the catecholamine response to surgical stress. These studies suggest that epinephrine included in local anesthetics is rapidly absorbed and results in measureable circulatory changes. While these changes are well-tolerated in our subjects who have been screened as healthy and free of systemic disease, they may not be so innocuous in the elderly or cardiovascular risk patient.

A parallel study has evaluated pituitary release of beta-endorphin into the circulation during surgical stress. In a pilot study, blood samples were collected prior to and during oral surgery. A significant increase in circulating beta-endorphin levels was seen during surgery which was associated with subjects' intraoperative report of anxiety and discomfort. Preliminary results suggest that increased intraoperative levels of beta-endorphin result in lower levels of postoperative pain. Interestingly, administration of a narcotic analgesic, which is known to suppress beta-endorphin release, results in greater levels of postoperative pain. These findings suggest a role for the pituitary release of beta-endorphin in modulating postoperative pain. Investigation of the relationship between surgical stress, pituitary secretion of beta-endorphin and postoperative pain is clarifying endogenous mechanisms of pain inhibition. Better understanding of these dynamic processes will likely result in more rationale pharmacological approaches to the management of pain.

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

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NEUROBIOLOGY AND ANESTHESIA BRANCH

Intramural Projects

PROJECT NUMBER	INVESTIGATOR	PROJECT TITLE
Z01 DE00020-18	Stephen Gobel	Studies of Organization of Main Sensory & Spinal Trigeminal Nuclei
Z01 DE00031-15	Frederick J. Brown	Computer Interfacing of Neurophysiologic & Laboratory Instruments
Z01 DE00132-09	Raymond A. Dionne	Evaluation of Intravenous Sedation for Ambulatory Oral Surgery
Z01 DE00133-09	Richard H. Gracely	Assessment of Pain & an Evaluation of Pain Control Agents
Z01 DE00246-06	G. Duncan	Masseteric Exteroceptive Reflex & Sensory Responses
Z01 DE00247-06	Gary Bennett	Cytomarphology of Functionally Characterized Spinal Cord
Z01 DE00276-05	Richard H. Gracely	Narcotic and Brain Stimulation Analgesia
Z01 DE00286-04	Raymond A. Dionne	Evaluation of Etidocaine & Flurbiprofen
Z01 DE00288-04	Maryann T. Ruda	Neuropharmacological Characterization
Z01 DE00291-04	J. Oliveras	Neural Correlates of Behavior in the Monkey Medullary Dorsal Horn
Z01 DE00312-03	Marvin J. Hoffert	Physiology, Morphology & Immunocytochemistry of Spinal Dorsal Horn
Z01 DE00313-03	Richard H. Gracely	Conjoint Measurement Analysis of Metric and Nonmetric Stimuli
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