

ANNUAL REPORT  
OF  
PROGRAM ACTIVITIES  
NATIONAL INSTITUTE OF DENTAL RESEARCH  
Fiscal Year 1980  
Parts I, II, III

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
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PART I

(10/1/80) NATIONAL INSTITUTE OF DENTAL RESEARCH

ANNUAL REPORT

OFFICE OF THE DIRECTOR

October 1, 1979 - September 30, 1980

*This document was prepared for administrative use at NIH. The comments and declarations of its contributors are their own and do not necessarily represent an official statement of the Institute.*

Compiled By

Dental Research Data Officer

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National Institutes of Health

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REPORT OF THE DIRECTOR

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

October 1, 1979 - September 30, 1980

The National Institute of Dental Research continues to support the major portion of all the oral health research in this country. The mission of the Institute comprises a wide range of research interests, from studies of basic mechanisms in health and diseases to the development and application of preventive and therapeutic measures. Areas of activity are concerned with problems in dental caries, periodontal disease, cranio-facial anomalies, soft tissue stomatology and nutrition, restorative materials, and pain control and behavioral studies. Research is supported through Intramural, Extramural, and Collaborative programs.

A National Caries Program has been established as a targeted effort and support is also given to several Dental Research Institutes and Clinical Periodontal Research Centers throughout the United States. In addition, the Institute gives major support to the training of investigators, particularly at the post-doctoral level, for the conduct of dental research.

Progress in these various programs is summarized in Parts II through V of the Annual Report, and the activities of the Office of the Director are described in the present section.

The Office of the Director is responsible for overall policy determination and general management of the Institute. This is achieved with the support of various offices, including planning and evaluation, financial management, personnel, research data and data processing, scientific and health reports, equal employment opportunity, and collaborative research. Summaries of these activities follow in the rest of this section.

The conduct of the general managerial duties of the Director now requires considerably more time to be devoted to keeping abreast of and participating in the governance of NIH as part of the Public Health Service and the Department of Health and Human Services. As a result of the increasing complexity of management and the escalating demand for committee work, the Director attended almost 200 meetings during the year, serving as organizer and chairman for many of them. These ranged from sessions of the small staff of NIDR, and the Bureau-Institute-Division Directors of NIH, to advisory councils and committees for extramural and intramural programs, to planning and evaluative groups, to various special long and short range committees. In addition, the Director participated in a two-day retreat of the B/I/D Directors with the Director, NIH, which was devoted to a consideration of a broad spectrum of policy issues, and also attended a two-day EEO training course for supervisors.

During the year the Director was invited to make various public presentations on NIDR programs as well as scientific subjects dealing with personal expertise. Lectures on the NIDR programs were presented to the Alpha Omega Dental Fraternity, the Northern Virginia Dental Society, and at several dental schools. Current trends in research support were discussed at the Northeastern Ohio Medical College. A special lecture on clinical research was given at the dedication of the W. D. Miller Clinical Research Center at the University of Pennsylvania. The W. D. Miller Lecture was given before the Pierre Fauchard Academy at its annual meeting in St. Louis. A talk on forensic odontology was presented to the Ash Pathology Group in Washington, D.C., and two tapes on research highlights were made for the "Open Wide" series at TV-45 in Baltimore.

The Director also participated in meetings of various professional and research organizations, representing NIDR and performing various administrative or committee functions. These included the annual meetings of the American Dental Association, the International and American Associations for Dental Research, the American Association of Dental Schools, and the American Academy of Forensic Sciences. Committee meetings included an ad hoc advisory group on administration of the American Fund for Dental Health, the American Dental Association's (ADA) Council on Dental Health, the ADA National Advisory Committee on Fluoridation, and the ADA Committee on Legislation, and the Boards of Directors of the American Board of Forensic Odontology and the Forensic Sciences Foundation. The Director also attended the triennial International Conference on Oral Biology, and continued to serve as a permanent member of the Oral Research Advisory Group of the World Health Organization. The latter group met in East Germany this year, in order to gain fuller participation of various European countries in discussions of advances in dental research, with special attention on epidemiology, prevention, nutrition, and behavioral science.

The Institute also served as host for numerous foreign visitors, including scientists from Great Britain, Scandinavia, China, Spain, South Africa, Rhodesia, France, and Libya.

SPECIAL ASSISTANT TO THE DIRECTOR

As Planning Officer for the Institute, the Special Assistant coordinated and prepared the final submission of the NIDR Research Plan, FY 1982-84. In an effort to formulate a long-range plan for the Institute, four program branches --Pain Control and Behavioral Studies, Restorative Materials, Soft Tissue Stomatology and Nutrition, and Dental Research Institutes and Centers--were assisted in developing the goals, objectives, and evaluation criteria for their areas. The following consultants assisted the staff in completing the work on this project: Dr. Donald Kruper, University of Pittsburgh; and Drs. Richard Norman and Leo Sreebny, SUNY at Stony Brook. Panels of scientists enlisted to refine the planning elements held several meetings between January and August 1980.

The evaluation of the NIDR National Caries Program was completed and a report submitted to the Director. Reports were made to Council and the Subcommittee on Dental Caries of the NIDR Program Advisory Committee in November 1979. Assessment of the utilization of the findings is being made by the National Caries Program, its advisory committee, and the Director, NIDR.

During the year, external consultants assisted staff in formulating the charge for the Director's evaluation of the craniofacial anomalies research activities of the Institute. An RFP for a support services contract was designed and advertised and a contract is anticipated this fall. A chairman of the scientific peers has been chosen and panel selection activities are proceeding on schedule. The assistance of John Wilson, Data Processing Officer, has been invaluable in handling this project.

The Special Assistant prepared the NIDR Evaluation Plan for FY 1981 in which tentative plans for the assessment of activities associated with Dental Research Institutes and Centers were proposed. A meeting was convened in April 1980 of past and current Center Directors to refine the goals, objectives, and evaluation criteria and to discuss evaluation questions and design issues. Some of the major issues relevant to the Centers for the 1980's--which might guide the agenda for assessment--were discussed with the NIDR Special Grants Review Committee in March 1980 and with Council in May 1980.

Due to the expanded workload, particularly in the area of evaluation activities, the office recruited for an Evaluation Officer. Dr. Dushanka Kleinman was selected and joined the staff on May 1, 1980.

During the year comments have been prepared on a variety of issues, including legislative proposals from the House, the Senate, and the Administration on NIH bills of various HHS health research planning documents, and on prevention initiatives. The Special Assistant served during the year on the Oversight Committee for the Office of Medical Applications Research project, "Evaluation of NIH Consensus Development Processes," and on the NIH/OD Evaluation Oversight Committee on Science-Base Activities. A seminar for the Grants Associates Program was given describing NIDR Planning and Evaluation activities in February 1980.



Among the duties of the Special Assistant is the responsibility for advising the Director in matters relating to the support of the social and behavioral sciences. Consultation continues to be provided to the Pain Control and Behavioral Studies Program as well as to the National Caries Program. During the year, seminars, lectures, papers, and activities in this area included:

Observer representing International Sociological Association,  
Directing Council of Pan American Health Organization, October 1979.

"A Cross-Cultural Look at Children's Attitudes Toward Dental Care,"  
Meeting of Federation Dentaire Internationale, Paris, France, October  
1979:

"Federal Role in the Socio-dental Sciences," Walter Reed Army Institute  
of Dental Research, December 1979:

"Setting Priorities, Goals and Objectives for the Inclusion of Dentistry  
in the Health Systems Plan," Southeastern Association of Health  
System Agency Executives, Clearwater, Florida, February 22-23, 1980:

"Dentistry and the Behavioral/Social Sciences: An Historical Overview,"  
National Research Conference on Oral Health Behavior, Airlie Conference  
Center, Warrenton, Virginia, April 20-22, 1980;

Guest lecturer on social sciences and dentistry, Colombian Dental  
Federation meetings, Cali, Colombia, May 13-15, 1980;

"Theory and Practice of the USPHS/WHO International Collaborative  
Study of Dental Manpower Systems in Relation to Oral Health Status,"  
symposium on the Japanese Dental Care Delivery System, Tokyo Medical  
and Dental University, Tokyo, Japan, June 4, 1980;

"International Comparisons in the Provision of Oral Health Care,"  
Centenary Celebration of the British Dental Association, London,  
United Kingdom, July 10-11, 1980;

"Motivation for Prevention," Pan American Health Organization,  
August 26, 1980;

Socio-dental Indicators Committee of Behavioral Scientists in Dental  
Research--progress report, Oral Research Advisory Committee of WHO/Oral  
Health Unit, Erfurt, German Democratic Republic, September 1980;

"Introduction to the International Collaborative Study of Dental  
Manpower Systems in Relation to Oral Health Status," Federation  
Dentaire Internationale, Hamburg, Federal Republic of Germany,  
September 2-7, 1980;

Coeditor, Special Issue on Social Sciences and Dentistry, Social  
Sciences and Medicine; reviewer for this journal and for the Journal  
of Public Health Dentistry.

Organizational commitments honored during the year follow:

Member, Committee on Dental Options for National Health Insurance, Institute of Medicine, NAS, 1977-80:

Nominations Committee, American Association for Dental Research, 1979-80;

Chairperson, International Relations Committee, International Association for Dental Research, 1979-1980:

Program Committee, 8th International Congress on Oral Biology, Tokyo, Japan, June 1-3, 1980:

Program Committee for 7th International Conference on Social Sciences and Medicine (scheduled for June 1981):

Coordinator, D.C.-Baltimore area members of Behavioral Scientists in Dental Research (monthly meetings):

Consultant to Federation Dentaire Internationale (Scientific Assembly Committee and Commission on Oral Research and Epidemiology).

Committee assignments in the Department included the NIDR Awards Committee, PHS Dental Activities Task Force, and the U.S. Coordinating Committee for PHS-PRC Cooperation in Public Health and Health Services Research (Oral Health). The Special Assistant serves as consultant to the Health Resources Administration and the National Center for Health Services Research for purposes of the USPHS/WHO International Collaborative Study of Dental Manpower Systems in Relation to Oral Health Status. Consultation also has been provided to the Office of the Chief Dental Officer, PHS, on the subjects of fluorosis and dental health services research.

Various duties associated with coordinating support functions for the Director include preparing: materials for the Director's reports to the National Advisory Council; materials on international health activities; commentaries on documents generated by other governmental agencies; and selected correspondence responses for the Director's signature.

#### OFFICE OF SCIENTIFIC AND HEALTH REPORTS

The Office of Scientific and Health Reports (OSHR) is responsible for developing and maintaining the information program of the National Institute of Dental Research. OSHR disseminates information on the Institute's research studies and other activities to the scientific and dental communities, educators, Congress, members of the communications media, and the general public.

Using NIDR and NIH information channels and direct media contact, OSHR has continued, during the past year, its intensive efforts to increase the understanding of oral diseases and of known preventive measures.

A major personnel change occurred in the office this year with the departure in May 1980 of the Information Officer, Mrs. Shelbia Lengel, who assumed new duties as the Director of Public Information, Health Care Financing Administration. Mr. John Patterson, NIDR's Executive Officer, served as Acting Information Officer until September when Dr. Kenneth Lynn, who is also NIDR's Dental Research Data Officer, became the Acting Information Officer.

In FY 1980, an OSHR staff member, Mrs. Hilah Thomas, received recognition for her contributions to the goals and activities of the American Medical Writers Association by their election of her as a fellow.

Staff members served on the NIH Information Training Committee, the Printing Liaison Committee, the NIH Nutrition Education Committee, and NIDR's EEO and Awards Committees.

### Audiovisual Activities

Continuing the office's efforts begun in FY 1979 to encourage children to eat between-meal snacks that are non-cariogenic, OSHR this year produced two new 30-second public service announcements (PSAs) for TV, both aimed primarily at 6 to 12 year olds. The first one, "Notes on Good Snacking" was distributed in May 1980 to 750 TV stations. Within one month, 227 of the 268 stations that responded indicated their plans to provide free air time for this PSA. A total of 3,441 telecasts were reported. An additional 1,455 telecasts are estimated; the total viewing audience is estimated at 254,536,900. The second PSA, "Magical Munching," will be released this fall.

To date, a large volume of requests for an NIDR leaflet advertised in the first PSA has been received. A second leaflet carrying the same message, but aimed at teenagers, is in preparation. A series of posters and stickers depicting this theme has also been printed as part of this major OSHR project.

The office is preparing four radio PSAs. These spots, aimed at a teenage audience, feature popular recording artists promoting the concept of non-cariogenic between-meal snacks.

Health promotion kits have been developed by OSHR to encourage the use of non-sugary snacks. These will be sent to public school educators, dental and other health professionals, health clinics, dental journals, child health organizations, family magazine editors, and other groups concerned with children's health. The kits include samples of the leaflets, posters, stickers, public service announcements for the print media, and advertisements for children's journals.

A PSA produced earlier by this office, also dealing with the wise choice of snacks, received exceptionally wide exposure from the networks: ABC showed it immediately preceding a day's coverage of the 1980 Winter Olympics, while CBS aired it before the Evening News with Walter Cronkite. In addition, many local TV stations throughout the country provided air time for it.



OSHR also coordinated a radio and three television interviews for NIDR scientists. OSHR staff reviewed and edited: nine radio spots prepared by the NIH Division of Public Information, on various subjects including periodontal disease, plaque, fever blisters, and canker sores; and the script of a slide show presentation, This is NIH.

The office continued to serve as coordinator for developing, scheduling, maintaining, and storing of all NIDR exhibits. The new NIDR exhibit, "Clinical Evaluation of Restorative Dental Materials," built in collaboration with the Restorative Materials Branch, was displayed at seven major dental meetings, including that of the American Dental Association where the exhibit won an award in the Scientific Exhibits category.

The National Caries Program exhibit on self-applied fluoride tablets and mouthrinse programs for schools, was shown at 8 national meetings. A smaller exhibit on the same subject was displayed at 13 state and local dental meetings. Program staff represented NIDR at all of this year's exhibits except one where OSHR staffed the exhibit.

### Media

A major effort of OSHR this year focused on the NIDR/NIH Consensus Development Conference on Removal of Third Molars, held in November 1979. For this event, OSHR prepared the announcements and program brochure, handled the meeting's publicity, ran the press table during the conference, and arranged an informal press conference following the meeting to enable members of the press to interview the conference co-chairmen and NIDR administrators. Numerous press inquiries concerning the meeting resulted in several newspaper articles and a TV report. More than 900 requests for the conference's summary have already been received and filled.

In a continuing endeavor to alert the public to news of or research advances by NIDR scientists and grantees, OSHR this year issued four departmental press releases. In addition, press summaries of papers presented by NIDR scientists at the Federation of American Societies for Experimental Biology, the American Association for the Advancement of Science, the International Association for Dental Research, and the American Public Health Association were prepared for use in the meetings' press rooms.

The office continued to serve as the contact point for science writers of both the print and electronic media. In FY 1980, OSHR provided information or photographs to 88 writers associated with 42 different publications and reviewed several manuscripts for national publications including Vogue, Family Health, Children Today, and Good Housekeeping.

OSHR arranged for interviews and photographs of institute scientists for an extensive 11 page article on tooth decay which appeared in the American Chemical Society's Chemical and Engineering News. The office provided assistance for the preparation of the report which highlighted NIDR research activities.

Information and photographs of NIDR's research on diabetes and caries vaccine were provided to the New Book of Popular Science, an annual supplement to Science Encyclopedia.

The office also furnished numerous articles on NIDR research accomplishments, programs, and activities to the NIH Record, News and Features, and the "Search for Health" columns. Press summaries were prepared for two NIDR scientists who participated in NIH Science Writers Seminars.

### Publications

The public's need for readily understandable information in the area of oral and dental health is underscored by the requests for more than one-half million NIDR publications in the past year. The majority of these were mailed by OSHR through a contract mailing service, St. Elizabeth's Transitional Workshop. The Consumer Information Center in Pueblo, Colorado distributed 180,000 copies of Rx for Sound Teeth, while the NIH Visitors Center received 18,000 NIDR publications for distribution.

Because of this large volume of requests, several pamphlets had to be reprinted in FY 1980. These were: Malocclusion, Seal Out Dental Decay, Canker Sores and Fever Blisters, Tetracycline Stained Teeth, and Tooth Decay. Following a listing in Free Stuff for Parents, a large number of requests were received for several of NIDR's pamphlets.

In June 1979, Good Housekeeping published an article on oral ulcerations for which OSHR provided information. NIDR's pamphlet Canker Sores and Fever Blisters was mentioned in that article. To date, 15,752 requests for this publication have been received, more than 5,000 of them in FY 1980.

The Institute publication NIDR Research News provided 21 items on research accomplishments and NIDR activities to science writers and editors of state and county dental journals. Editors of these journals use this material extensively in informing their readers of NIDR research advances. This publication is also sent to dentists, members of dental societies, universities, members of the National Advisory Dental Research Council, and the NIDR Programs Advisory Committee.

The NIDR Fact Sheet was revised during FY 1980. This handout which describes the organization and programs of NIDR is distributed to visitors to the Institute and during NIDR exhibits at scientific and dental meetings. Work is proceeding on a more complete description of NIDR in the form of an Institute brochure.

A leaflet Sugar and Tooth Decay was prepared and submitted to the NIH Nutrition Education Committee. This publication is one of the NIH series of leaflets on health and nutrition which will be reproduced and distributed by large supermarket chains throughout the country.

This office continues to provide concept clearance for all Institute publications and has provided editorial services and copy preparation



for the proceedings of two NIDR conferences during the year. These were: Feedback Control of Exposure Geometry in Dental Radiography and Clinical Evaluation of Dental Materials.

NIDR Abstracts continues to be published containing abstracts of current papers published by NIDR scientists. This is sent to libraries of dental schools and universities, members of the Institute's council and advisory committees, and U.S. and foreign researchers.

To meet the long-standing need for a handbook that will provide all NIDR employees, particularly new ones, with an overview of the Institute, OSHR this year prepared such a booklet which will be published shortly. It contains an organizational staff listing, a description of programs, services, and facilities, as well as practical information on parking regulations, payslips, training, and career development.

#### Editorial, Public Inquiries, and Other Activities

OSHR prepared materials for use by the NIDR Director at the NIH appropriations hearings and also provided the Institute's input for NIAMDD's special reports to Congress on research on digestive diseases and cystic fibrosis. The office prepared the Institute's input for a variety of NIH and DHHS reports including: Prevention 79, the annual report of the NIH Office of Disease Prevention and Health Promotion; the Fogarty Center's Annual Report of International Activities; the NIH Almanac; the Public Health Service Report to Congress; The DHEW Nutrition Information and Education Inventory; the NIH Scientific Directory and Annual Bibliography; and the PHS Recruitment Manual.

OSHR maintained close liaison with the International Association for Dental Research and the American Dental Association in presenting current information to the public. Material was provided to the ADA on a regular basis for inclusion in the Journal of the American Dental Association. News of NIDR research findings was also distributed to the general medical profession through articles published in the NIH column of the Journal of the American Medical Association. One of these, describing a new head restraining device that makes dental treatment of cerebral palsy patients easier, generated a large number of requests from physicians wanting to obtain the device.

OSHR functions as the clearance center for all manuscripts and abstracts written by Institute scientists and administrators. This year, the office processed 262 manuscripts and 144 abstracts. A staff member also provided copy editing as requested of a number of scientific manuscripts.

In addition to providing information and material on request to other NIH components, OSHR reviewed: several publications for the NCI which discussed the effects of cancer treatments on oral and dental health; a pamphlet for NASA on oral hygiene; and a joint publication of HHS and USDA on nutrition guidelines.

Responses were prepared for use by members of Congress to reply to constituents' inquiries concerning dental research and treatment, and OSHR provided more than 5,000 copies of NIDR publications for use in these replies.

OSHR responded to telephone and written inquiries from various groups including: professional associations; Federal, State, and County health agencies; journals; dentists and health professionals; and the public.

Tours of the NIDR laboratories and visits to the staff were arranged by OSHR for 218 individuals this year. These included both American and foreign dental students and practitioners.

In summary of the highlights of FY 1980 activities, OSHR produced 2 new public health service announcements for TV and 4 for radio; prepared 2 new leaflets, reprinted 5 existing pamphlets, and initiated 2 new publications; prepared posters, stickers, PSAs for the print media, and advertisements for children's journals on the non-cariogenic snack theme; coordinated a radio and 3 TV interviews for NIDR scientists; edited 9 NIH radio spots; handled publicity, programs, and press relations for the Consensus Development on Removal of Third Molars; issued 59 press items (including summaries and articles for NIDR Research News, NIH Record, News and Features and "Search for Health" columns; provided information and assistance to 88 science writers from 42 publications; reviewed and edited manuscripts for magazines, other NIH components or agencies, and Institute scientists and administrators; prepared or provided assistance in preparation of numerous internal, NIH, DHHS, and congressional reports; responded to 19,458 written and telephone requests for information or specific publications; wrote 1,003 letters of response; arranged for 3 NIDR exhibits to be shown at 28 meetings; handled the distribution of 556,114 publications; cleared 262 manuscripts and 144 abstracts; served as concept clearance center for all Institute publications and audiovisual activities; arranged interviews and tours for 218 visitors to NIDR; provided editorial and copy preparation for publications of proceedings of 2 NIDR conferences; and continued to provide communications assistance to all program areas of the NIDR.

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As a continuing requested service, the specialist (Mr. John Small) has, throughout the year, provided information about health benefits, health issues, legal matters, and major political developments concerning fluoridation to various U.S. and foreign agencies, dental societies, and congressional subcommittees.

In May 1980, at the request of the American Dental Association, the specialist provided technical information for the court defense of the State of Illinois law requiring fluoridation of public water supplies. A similar service was provided to the State's attorney for South Dakota in the challenge to their fluoridation law. The specialist also furnished reports and assistance to the Strathclyde Regional Health Council in Glasgow, Scotland. A court suit brought against the Council



by opponents of fluoridation there is considered by British health authorities to be a key test of their fluoridation initiatives.

At the request of the Maryland State Health Department, the specialist served as PHS liaison person for the various activities resulting from the fluoride spill at the Annapolis water treatment plant in November 1979. In this capacity, status reports, technical assistance, and information were provided to government officials, journalists, and dental and public health organizations. Technical and editorial assistance on various documents dealing with the quality of dialysis water was provided to the Office of the Assistant Secretary for Health, the Mayo Clinic (which is organizing a committee to advise the National Kidney Foundation on their position on fluoride in dialysis), the Association for the Advancement of Medical Instrumentation, and others.

The specialist provided advice to EPA and the University of Texas, concerning development of the Texas dental fluorosis study until October 1979, when all NIDR protocol suggestions were adopted and the first progress report was issued.

At the request of the Minnesota Department of Health, the specialist coordinated PHS responses to a preliminary report on fluoride and health by an environmental review committee in Quebec, Canada, and assisted the State dental staff in publication and distribution of the critique of the report to dental and public health groups.

The specialist served as a technical resource person for the Chief Dental Officer, PHS, in discussions with EPA officials and the Assistant Secretary for Health on EPA's enforcement of water quality regulations that limit fluoride concentrations.

Upon review of a comprehensive publication on fluoridation procedures by an equipment manufacturer, the specialist provided comments to the manufacturer and to a Committee of the American Water Works Association.

The specialist provided information on fluorides and health for Congressional staff reports and responded to inquiries from members of Congress, other government officials, the public, and several foreign countries. Regular reviews of the dental and medical literature were conducted and information exchanges with health and environmental agencies and professional groups were maintained.

The Specialist participated in program planning and was an invited speaker at a fluoridation workshop in Tucson, Arizona, sponsored by the Indian Health Service for tribal representatives and community health service and water service personnel. At the request of the Maryland State Dental Director, Mr. Small also spoke on fluoridation at a regional meeting.



## THE FINANCIAL MANAGEMENT OFFICE

The Financial Management Office (FMO) serves as the Institute's center for budgetary data and related activities. FMO's financial planning and budgetary activities, which are divided between its budget formulation and execution cycles, represent the bulk of the office's operations. The FMO formulates and presents to Congress budgetary estimates which will support current and future program plans and operating expenses. The office supervises the apportionment, allocation, and allotment of the Institute's final appropriation.

In FY 1980, FMO continued to formulate the Institute's zero-base budget in a manner that had not been used prior to 1979. Budgetary submissions and program dollars were apportioned within Basic, Applied, and Developmental (BAD) research categories, as opposed to the lengthier Science-Base, Application, Transfer, and Training (SATT) structure of two years ago. The FMO's emphasis on the concise and informative BAD budget structure resulted in a highly manageable financial program.

The FMO continued to monitor the Institute's increasing contributions to trans-NIH activities in FY 1980 and disseminated to the Congress and other agencies detailed information in the areas of diabetes, arthritis, nutrition, and disease prevention. The FMO responded to special requests for program data from Federal and non-Federal agencies, the OMB, and Congress, in addition to preparing annual and special reports to Congress.

Full staffing and increased office efficiency were achieved through systematic analyses of the office's personnel and administrative functions. In FY 1980, position descriptions were updated and categorized into discrete areas of responsibilities such as planning, presentation, formulation, and execution. The office continued to train its employees on the job and through outside courses to enhance each employee's career development and also as a means of increasing the office's efficiency.

The FMO is purchasing an integrated word processor to facilitate its information dissemination and budget presentation responsibilities. In addition, WYLBUR computer display terminals will be linked to the office's new information systems to save time and money in generating and updating budgetary reports.

In FY 1980, the FMO trained one NIH Stride Program assignee and two NIH management interns in accordance with the FMO's own commitment to enhance the overall NIH resource pool of budget analysts and officers. The FMO anticipates training more such individuals during the next fiscal year.

## PERSONNEL OFFICE

The Personnel Office serves as the focal point for Institute personnel management services. During the past year, Personnel Office efforts have focused on internal reorganization, communication with Institute employees, classification and pay administration, placement of employees

affected by the Three-Year Classification Review, implementation of Senior Executive Service and Merit Pay Systems, and Affirmative Action.

A major change took place in the Personnel Office in August, 1980 with the separation of the NIDR Personnel Officer function from that of the National Institute of Neurological and Communicative Disorders and Stroke. With the assignment of a full time Personnel Officer to the Institute, it was possible to have that individual assume Institute-wide management analyst responsibilities. The NIDR strongly believes that both the Personnel Management function and the Management Analysis function can be one management responsibility, especially in such areas as organizational structure, methods and procedures, staff planning and organizational relationships, and delegations of authorities throughout the Institute. This change should strengthen the services provided to all Institute employees.

In an effort to communicate better with NIDR employees and to improve service to them, the Personnel Office Staff has scheduled regular visits to the Westwood Building, the Park Building, and Building 30. The Staff also hopes to initiate regular informal sessions on personnel matters at the various employee locations.

In the area of pay administration, the classification of positions continues to be an important issue in the Institute as well as at the NIH. Questions raised during the year concerned the classification of positions in several occupational series. Members of the Personnel Office have worked with Institute and NIH Management Staff to resolve many of these issues. The Personnel Office has also taken a positive role in commenting on proposed Factor Evaluation Standards (FES) in an effort to assure that Institute employees will be given credit for all aspects of their jobs as new standards are published and implemented. Another area of pay administration is the inclusion of dentists under the Physicians Comparability Pay Program. Personnel Office staff worked closely with managers of the Institute to provide information to the Department supporting the comparability allowance for dentists. This issue should be resolved shortly.

A major NIH initiative during FY 1980 was the NIH Training and Placement Program (TPP). The goal of this Program was to assign to new positions those employees in positions identified as overgraded during the Department's Three-Year Classification Review. The Personnel Office Staff worked closely with NIDR employees and supervisors to identify employee qualifications and interests in order to accomplish this. For those employees who have not found new assignments, the Staff has been working through the NIH Placement Program for Retained-Grade Employees.

Full implementation of the Senior Executive Service (SES)/Senior Scientific Service (SSS) and the Merit Pay System is scheduled for October 1, 1980.

Approximately 50 NIDR employees are affected by these two systems. The key elements of SES/SSS and Merit Pay are incentives and accountability. It is in the implementation of these systems that one should see the



most significant impact of the Civil Service Reform Act of 1978. In both systems the performance appraisal becomes a key element. During the year, each NIDR employee subject to one of these two systems was required to develop a Performance Plan which identified major job responsibilities, goals and objectives, and standards of performance. The identification of employees for these two systems, the development and presentation of training sessions, assistance in developing performance plans, and the submission of reports required by NIH and the Department regarding implementation of the systems have all been developed or coordinated by members of the Personnel Office.

The Personnel Office continues to work closely with the NIDR EEO Coordinator, the NIDR Director's Advisory Committee, and supervisors in supporting affirmative action and furthering equal opportunity goals. Major activities have centered around working with members of the Advisory Committee on supervisory training and incentive awards. Personnel Office staff have also worked with supervisors in the establishment, selection, and development of training plans for an NIH Stride position and an Institute Individual Career Development opportunity. The Staff has also worked with the NIDR EEO Office in supplying data for reports, helping to resolve EEO complaints, developing Federal Equal Opportunity Recruitment Program (FEORP) goals, and hiring minorities and women for the Summer Employment Program. We are pleased to note that summer hiring goals were exceeded again this year.

During the year, Personnel Office staff participated in several trans-NIH activities. These included membership on the NIH Administrative Training Committee, the Committee on the Continuing Education of Division of Personnel Management Staff, the Task Force on the Classification of Grants Support Positions, and the Task Force on Clerical Support Recruitment. The staff has also participated in activities such as the NICHD Career Development Course and the NIH Minority and Women Recruitment Program.

#### DENTAL RESEARCH DATA OFFICER

Through the operation of a specialized dental research information center, the Dental Research Data Officer has continued to provide NIDR staff and other users with a wide variety of information related to dentistry and dental research. While subjective and statistical data related to the activities of the Institute are a primary responsibility of the office, it receives requests for information in other areas of dentistry as well. In addition to meeting the Institute's needs, the office also served a large number of other Federal (53 percent) and non-Federal (16 percent) users as well.

The Dental Research Data Officer also serves as the Privacy Act and Freedom of Information Act Coordinator for the Institute. This includes: the determination of those requests to which the Acts apply; response to those requests or referral to an appropriate responder; consultation with legal counsels on matters of patents and copyrights; and a number of related tasks. The latter this year included the development of a new system of records to cover NIDR-supported contractors: the updating

of Purpose and Safeguard statements for publication in the Federal Register; and the issuance of several PA/FOIA Advisories for the staff.

There has been an increase in both Privacy Act and Freedom of Information Act requests this year. The FOIA requests have increased by about 25 percent. The cost to NIDR in responding to these requests in FY 80 was about \$7,000, less than one-third of that amount being recovered by the Government. That figure does not include staff time lost from other activities.

Various ad hoc requests for data this year dealt with such subjects as alcoholism, the disabled and handicapped, drug abuse, germ plasm banks, interferon, ionizing radiation, marijuana, and pesticides. The only significant change in the number and distribution of these requests has been an increase in the number of non-Federal requestors. This may be due to the office's greater visibility as a result of listings in specialized information directories and publications and referrals by NIDR staff.

A number of major changes have affected our printed reports and publication activities. One of the most obvious is due to the Government-wide change in paper size from 8 X 10 1/2 inches to 8 1/2 X 11 inches. In addition, this year all of our DRDO covers will incorporate the NIDR logo. The GPO-published reports, Dental Research in the United States and Other Countries (ISSN 0147-264X) and National Institute of Dental Research Programs (ISSN 0360-7753), will continue with the same cover design and size as last year.

The Index of grants and contracts, compiled for us by the Division of Research Grants, was combined with the Indexes, part VI of the Annual Report for FY 79. While this delays the availability of that part, the consolidation of the two should make the Indexes more useful and less expensive than two separate printings.

The Smithsonian Science Information Exchange (SSIE), with whom we have contracted since 1969 for the preparation of Dental Research in the United States and Other Countries and for other services, will become a Government agency October 1, 1980. While certain details of the transfer are not yet clear, only the method of payment should change, from contract to interagency agreement.

The DRDO, which depends on SSIE for its assessment of dental research not supported by NIDR, has worked with the Exchange to promote its services and to develop its data base. In a cooperative effort to reach a new group of investigators, especially those without Federal support for their research, we monitored the SSIE exhibit at the annual meetings of the American Association of Dental Schools and the American Association for Dental Research. SSIE's new policy of charging investigators to register research projects not sponsored by the U.S. Government may greatly diminish the office's efforts to collect dental research projects in other countries.



At the request of the Associate Director for Extramural Programs, this office compiled (in a matter of weeks) an orientation manual for members of the National Advisory Dental Research Council. The manual, which had only limited circulation, has been useful to a large number of individuals and we highly recommend a more professional rendition with provisions for updating.

The selective dissemination of information (SDI) to NIDR staff continues to operate in two areas: (1) ongoing research and (2) technical reports. Ongoing research, registered with the Smithsonian Science Information Exchange, is presorted and distributed monthly to those categorical program areas with similar research interests. Microfiche copies of technical reports that result from Government-supported research contracts are received in the office, catalogued, and listed by accession number for distribution to NIDR staff about once a month. A supplemental effort to include abstracts to those technical reports will be discontinued this year for lack of need and its relatively high cost in time and effort.

The National Library of Medicine has approved online access to approximately 18 computerized data bases, including MEDLINE, SDILINE, and TOXLINE. Utilization of this system by investigators in remote off-campus locations should be most valuable. This office will be responsible for the system's coordination, payment of fees, and training of users. The service should be operational for Westwood and Park Buildings by 1981 and will be expanded to include the rest of NIDR as soon as possible.

Dr. Kenneth Lynn served as Chairperson of the Dental Libraries Special Interest Group of the Medical Library Association at its annual session. He also attended, as a liaison person, the Dental Libraries Group of the American Association of Dental Schools at its annual session.

Mary Ann Williamson served this year as Chairperson of the NIDR EEO Advisory Committee.

#### OFFICE OF COLLABORATIVE RESEARCH

OCR's major responsibilities include the initiation, review, business management and monitoring of ongoing R&D contracts and agreements as well as the closeout of completed contracts. During FY 1980, the Institute had a total of fifty active contracts and interagency agreements. On the average, there were approximately thirty to forty active contracts and agreements at any given time. The office issued fifteen Requests for Proposals (RFPs), eleven for new actions, and four for renewals. The RFPs for new activities included five competitive actions and six interagency agreements. Review and monitoring activities involved seven project site visits, five RFP review meetings at NIDR, and four telephone conference call reviews. Eighty-three consultants were used in these activities. Six new contracts, six renewal contracts, and seven interagency agreements were awarded. In addition, thirty incremental funding actions and other contract modifications were made. Approximately \$4.2 million was expended for the conduct of these activities.

During the year, the Office of Collaborative Research decreased its staff slightly through attrition, consistent with the recent decrease in R&D

contract activities. This action should result in increased productivity measures for the office in future years. An HHS initiative during FY 1980 that will have a significant impact on the workload calls for major increases in documentation and reporting of planning efforts for the initiation of contracts. The office has completed and submitted its first planning report for FY 1981 contract awards. Through these activities, the office hopes to achieve a better workload balance throughout the year.

In addition to his responsibilities for the management of the office, the Associate Director for Collaborative Research served on a number of advisory committees and working groups including: Office of Medical Applications of Research Advisory Committee; OD Evaluation Oversight Committee on Application and Technology Transfer; Committee to Implement GPRST Recommendation No. 38 (Council Orientation Subcommittee); Chairman, Study Group on Review and Evaluation of NIH R&D Contract Proposals; NIH Inventions and Patents Board; NCHCT Planning Committee for Assessment of Dental Radiology; NIH Contracting Officers' Standards Committee; and OMAR working group to define emerging technologies. Although these activities have been somewhat time consuming, they have provided an opportunity to present NIDR's views on emerging policies at an early point in their formulation. These or similar activities are likely to continue in the future.

#### EEO PROGRAM

NIDR's EEO Program activities during FY 1980 encompassed aspects of recruitment, training, contract compliance, support to minority schools, reports and analyses of the Institute's EEP profile and efforts, the assignment of collateral EEO duties, and recognition of EEO efforts.

#### Recruitment and Selection

Recruitment efforts included establishing contacts at minority schools, sending letters to those contacts about summer employment opportunities, and encouraging contacts through NIDR staff attendance at the National Dental Association (NDA) Meeting, the Federally Employed Women, Inc. (FEW) Conference, and the Minority Biomedical Support (MBS) Symposium.

The employment goals for the summer undergraduate program set for NIH were 16% for minorities and 39% for women. NIDR employed 14 undergraduate students, of whom 29% were minority and 64% were women. Similarly, the NIH goals for the summer graduate program were 12% for minorities and 26% for women. NIDR employed 8 graduate students - 38% minority and 63% women. Minorities in the two programs included one black man, three black women, one Asian man, one Asian woman, and one Hispanic woman. Of eight dental students employed in all summer programs in the Institute, three were minorities - one black man and two black women. Hence, the Institute exceeded the NIH selection goals.

The Institute continued to employ students under the Cooperative Education Program and brought the total number who have been thus employed to twelve.



Improvements in representation of minorities and women included the appointment of a black female dentist as a Staff Fellow, the employment of a non-minority female dentist in the Commissioned Corps and the selection of a black female for a Stride position in the administrative series.

### Training

The results of a supervisory training questionnaire developed by the EEO Advisory Committee were used to develop recommendations on training for NIDR supervisors. The first phase of those recommendations consisted of two sessions on EEO training for supervisors conducted by McClure-Lundberg Associates, Inc. in June and September, 1980. Seventy Institute supervisors attended these meetings.

The EEO Coordinator worked with the NIDR group to complete the NIDR Training Policy, and the EEO Advisory Committee commented on all drafts of the policy.

The Institute's Secretarial Training Advisory Group coordinated two training sessions during the past fiscal year - one on "Time Management and Managing Stress" in October, 1979 and another on "Power is Given - Not Taken" in April, 1980 during National Secretaries Week. Both workshops were conducted by Ms. Kip Potts, a career management consultant.

### Discrimination Complaints

The Institute was involved in two complaints of discrimination during the past year. One, a formal complaint filed and investigated prior to FY 1980, was resolved during this year with a signed, informal adjustment prior to a proposed disposition from NIH. The second, filed as a formal complaint in FY 1980, was informally resolved with a signed agreement prior to an investigation.

### Contracts, Grants, Public Advisory Bodies

The NIDR EEO Coordinator continued to serve as the Institute's Contract Compliance Coordinator. The Coordinator worked with the NIH Contract Compliance Officer and the NIDR Associate Director for Collaborative Research to identify and schedule Institute project officers for training in NIH's Contract Compliance Program. Twenty-four of twenty-six project officers completed the training. On July 1, 1980, project officers began to administer the NIH Abbreviated Check List during site visits to the principal investigator on contracts.

The Institute continued to fund research related to its programs through its cooperative agreement with the Division of Research Resource's Minority Biomedical Support Program.

The percentage of minorities in the NIDR's four public advisory bodies increased from 20% to 29% and the percentage of women increased from 28% to 35%. Minorities on these bodies include black, Asian and Hispanic men and women.



## Other Program Activities

In January of 1980, and EEO Report of accomplishment and planned actions was discussed with the Director, NIH when he reviewed the NIDR Forward Plan.

As required by EEOC and OPM under the Federal Equal Opportunity Recruitment Program (FEORP), an analysis was made of underrepresentation by race and sex in NIDR's staff as of September 30, 1979, in grade level bands and in selected occupations, compared with civilian labor force data. Institute goals were established for grade-level bands pending the implementation of the NIH FEORP Plan.

The NIDR EEO Coordinator served in a collateral assignment for seven months as the Chief of Staff to the Task Force to Evaluate the NIH Division of Equal Opportunity (DEO). The Institute contributed to this Task Force through a report from the NIDR EEO Advisory Committee and by completing a questionnaire on the Institute's EEO Program. Two other NIDR staff members made statements to the Task Force - the Institute's Scientific Director, representing the Review Panel for the NIH Conflict and Cooperation Study, and the Institute Director's Secretary, representing the NIH Office Support Staff Coordinating Committee. After the NIH DEO Task Force Evaluation, the NIDR submitted a ten-year report on EEO efforts and accomplishments as requested by the Director, NIH, from all NIH Bureaus, Institutes and Divisions.

The assignment of collateral duties in EEO to NIDR staff includes appointments of the EEO Counselor, the Delegate and Alternate to the NIH Women's Advisory Committee (WAC), and the representatives to the Institute's EEO Advisory Committee. During FY 1980, the terms of the previous Delegate and Alternate to the NIH WAC expired, and the Institute's previous EEO Counselor resigned from the Institute. In both instances, after suggestions were requested from all institute employees, the NIDR Director submitted nominations to the NIH Division of Equal Opportunity on the basis of recommendations of the NIDR EEO Advisory Committee. The new Delegate and Alternate to the NIH WAC were appointed. The appointment of a new EEO Counselor will be made after October, 1980. Staggered appointments of two-year terms to the NIDR EEO Advisory Committee are made by the NIDR Director each October after elections are held in one-half of the geographic areas.

The NIDR EEO Advisory Committee submitted nominations for the EEO Special Achievement Award to the Institute Director after suggestions were requested from Institute employees. The Institute's annual awards ceremony is scheduled for early FY 1981.

## OFFICE OF DATA PROCESSING AND ANALYSIS

### Scientific Systems Section

A major project of this section during the past fiscal year was the hardware upgrade and software conversion for the Diagnostic Methodology Section. Their computer system was moved from a cramped office into a renovated room to provide proper electrical, cooling, and humidity

controls. To provide additional computation power, the central processing unit (CPU) and memory were replaced by a faster, more reliable CPU with semiconductor memory and a floating point processor. Several peripheral options were replaced by newer, space efficient boards so as to reduce the load on the I/O bus and improve the thruput of the system. Major software changes were designed and implemented in order to change the system from a single user system to a multi-user system. This change greatly enhanced the availability of the system to the researchers.

The section continued its mission in the realtime data acquisition area by interfacing a spectrophotometer to the DEC PDP 11/70 computer. New technology was employed in this project by designing and fabricating a microprocessor-based interface board that is housed within the cabinetry of the spectrophotometer. The interface is controlled by software in read only memory (ROM) chips on the board. The ROM chips were programmed using a special hardware device called a "prom burner" which is controlled by the DEC 11/70 computer.

During the past year, more emphasis has been placed on data analysis. To accommodate the ever-expanding requirements in this area, the BMDP collection of statistical programs has been installed on our system. Also, three different graphics and plotting devices are now supported to allow people to visualize the relationships in their data. Researchers can perform a quick visual analysis of their data by obtaining a graph on a CRT terminal. For hard copy output of the graph, they can generate graphs on the Versatec printer and/or the Zeta plotter. A recently purchased software package called RS/1 will soon be installed on the system to further enhance the above capabilities.

Another area of improvement that will soon be visible is the data entry and validation capability of the system. The dental clinic, for example, routinely performs studies that involve many patients and considerable data for each patient. In the near future, some studies will be allowed to enter their data directly into the computer using alphanumeric video terminals which can be programmed to display forms that normally would be on paper. This mechanisms will reduce paperwork eventually and will reduce the number of errors in data recording.

A major thrust of the Scientific Systems Section during the past year has been to increase the reliability of all the computers that this section supports. For example, efforts are under way to standardize the configuration of our satellite computers as much as possible. This increases the efficiency of the manpower by increasing commonality. Configuration worksheets have been prepared on all the systems to improve the hardware documentation. Preventive maintenance schedules for each system are being prepared to chart the work for twelve-month periods. Special software is being used to log hardware errors so as to correct problems before they become major. Furthermore, we are just beginning to expand these efforts into performance evaluation order to acquire hard data that will allow us to make recommendations regarding system expansion or reconfiguration. These efforts will continue into next year, and we are excited by the potential in this area.



Currently, the section is completely redesigning the system for the Neurobiology and Anesthesiology Branch which has been developed over the past decade. New technology, major new requirements, and new initiatives led to the decision to develop a new, more powerful system using distributed processing techniques and microprocessors. This complex project will not be completed for many months due to limited manpower, but the capabilities that it will provide are enormous.

#### Management Information Section

Within the last fiscal year, the Management Information Section (MIS) continued its efforts in meeting the informational requirements of the NIDR staff in the areas of grants, contracts, and financial data.

While all data systems have been enhanced, the grants system of performing daily global updates was significantly improved and automated. These changes in the updating process have resulted in noteworthy savings in terms of updating cost and time, while producing greater data file integrity. Although the grant file is accessed in a "batch" environment, these refinements have produced a near real-time capability.

Specialized reports have been developed in support of the National Caries Program (NCP) using the Division of Financial Management allotment ledger master file and the open document file. One series of reports provides consultant financial tracking and the other series displays financial expenditures by month and object class with remaining balances.

The NIDR extramural travel, contracts, intramural and personnel files have continued to enjoy heavy utilization both in terms of ad-hoc requests and monthly reporting. It is anticipated these files will be even more heavily accessed with the advent of increasingly stringent budget tracking mandates.

The NIDR graphics capability should be considerably increased during the next fiscal year. The Division of Computer Research and Technology (DCRT) will install the TELLAGRAF software package in early FY 1981. Previously we had utilized the Parklawn Computer Center; however, DCRT is a much larger system and will supply expanded capabilities.

The committee formed to study NIDR policy and requirements in the area of word processing systems has been quite active within the past fiscal year. A number of vendors have given demonstrations of hardware capabilities and limitations. Future plans call for continued study and acquisition of word processing equipment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00030-13 OD		
PERIOD COVERED October 1, 1979 - September 30, 1980				
TITLE OF PROJECT (80 characters or less)  Laboratory Automation Optimization of Computer Resources in Dental Research				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT				
PI. COPI: Other:	Terry P. Medlin Sheila A. Taylor Leidy D. Zern, Jr. Eugene R. Coley Patricia P. Friedman Kathleen Jo Powers Murray Oltman Mary Mendelis	Supv. Computer Specialist Computer Programmer Elec. Engineering Tech Computer Operator Computer Programmer Computer Programmer Elec. Engineering Aid Clerk-typist	OD OD OD OD OD OD OD OD	NIDR NIDR NIDR NIDR NIDR NIDR NIDR NIDR
COOPERATING UNITS (if any)  None				
LAB/BRANCH Office of the Director, Office of Data Processing and Analysis				
SECTION Scientific Systems Section				
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD 20205				
TOTAL MANYEARS: 3.75	PROFESSIONAL: 1.6	OTHER: 5.35		
CHECK APPROPRIATE BOX(ES)				
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER				
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords)				
<p>A main thrust of this project during the past year has been the goal of improving the data analysis and data presentation capability of the system. In the past year, two additional devices for <u>data graphing</u> have been made available to the research scientist. Further, a new <u>data management</u> package which combines analysis with graphing capability will soon be added.</p> <p>An enhanced package for <u>behavioral research</u> is currently being implemented. Work is also continuing in the area of computer performance evaluation.</p>				



Computer utilization continues to expand and change direction. Several new people began to use the computer and a new thrust into the area of data analysis and data presentation tools was experienced. Additional methods of data presentation were added and a significant package for data management, analysis, and presentation was added.

A totally new and greatly expanded system for controlling behavioral experiments, collecting the realtime data, and performing various kinds of analysis is currently in progress. A blend of microprocessors and satellite minicomputers are being used in this project in order to increase the speed and reliability of the total system.

The capability and usefulness of the image processing system was substantially increased this year. Formerly a single user system, the system now supports five users who may simultaneously be performing a mixture of image processing, digitization, or program development. Additional work on this system in the future will lead to further improvements since performance evaluation tools are currently being developed to allow one to determine how to optimally configure specific hardware and software components.

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PART II  
NATIONAL INSTITUTE OF DENTAL RESEARCH  
ANNUAL REPORT

NATIONAL CARIES PROGRAM

October 1, 1979 - September 30, 1980

*This document was prepared for administrative use at NIH. The comments and declarations of its contributors are their own and do not necessarily represent an official statement of the Institute.*

Compiled By  
Dental Research Data Officer  
National Institute of Dental Research  
National Institutes of Health  
Bethesda, Maryland





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# Section I

## REPORT OF THE ASSOCIATE DIRECTOR

STRATEGY AREA I:  
Combatting the Microbial Agent

STRATEGY AREA II:  
Increasing the Resistance of the Teeth

STRATEGY AREA III:  
Decrease the Cariogenic Potential of the Diet

STRATEGY AREA IV:  
Improved Delivery and Acceptance of  
Caries Preventive Methods





# National Caries Program

## Report of the Associate Director

Since it began in 1971, the National Caries Program has been characterized by the breadth of its activities, which have been concerned with virtually every aspect of the questions of what causes dental caries and how the disease can be most effectively and economically prevented. This year's Annual Report is especially illustrative of the diversity of the NCP. Advances have been made in understanding the mechanisms of adhesion and the reasons for the virulence of certain caries-inducing microorganisms, in the testing and refinement of techniques for improving tooth resistance and modifying the diet, and in the successful promotion of caries prevention regimens to large segments of the public. In particular, the report demonstrates how the NCP simultaneously functions, as it must, in the research laboratory, the clinic and the community, and in the highly visible arena of dental public health.

How microorganisms adhere to tissues has been a subject of intense research interest recently in many areas of biomedical research. This is an area in which NCP-supported scientists have frequently been the leaders. Recently, NCP grantees have reported substantial new information suggesting that a class of enzymes, glucosyltransferases (GTF), are a central factor in the adherence of Streptococcus mutans to enamel and, through a somewhat different mechanism, in the agglutination of these bacteria in dental plaque. Further, experiments with mutant forms of S. mutans indicate that the GTFs may be critical and sufficient factors to explain observed variations in the cariogenicity of S. mutans and that this virulence may be subject to alteration. The possibility of using GTF-derived antigens in a vaccine against caries, previously explored empirically in animals, now appears to have a sound theoretical basis.

Current clinical research activities of the National Caries Program are focussed on the identification of the most cost-effective topical fluoride regimens of those now available for general use, and on the possibility that combinations of these regimens may have enhanced therapeutic effects. Preliminary data from a study on the effect of fluoride mouthrinsing in optimally fluoridated communities are especially interesting, as they suggest that this self-applied fluoride regimen significantly increases the preventive benefits conferred by water fluoridation.

Recently, research on the role of the diet in the etiology of dental caries has sharply expanded due, no doubt in part, to a heightened interest in the subject on the part of consumer groups and regulatory agencies. A major obstacle to progress in this area has been the lack of data to establish which foods contribute most to the cariogenic potential of the American diet. It is neither experimentally feasible nor ethically possible to generate such information from human trials. Therefore, as mentioned last year, the NCP has given very high priority to research, in our own laboratories, to develop a reliable animal system to establish the relative cariogenic potential of specific foods. These efforts have been successfully concluded. A complete protocol for a food screening system was developed and tested and has been shown to yield reproducible results. A report of this research was published in May 1980, and the system is already in use in several other laboratories.

No matter how successful the research efforts of the NCP to identify and develop cost-effective methods to prevent caries, there can be no beneficial effects on the public health unless these methods are widely used. Therefore our efforts to disseminate information and promote new preventive programs were expanded further during the past year. With a very modest expenditure of staff time and budget, we were able to supply information on practical caries prevention programs in response to over 5,000 requests. More significantly, the number of children estimated to be participating in school-based self-applied fluoride programs increased to over 12 million, compared to 10 million a year ago.

An unexpected and welcome development during the year was the decision of the European Organization for Caries Research (ORCA) to hold its 1982 Annual Congress in the U.S. in recognition of the tenth anniversary of the National Caries Program. This unprecedented gesture by one of the world's most prestigious dental research organizations is a distinct honor for the NCP, and emphasizes our continuing close collaboration with the international caries research community.

The following sections of the report briefly summarize the progress and current status of research and development in the four Strategy Areas of the National Caries Program.

## STRATEGY AREA I: Combatting the Microbial Agent

Glucosyltransferases (GTF) are important factors in determining the ability of cariogenic organisms such as Streptococcus mutans to attach to tooth surfaces and develop dental plaque. Acid production by these organisms, in close proximity to the tooth, results in enamel dissolution and formation of carious lesions. The possibility of preventing caries by blocking GTF-mediated adherence of cariogens has prompted numerous NCP supported studies. This section of the report summarizes the current status of these investigations.

It is apparent that GTF-mediated adherence and plaque formation are considerably more complex processes than they were thought to be only a few years ago. Fundamental information on the biochemistry and physiological importance of these enzymes is required before this avenue can be exploited fully. The majority of studies have been conducted with S. mutans but information gained from other streptococci, actinomyces and Leuconostoc mesenteroides has provided valuable leads to understanding the role of GTF in adherence of cariogenic microorganisms.

GTFs have been categorized according to their ability to produce water-soluble or water-insoluble glucose polymers or glucans from sucrose. The solubility of the polysaccharide produced is related to the proportions of  $\alpha$ -1,6 and  $\alpha$ -1,3 linkages between adjacent glucose moieties. The  $\alpha$ -1,6 linkages predominate in water-soluble glucans or dextrans and the  $\alpha$ -1,3 linkages predominate in insoluble polymers known as mutans. The mutans are sticky and adhere to smooth surfaces of teeth, to other mutan molecules and to plaque microorganisms. Branches occur in the polyglucose chains due to mixed linkages. Analysis of glucan from a particular strain of S. mutans showed that it contained about 60%  $\alpha$ -1,3 linked glucose molecules, 30%  $\alpha$ -1,6 linked glucose molecules and the remaining molecules were involved in  $\alpha$ -1,3 and  $\alpha$ -1,6 branch linkages. Two glucans synthesized by a strain of L. mesenteroides have been examined recently. One is a conventional dextran with 95%  $\alpha$ -1,6 linked glucose and 5%  $\alpha$ -1,3 linked branch points. The second glucan is novel, having glucose units alternately linked by  $\alpha$ -1,6 and  $\alpha$ -1,3 bonds; it is completely resistant to dextranase. Grantees have also shown that the analogous polysaccharide, fructan, is synthesized from the fructose moiety of sucrose, but fructans are not thought to be as important as glucans in adherence.

GTFs have also been categorized on the basis of their association with the microbial cell surface or from their occurrence in the culture media. The evidence indicates that cell-associated and cell-free forms of the enzyme are different states of the same protein. Cell-associated GTF is not a precursor of the extracellular enzyme, which is secreted by the organism; rather, it seems likely that the converse may be true. Several investigators are examining the novel mechanisms by which S. mutans binds glucans and GTF. The ability to bind GTF is characteristic of S.



mutans. Small but significant amounts of cell-associated GTF occur on cells grown in the absence of sucrose. Increasing the sucrose concentration of the growth medium results in an increased proportion of the GTF associated with the cell. This cell-associated GTF synthesizes insoluble glucans, which remain associated with the cells. Grantees hypothesize that this cell-associated glucan then binds additional GTF from the medium, which in turn synthesizes more cell-bound glucan leading to formation of adherent masses of polysaccharide. The finding that addition of dextranase to cells grown in sucrose-containing medium eliminates cell-associated GTF lends support to this hypothesis. In addition, heat treatment of sucrose grown cells, which destroys their enzymatic activity, did not prevent their ability to bind extracellular GTF. This stability of the GTF-binding factor to heat emphasizes its carbohydrate, rather than protein, nature. The recent demonstration of GTF and glucan binding to lipoteichoic acid provides an additional explanation of how these materials may attach to S. mutans. Lipoteichoic acid is a major component of the bacterial cell surface.

Studies supported by NIDR more than ten years ago showed that incubation of high molecular weight dextran (>100,000) with S. mutans resulted in agglutination. No agglutination occurred if cells were heated prior to incubation. Other species of streptococci and L. mesenteroides, which synthesize dextran, are not susceptible to agglutination by dextran. More recent studies by NCP supported scientists indicate that different serotypes of S. mutans vary in their response to dextran. Dextran binding factors, devoid of GTF, dextranase and invertase activities, have been isolated from streptococcal culture supernatants and cell sonicates by several investigators. They were inactivated by heat, trypsin and dextranase, indicating that they are glycoproteins; their ability to bind specific carbohydrates and the blocking of agglutination by Concanavalin A indicates that they are lectins. Studies on semi-purified factors showed that divalent cations are required for dextran binding activity and that each mole of lectin binds three moles of dextran (MW 175,000). In contrast, GTF binds only one mole of dextran per mole of enzyme. A mutant of S. mutans, which was cariogenic but failed to agglutinate in the presence of dextran, was shown to have normal levels of GTF but exhibited only trace amounts of dextran binding lectin. Thus, although agglutination and adherence of S. mutans are distinct processes, they are clearly related through dextran derived from GTF. After adherence has occurred agglutination contributes to plaque accumulation on tooth surfaces.

Several investigators are attempting to purify GTF for use in a variety of applications. The properties of the enzymes depend upon the species, strain and serotype of the organisms, on the culture media used and on the isolation and purification methods used. The enzyme is constitutive since growth in the presence of glucose, fructose, mannitol and sorbitol produced the same levels of enzyme as growth in sucrose-containing medium. There appear to be multiple forms of GTF with different molecular



weights and specificities. The tendency of GTF to aggregate may account for these different forms and has led to difficulties in enzyme purification. Treatment of a high molecular weight form of the enzyme with dextranase or high salt concentrations dissociated it into lower molecular weight sub-units, which retained enzymic activity. The product of the sub-unit enzyme was more soluble than the product of the parent preparation. Components of culture media, including sucrose and dextran, cause aggregation of the enzyme, and may invalidate kinetic experiments to characterize the enzyme unless rigorous procedures are followed to eliminate these factors. Several purification procedures have been devised but none of these yield a pure enzyme, as judged by classical criteria. Purified preparations always contain carbohydrate of which glucose is the principal component. However, several pieces of evidence, including the requirement for dextran as an activator or primer and the failure of anti-dextran globulin to inhibit activity, indicate that the carbohydrate component of this glycoprotein is not dextran.

Two mechanisms have been proposed to account for the elongation of glucan chains by GTF. In the first, glucosyl moieties of sucrose are added in stepwise fashion to the non-reducing end of the glucan, which is attached to the enzyme by non-covalent bonding. In the second, the chain is lengthened by insertion of the glucosyl moieties at the reducing end of the chain, which is bound to the enzyme covalently. Precedent exists for each of these mechanisms and experiments to determine unequivocally which mechanism is operative have proved impossible to devise. Starting with *S. mutans* grown in a chemically defined medium, a grantee has obtained chromatographically pure GTF from the cell-free culture broth. The preparation synthesized water-soluble but not water-insoluble dextran and was devoid of invertase, levansucrase, dextranase and phosphorylase activities. It showed an absolute requirement for dextran; nanomolar concentrations of dextran were effective in activating the enzyme. In the absence of dextran, sucrose hydrolysis occurred but dextran was not synthesized de novo. The investigator presented evidence that sucrose is hydrolyzed at the same enzymic site as dextran synthesis. His studies support a mechanism of GTF action in which the two substrates, sucrose and dextran<sub>(n)</sub> are not obliged to bind to the enzyme in a specific order, a covalent glucosyl intermediate participates and one or both of the products, dextran<sub>(n+1)</sub> and fructose, may leave the enzyme before the substrates are bound. Several other workers have reported that dextran stimulated glucan synthesis by GTF. The requirement for dextran has been cited as evidence favoring a primer function for dextran and the stepwise mechanism for elongation at the non-reducing end of the chain. However, other investigators showed that dextran, with its non-reducing ends modified to make them unreactive, was still an effective activator of GTF. They questioned whether dextran functions as a primer and suggested that activation must occur by a different mechanism. In another laboratory, GTF preparations from *S. mutans* synthesized a series of oligosaccharides of increasing size, in the absence of dextran. Since these oligosaccharides

competed with the dextran provided as an acceptor, this investigator proposed that the glucan synthesis proceeds by stepwise transfer of single glucosyl units rather than by the insertion mechanism advocated by other workers. Development of improved enzyme purification methods may resolve these discrepant findings and various interpretations.

A grantee found that glucan formation by a crude preparation from S. mutans was stimulated by phosphoglycerides as well as by dextran. Stimulation by lipid and dextran was additive. The effect on water-insoluble glucan production was greater than that on soluble glucan synthesis. Subsequent studies on growing cells of S. mutans indicated that a lipid synthesis inhibitor suppressed production of extracellular GTF. This effect was not due to a direct effect on the enzyme or on release of enzyme bound to the bacterial surface. The investigator postulated that secretion of GTF by the cells requires lipid and that inhibition of lipid synthesis impairs GTF secretion into the extracellular phase.

Recently, a NCP supported laboratory reported the isolation of six S. mutans derived GTF's. They could be classified into three groups, depending on their requirement for primer dextran and the solubility of the glucan produced. Two enzymes had no requirement for dextran and synthesized soluble dextran; two others also required no primer dextran but synthesized water-insoluble glucan, which chemical staining indicated contained a mixture of  $\alpha$ -1,6 and  $\alpha$ -1,3 linkages; finally, two enzymes had an absolute requirement for acceptor dextran and synthesized insoluble mutan, which was extremely rich in  $\alpha$ -1,3 linkages. A mutant of S. mutans, which was incapable of plaque formation, was noncariogenic and exhibited some ability to form insoluble glucans, proved to be lacking the latter two  $\alpha$ -1,3-mutan producing enzymes. This suggests that these mutan producing enzymes are key virulence factors and that glucan required in the early phases of adherence of the organism to smooth surfaces is inadequate for subsequent development of cariogenic plaque.

The existence of multiple GTFs with different specificities, which could act in concert, may provide one explanation of the origin of branched linkages in glucans. Several other mechanisms have been proposed. One of the earliest suggestions was that branching enzymes acted on preformed linear chains. The requirement for either exogenous sucrose or for dextranase to hydrolyze glucans before branched glucan synthesis occurs makes this mechanism unlikely. The proposal that a single GTF capable of forming glucosyl linkages with terminal and non-terminal glucose moieties continues to receive some support. A grantee's preparation of highly purified GTF from S. sanguis, which catalyzes formation of glucan containing  $\alpha$ -1,3 and  $\alpha$ -1,6 linkages in the same ratio as the crude enzyme, favors such a mechanism. The insertion mechanism offers an attractive explanation of how branching may occur. As described earlier, in this mechanism dextrans are elongated by insertion of glucosyl moieties of sucrose between



the enzyme and the reducing end of the chain. Periodically, attachment of the chain to the enzyme is interrupted by insertion of water, rather than a glucosyl moiety, resulting in liberation of the glucan. A branch point would be introduced when a dextran rather than a glucosyl moiety is inserted to form an  $\alpha$ -1,3 linkage. Using radiolabelled dextran and sucrose with an immobilized GTF preparation, evidence was obtained that branch linkages are formed when the C-3-hydroxyl of a glucosyl moiety of dextran acts on the C-1 of the reducing end of a dextran-GTF complex. This displaces the dextran from the GTF and an  $\alpha$ -1,3 linkage is formed.

Studies with mutants of S. mutans, by contractors and grantees, have been useful in establishing the connection between GTF and virulence of this organism. One mutant, with elevated levels of GTF, demonstrated abundant synthesis of water-insoluble glucan and, as a consequence, greater plaque-forming and caries-inducing activities than its parent strain. Another mutant, which was completely devoid of GTF adhered poorly, produced little plaque and was avirulent in gnotobiotic rats. Continuous feeding of GTF, but not of water-insoluble glucan, produced caries in gnotobiotic rats infected with this mutant. Caries activity was augmented by addition of both GTF and water-insoluble glucan to the diet. A GTF deficient mutant was found to retain its ability to attach to rat teeth and to saliva-coated hydroxyapatite. This confirms that initial attachment of S. mutans to the teeth is not dependent on sucrose and GTF. Initial attachment is thought to be due to electrostatic forces between the bacterial and tooth surfaces and/or binding of the bacteria to glycoproteins of the salivary pellicle covering the teeth.

S. mutans possesses several distinctive characteristics which make it especially adept at causing tooth decay. These include the abilities to colonize the teeth; to synthesize adherent polysaccharide; to produce acid from sucrose; to survive in an acidic environment, and to store intracellular polysaccharides, which can be converted to acid during periods of sucrose deficiency. Preventive techniques targeted at any of these factors offer promise of reducing caries. Elimination of GTF-mediated production of adherent polysaccharide is a particularly attractive possibility. The information gained from NCP sponsored studies on this virulence factor suggest some specific methods for its control, which would create minimal alterations in oral ecology. The Program is already supporting several projects with this objective. These include synthesis of compounds which will specifically inhibit GTF, efforts to prevent plaque formation and accumulation by using enzymes which hydrolyze GTF products and induction of specific immunity to S. mutans by vaccination with GTF.

Analogs of the two substrates of GTF, dextran and sucrose, have been tested as enzyme inhibitors. Partially oxidized dextrans from L. mesenteroides were effective inhibitors of soluble GTF from S. mutans. Unfortunately, they had no effect on cell bound fractions of GTF or on adherence and growth of cariogenic strains

of S. mutans. The high molecular weight of these dextrans may have prevented them from penetrating preformed polysaccharide masses. Lower molecular weight compounds were tested by other investigators. Sucrose derivatives halogenated in the 6 and 6' positions were synthesized and found to be effective GTF inhibitors. Hexoses substituted in the 6 position were also inhibitory. 6-deoxy-6-amino- $\alpha$ -methylglucose, 6-deoxy-6-amino- $\beta$ -methylglucose and 6-deoxy-6-amino- $\alpha$ -methylmannose were highly effective inhibitors of the synthesis of insoluble glucans from sucrose by GTF. These compounds are promising agents for preventing caries but their effectiveness in vivo remains to be tested. NCP supported scientists and others have shown that dextranase prevents plaque formation and inhibits development of caries in laboratory animals. The enzyme was ineffective when applied in a gel to children's teeth. Differences in the composition of plaque from humans and laboratory animals may account for this disappointing result. A NCP contractor recently found that proteolytic enzymes were more effective than dextranase in solubilizing human plaque. A combination of the proteinase, thermolysin, and dextranase were as effective as fluoride in reducing caries in rats. Improved procedures for treating human teeth with the enzymes may be needed to develop a satisfactory preventive method.

The possibility of preventing caries immunologically has been pursued for nearly a decade. The NCP has supported studies showing that antisera to S. mutans whole cells reduced adherence in vitro and inhibited extracellular GTF. Antibodies developed to partially purified GTF preparations were effective inhibitors of sucrose-dependent colonization of smooth surfaces; antibodies against enzyme preparations which synthesized insoluble glucans were more effective than those against preparations which synthesized soluble polysaccharides. Immunization of laboratory animals with S. mutans whole cells induced salivary antibodies and conferred immunity to caries. However, grantees reported that unidentified components of S. mutans whole cells may cross-react with human heart tissue, making use of a defined antigen desirable. Since GTF preparations were devoid of this cross-reactivity, some investigators have concentrated their attention on GTF as a potentially safe antigen. Their findings are extremely promising. S. mutans GTF, capable of synthesizing insoluble polysaccharide, was bound to insoluble polysaccharide for use as an antigen. Oral administration produced salivary antibodies and reduced caries scores in rodents. The antibodies conferred cross-protection against different serotypes of S. mutans, suggesting that development of a vaccine for humans may only require the use of GTF prepared from only one strain of the organism.

The recent developments in recombinant DNA and somatic cell hybridization techniques may circumvent many of the difficulties encountered in previous attempts to elucidate the role of GTF in adherence. For example, cloning of S. mutans genes, which determine virulence, into nonpathogenic hosts and the availability of



monoclonal antibodies to virulence factors, including GTF, will permit detailed analysis of each step in the adherence process. Monoclonal antibodies will also facilitate purification of antigens, such as GTF, for use in a safe and effective caries vaccine. The NCP plans to encourage and support projects using these promising new techniques.

During the year 73 grants, 11 contracts and 31 direct operations projects were active in Strategy Area I, representing 56 percent of the National Caries Program research projects.

## STRATEGY AREA II. Increasing the Resistance of the Teeth

The activities of the NCP in Strategy II are characterized by being largely at the level of clinical and field testing of agents. Because these projects must be executed in a prescribed way and interpreted carefully they generally are carried out by staff directly or conducted through contracts. Consequently, these projects entail major investments of staff time. NCP's clinical trials and demonstrations in Strategy II are listed below so that the extent of staff's involvement can be seen. In addition, in this year's Report progress in the development of pit and fissure sealants is reviewed.

### -Fluoride Regimens-

During the last few decades clinical investigators have tested a number of topical fluoride regimens to prevent caries. They have applied fluoride to the teeth in gels using custom-fitted mouth trays; they have incorporated it in tooth paste, chewable tablets and in oral rinse solutions, and in almost all cases they have found effectiveness against caries. Because of differences in these clinical trials in the way in which caries was measured and in other factors the reported effectiveness of the agents cannot be compared with confidence. To overcome this problem the NCP is evaluating most of the noteworthy topical fluoride regimens under side-by-side conditions in carefully controlled clinical trials. These studies are listed below.

- . At Eastman Dental Center weekly rinsing with dilute fluoride solution is being compared with and without prior tooth cleaning.
- . At the State University of New York, Stony Brook, semi-annual topical treatment with acidulated phosphate fluoride gel is being compared with and without prior tooth cleaning. These two studies are important because cleaning increases delivery cost and complexity; on the other hand plaque may provide a beneficial reservoir for fluoride.
- . At the University of Florida, school-based mouthrinsing with fluoride is being tested in the two concentrations, 0.05 and 0.2% NaF, and at daily and weekly intervals.
- . In Pittsylvania Co., Virginia, neutral NaF and acidulated phosphate fluoride are being compared as the active agent in both rinses and tablets.
- . At Eastman Dental Center and the University of Texas Health Science Center, Houston, SnF<sub>2</sub> and NaF are being compared in daily mouthrinse programs.

In addition to the above studies to obtain reliable, comparative information on currently described variations of the same regimen, NCP staff is carrying out or supporting studies to establish whether certain regimens used in combination possibly have significantly higher effectiveness or to establish the optimal duration of school-based programs. Some of these are listed below.

- . At the University of Connecticut Health Center a study has just been completed in which three important approaches to caries prevention have been compared singly and in combination with respect to effectiveness and cost. These approaches are based on topically applied concentrated fluoride at 6-month intervals, topically applied dilute fluoride by mouthrinsing at weekly intervals, and sealing pits and fissures of the occlusal surfaces of the teeth. In these trials personnel costs accounted for the largest portion of expenditures. It was also found that costs were not necessarily strictly additive when two or more procedures are combined.
- . In Wayne Co., N. Carolina, NCP staff are measuring the caries reduction that children obtain if during all the years of elementary and highschool they chew and swallow a fluoride tablet on a daily basis.
- . In Seagrove, N. Carolina, Program staff are conducting a similar evaluation of benefits of long-term attendance at schools with fluoridated water.
- . In three counties in Virginia, Program staff are examining the level of benefit achieved if children attend schools with fluoridated water only during early years of schooling.
- . At the NIH Clinical Center, NCP staff conducted a short clinical assessment of an intraoral device for maintaining desired levels of fluoride in the saliva and plaque. The device worked extremely well so that salivary levels of fluoride were maintained slightly above 1 ppm for a month whereas baseline levels had been approximately .05 ppm. A large and sustained increase in plaque fluoride levels was also observed.
- . At Biddeford Maine, a low-fluoride area, and Des Moines, Iowa, an area with optimal levels of fluoride, staff are comparing school-based daily and weekly fluoride rinse programs.
- . In Nelson Co., Virginia, NCP staff are evaluating the combined effectiveness of three of the most effective fluoride based measures--chewing and ingesting a fluoride tablet on a daily basis, oral rinsing with dilute fluoride on a weekly basis and use of a fluoridated dentifrice.

Finally, it should be pointed out that this year staff is concluding the last 5 of the Program's 17 demonstrations of caries prevention by community-run programs of fluoride mouthrinsing in schools. Overall, approximately 80 thousand children participated in the demonstrations in grades K-6(8). Simultaneously the Program has been carrying out an intensive campaign to make communities throughout the nation aware of these models and to assist them in establishing their own mouthrinse (or other self-applied fluoride) program. Both the demonstration of mouthrinsing in grades K-6(8) and accompanying promotional activities, described in part in



Strategy IV of this Report, have been highly successful so that currently about 14 million children are believed to be involved in school-based self-applied fluoride programs.

Though average participation in the demonstration projects in grades K-6(8) was nearly 83% it was expected that participation would decrease in highschool grades due to problems in delivering the rinse to participants and due to changes in the interests of older students. To examine this possibility and to identify ways that might be necessary to bring participation to satisfactory levels, the Program in 1977 expanded the demonstrations conducted by five contractors to include grades K-12. In April of the current year these contractors met with staff to discuss their results. As expected, the contractors reported that despite innovative measures to maintain participation it tended to decrease with each more advanced class and was particularly low if students had not previously been involved in rinse programs. The results clearly indicate the crucial importance of behavioral factors in the success of upper-grade health programs that involve group participation.

#### -Adhesive Sealants-

In 1975 National Caries Program staff completed five clinical trials independently investigating the effectiveness of caries prevention by sealing the occlusal surfaces of molar and bicuspid teeth with ultra-violet light cured bis-GMA adhesive resin. The "cost-effectiveness" of the procedure was determined, taking into account the length and number of clinical sessions entailed in examining, sealing, re-examining, and if necessary re-sealing teeth; the salaries of the clinical team consisting of a dentist and dental hygienist; and the numbers of new caries that would be expected to occur if the occlusal surfaces were not sealed. From these data the cost per surface saved was computed and found to range in the five trials between 15 and 26 dollars (in 1975 dollars). In examining these computations it was obvious that a major contributor to the cost was the amount of clinical time involved for each subject. Even if the computation was performed using the salary scale for a dental hygienist rather than a dentist the overall cost was only halved.

At a workshop in 1976 sponsored by NCP the high costs and their effect on acceptance of sealant programs was discussed. As a result it was concluded that to decrease the need for repeated, time-consuming examination of candidate surfaces that: (1) sealing of incipient lesions along with intact surfaces would have to be acceptable and (2) new sealants with improved retention and abrasion resistance would have to be developed. Furthermore, it was suggested that development of new sealants that could be self-applied and provide protection for all tooth surfaces might provide highly cost-effective caries prevention.

Since this workshop there has been considerable effort in a number of laboratories and research clinics to reduce the high cost of sealant programs. An immediate question is whether alternative commercially available sealants have significantly better properties than the ultra-violet light polymerized sealant used in the clinical trials described



above. To obtain data on this question NCPs Biometry and Community Programs Sections have assisted the Fairfax Virginia County Health Department in computing statistics from a clinical trial comparing a major self-polymerizing sealant with the light-polymerized sealant placed on the occlusal surfaces of teeth of opposing halves of each subjects mouth. Results showed that the percentage of treated teeth with fully retained sealant after 6 months and 2 years was 86 and 78 percent for the light-catalyzed resin and 96 and 92 percent for the auto-catalyzing resin.

A second approach to decreasing the cost of sealants is to make possible the sealing of early lesions bearing in mind, of course, that viable bacteria would be covered with the sealant. To assess this possibility investigators at Eastman Dental Center have studied for a number of years what happens to the bacteria underneath the polymer layer. The investigators report that two years after applying ultraviolet light hardened sealant the count of viable bacteria within the lesion drops to about 0.1% of the initial count. Additionally these investigators find that sealant placed over lesions seems to be well-retained. With somewhat similar objectives in mind, investigators at the Louisiana State University School of Dentistry have been exploring possibilities for developing a sealant expressly to penetrate the partially decalcified surface of early caries lesions, polymerize in situ, and block diffusion of substances involved in the caries process. During FY 1980 these investigators have developed three prototype systems and found that it was indeed possible to obtain infiltration of the monomers into the lesion.

In another approach to reducing the cost of sealant programs a team of investigators at the State University of New York at Buffalo are continuing to explore a family of compounds, termed polyphosphonates, that strongly adhere to tooth surfaces through chemical bonds. With these compounds, obtaining an adherent layer would not depend on interlocking a polymer with tabs etched into the enamel surface. Thus what is proposed is the elimination of all the steps of etching, washing and drying of tooth occlusal surfaces that require meticulous attention and contribute greatly to cost in conventional sealant programs. In place of these steps the investigators suggest that a brief swab of the clean tooth with a solution of the polyphosphonate would coat the enamel surface with a strongly adherent layer designed to reduce acid solubility and provide other desired functions.

During the year 36 grants, 15 contracts and 15 direct operations projects were active in Strategy Area II, representing 31 percent of National Caries Program research projects.

### STRATEGY AREA III: Decrease the Cariogenic Potential of the Diet

In last year's report the National Caries Program briefly described its Operational Plan for Strategy III. The Plan linked 11 major objectives which mutually support achievement of the goal, "American diets and dietary habits with decreased cariogenic potential." Of the 11 objectives two have high immediate priority and involve the main amount of activity in Strategy III at this time.

The first of these objectives is to develop information on safe, non-cariogenic sweeteners in anticipation of the possibility that one or more will be attractive for commercial development and eventually used in a wide variety of foods. This effort is set in the context that because of considerations concerning absolute safety, the four commercially-developed non-cariogenic sweeteners, cyclamate, aspartame, saccharin and xylitol will, at the most, probably be approved for only limited use. One must also be aware that partial elimination of sucrose or complete substitution with a non-cariogenic sweetener (xylitol) in human diets has dramatic effects on caries. Thus, it is clear that the goal of finding safe, alternative non-cariogenic sweeteners and stimulating the development of snack foods free of tooth destroying potential could have enormous benefits in caries reduction, particularly if coupled with interventions that simultaneously decreased the acid producing capacity of the oral flora and increased the resistance of the enamel.

Since its inception the NCP has been examining the few potentially non-cariogenic sweeteners that appear suitable for food use and has been searching for sweeteners among compounds having similar structure. Initially the Program contracted with the University of Pennsylvania to characterize a highly sweet protein, Monellin, and with Dynapol Corporation of Palo Alto, California, to synthesize analogs of neohesperidine, a highly sweet compound obtained from citrus rind, to improve its overall taste characteristics. In 1977 to focus extramural attention on sweeteners, the Program sponsored a major conference, "Sweeteners and Dental Caries," widely distributed the conference proceedings, and announced its interests in sweeteners in the NIH Guide to Grants and Contracts. These activities prompted submission of several sweetener-related research proposals and led in 1979 to grants to SRI International, Palo Alto, to study the metabolism of SRI Oxime V, a highly sweet cyclohexadiene, and to the University of California, San Diego, to synthesize sweet aspartame-like compounds with safe metabolic products.

During the past year the NCP has broadened its search through requests for proposals to synthesize new sweet compounds or to screen plant extracts for them. These procurements have led to contracts with Purdue University to examine sulfur analogs of common sugars for sweetness, with Research Triangle Institute to explore for sweetness among compounds with close structural similarities to aspartame, benzylthiorea, cyclamate, dihydrochalcone and saccharin, and with the University of Illinois to explore for other sweet compounds in the plant genus Stevia in which phyllo dulcin is found.

The second objective that the Program is intensively pursuing at this time is the development of a standard methodology that can be used by dental scientists and food technologists to compare the cariogenic potential of various food items including snacks and beverages. Direct Program involvement in this area has evolved from concern that none of the independently developed methods were sufficiently defined and tested to insure the high level of reliability and interpretability that is required for this crucial test. In 1976 the NCP compared results of animal tests of cariogenicity run similarly in several laboratories to identify sources of variation and to assess the degree of variability. After further assessment of the potential of available tests the Program selected two that, if fully developed and employed in concert, probably would efficiently distinguish cariogenic from non-cariogenic snacks.

The first of the tests is conducted with rats that are provided food in such a way that caries is the result of only the snack portion of the diet. To achieve this effect the animals are provided their basal diet by gavage and the snack portion of their diet by a programmed feeder. The complete protocol for this assay defines the use of internal standards of sucrose and describes exactly how rats are inoculated with S. mutans and how the test foods are prepared. To conduct the assay seventeen portions of either sucrose (the control) or the snack food are provided each day and the ratio of level of caries that results in the test and control situations is reported as the Cariogenic Potential Index. During the current year the assay has been tested with a variety of foods and found to provide reproducible, useful results. One intriguing result is that certain foods, such as cheese, when alternated with sucrose markedly decrease the level of caries. Another finding is that the nature of the snack with respect to sugar content determines the eventual microbial composition of the plaque.

The other assay being developed by NCP scientists involves clinical measurement of the amount of acid produced in dental plaque during consumption of foods containing fermentable carbohydrates. To obtain the information a small pH-sensitive electrode is mounted on a conventional intra-oral prosthesis. After several days to allow plaque to accumulate on the device, test foods are chewed under standard conditions and the production of fermentation acids is recorded. Though this assay has been used in Switzerland as a basis for requiring that certain snacks bear a warning concerning cariogenic potential, the test has not come into use in other countries because technical problems associated with the telemetry of data to external recorders and with contamination of the electrode surface by saliva and food substances have not been solved. Therefore, in addition to refining the test and preparing a standard protocol for it, NCP is involved in solving the technical problems of telemetry and in fabricating miniature pH electrodes that have a useful lifespan in the mouth.

During the year 11 grants, 5 contracts and 6 direct operations projects were active in Strategy Area III, representing 10 percent of National Caries Program research projects.



#### STRATEGY AREA IV. Improved Delivery of Caries Preventive Procedures

In establishing the National Institute of Dental Research, the 80th Congress gave it specific responsibilities including promotion of the useful application of the results of its research. This section of the Report reviews a variety of promotional and educational activities, carried out largely by Program staff, that comprise NCP's response to this Congressional charge.

These activities are based on two major principles. First we emphasize that municipal water fluoridation is the preferred public health approach to caries prevention. Second, where fluoridation of community water supplies is not feasible, we emphasize programs of self-application of fluoride designed for school settings. Though the target population for these programs is school-age children, because this is the age group most susceptible to caries, our educational activities are conducted through a variety of communication channels and addressed to a number of different audiences including health professionals, educators and parents as well as children.

In last year's Report we described the development of new exhibits on self-applied fluorides. In FY 1980 we took one of these exhibits to the annual meeting of the American School Health Association, the American Public Health Association, the Yankee Dental Congress, the Association of Teacher/Educators, the National School Boards Association, the National Education Association and the National Association of Pediatric Nurse Associates and Practitioners. There was a great deal of interest in this exhibit and staff was continually busy responding to requests for resource materials and answering questions concerning the implementation of self-applied fluoride programs.

In FY 1980 the other exhibits, developed to be distributed on a free-loan basis, were displayed a total of 112 days in a variety of settings including meetings of medical, dental, nursing and dental hygiene associations, meetings of state PTAs and school board associations, meetings of rural and public health groups, and hospitals and shopping malls. Two of these exhibits were designed for easy portability. For these we are receiving more requests than can be accommodated.

Also in last year's Report we described development of four films to be distributed on a loan basis. Promotional efforts to make known the availability of these films included: direct mailings, notices in health and educational publications, pamphlets accompanying the exhibits described above, and showings at film festivals dealing with health issues. The following table shows the current circulation of the films as reported to us by the film distributing agency.

<u>Title of Film</u>	Months in Circulation	Bookings	Showings	Viewers
The Daily Tablet for Healthier Smiles	7	158	164	2,375
The .2% Solution	8	231	316	7,313
Smilemakers: Self Applied Fluoride Programs in Schools	8	253	285	6,686
Prescribing Fluoride Supplements in Medical and Dental Practice	5	223	239	2,309
Totals for 4 Pictures		865	1,004	18,683

The films also are available from the American Dental Association and a number of other health and educational groups.

An earlier film, "Reading Writing and Rinsing" was distributed to television stations for local broadcasting. Data now available show that this film, which is on fluoride rinsing in schools, was broadcast 634 times to an audience estimated at over 11 million. Though television distribution has been discontinued recently, the film in 16 mm format may be borrowed from the American Dental Association.

Pamphlets and leaflets are another means of promoting self-applied fluorides that the Program uses extensively. As shown in the following table the Program distributed during the past year about 140,000 leaflets, mainly in bulk to program coordinators who send them home with parent permission slips or distribute them at parent and teachers association meetings. Of these the most popular leaflet is "Fluoride Mouthrinsing in Schools -- Protection for Children's Teeth", of which over 200,000 copies have been distributed. Permission has been given to several local programs to translate this leaflet into Spanish.

<u>Leaflet</u>	Number distributed
Fluoride Mouthrinsing in Schools... Protection for Children's Teeth	118,000
Fluoride Tablets: A Healthier Smile for School Children	7,200
A Healthy Start...Fluoride Tablets for Children in Preschool Programs	5,575
Fluoride to Protect Your Children's Teeth	8,523
Total	139,298

The publication, "Preventing Tooth Decay: A Guide to Implementing Self-Applied Fluoride Programs in Schools" also has been extremely popular. The NCP has distributed all of its 20,000 copies, the GPO has exhausted its supply, and we are currently revising and expanding it in preparation for a new printing.

In last year's Report we also described the development of nine posters designed to create interest in and emphasize the importance of fluorides in caries prevention. Some of the posters are designed for use in medical and dental clinics whereas others are designed for use in schools which have tablet or rinse programs. Over 205,000 of the posters have been distributed this year.

During the year Program staff have provided a great deal of help or consultation to community leaders on setting up and monitoring self-applied fluoride programs in schools; worked closely with other government agencies and offices with interest in these programs; frequently supplied background information on caries prevention to wire services or to writers for magazines such as Chemical and Engineering News and Family Circle; arranged a conference, "Dental Caries Protection in Public Health Programs" to provide a review of caries prevention research for dental public health personnel; and given over 35 lectures, workshops, and continuing education courses on techniques of caries prevention and on the National Caries Program in general. This is in addition to lectures at universities on research opportunities through grant support and presentations of research papers at national meetings.

Most of the work described above is carried out directly by staff or by personal service contracts. However, the five contracts for demonstrations of fluoride mouthrinsing in upper grade levels (see Strategy II), include research on delivery questions, and there are grant-supported studies on diffusion of health information and on factors that influence the adoption or non-adoption of self-applied fluoride regimens in U. S. schools. Approximately 3 percent of the NCP's discreet projects are in this area.



# **Section II**

# **DIRECT OPERATIONS**

The following reports describe NCP direct operations projects that were active in FY 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00029-13 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980 CT-0600057

TITLE OF PROJECT (80 characters or less)  
The effect of school water fluoridation on dental caries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR
Brunelle, Janet A.	Chief, BS	NCP	CPR	NIDR
Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR

COOPERATING UNITS (if any)  
Dental Health Division, North Carolina State Board of Health, Division of Water Hygiene, Environmental Protection Agency

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Community Programs

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS       (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluorides were added to the water supply of a school in Seagrove, North Carolina. The concentration of fluoride used was higher than the level considered optimal for the community water fluoridation in the geographic area. Children attending the Seagrove school live in an area without a central water supply and where the various sources of well water contain negligible levels of fluoride. Children are exposed to the higher fluoride level only while at school in an attempt to approximate the total fluoride intake of children who drink optimally fluoridated water on a full-time basis. Baseline dental examinations for dental caries were made prior to the installation of fluoridation equipment. Follow-up examinations were conducted after four, eight, and twelve years to determine the extent of caries protection as increasingly larger segments of the study population become continuously exposed to fluoridated water at school since entering the first grade. Results of the four- and eight-year examinations on full beneficiaries of the procedure showed decreases in caries prevalence of 30 and 40%, respectively, compared with baseline findings. On the eight-year examinations, an assessment of the prevalence of dental fluorosis showed that no children had any definite signs of the condition.



## 1. Project Description

### Objective:

The purpose of the study is to determine the decay preventive benefits derived by children who when at home drink water that is essentially devoid of fluoride but when at school consume water fluoridated at 7 times the level considered optimal for community water fluoridation in the same geographic area.

### Methods:

In 1968, fluorides were added to the water supply of a consolidated school (grades 1-12) in Seagrove, North Carolina. The target level of fluoride, 6.3 ppm, is seven times the level considered optimal for community fluoridation in the same geographic area. Children are exposed to the higher fluoride level in an attempt to approximate the total fluoride intake of children who drink optimally fluoridated water on a full-time basis. Prior to the installation of fluoridation equipment dental examinations using the DMF tooth and surface index were conducted on approximately 1100 children to determine baseline caries prevalence. Surveillance of the fluoride levels maintained is provided by school personnel under the supervision of the North Carolina State Board of Health. Follow-up examinations are conducted at four-year intervals to measure the extent of caries protection as increasingly larger segments of the study population become continuously exposed to fluoridated water at school since entering in the first grade.

### Major Findings:

Eight-year follow-up examinations were conducted in 1976. The interim data showed that children 6 through 14 years of age, the full beneficiaries of the procedure after eight years of exposure, had an overall 40% difference in age-specific DMF surface scores compared with those of their counterparts of the baseline. Examinations for dental fluorosis were also conducted on the 1976 examinations. None of the children examined exhibited any signs of the condition.

### Significance:

Currently, about 23% of the U.S. population resides in areas that lack central water systems. These persons are deprived the benefit of community water fluoridation. School fluoridation is an alternative method of preventing dental caries in children living in such area. The present study will help determine the optimal concentration of fluoride for school water fluoridation. Currently, a level of 4.5 times the optimum is recommended.

Proposed Course:

The final examinations after 12 years of school water fluoridation were conducted in 1980. Children in all grades (1-12) had been continuously exposed to the higher fluoride level at school since the first grade. The data are currently being tabulated to determine the full effectiveness of the procedure and to compare the benefits of school water fluoridation at 7 times the optimum with those at 4.5 times, the currently recommended fluoride level.

2. Publications

A final report after 12 years of school water fluoridation at 7 times the optimum will be prepared for presentation at a scientific meeting and subsequently submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-DE-00032-12 CPR															
PERIOD COVERED October 1, 1979 to September 30, 1980		CT 0060042															
TITLE OF PROJECT (80 characters or less) Effects of chewable fluoride tablets on dental caries in school children																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 40%;">Driscoll, William S.</td> <td style="width: 30%;">Clinical Investigator</td> <td style="width: 10%;">NCP</td> <td style="width: 10%;">CPR</td> <td style="width: 10%;">NIDR</td> </tr> <tr> <td>Heifetz, Stanley B.</td> <td>Clinical Investigator</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Brunelle, Janet Ann</td> <td>Chief, B Section</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR	Brunelle, Janet Ann	Chief, B Section	NCP	CPR	NIDR
Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR													
Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR													
Brunelle, Janet Ann	Chief, B Section	NCP	CPR	NIDR													
COOPERATING UNITS (if any) Wayne County Public School System, North Carolina																	
LAB/BRANCH Caries Prevention and Research																	
SECTION Community Programs																	
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINDERS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) The study was initiated in October 1969 with 1034 children in the first and second grades of nine schools located in Wayne County, North Carolina, an area that has negligible amounts of fluoride (F) in its supplies of drinking water. Following baseline dental examinations, in which the DMF surface index was used, the children were stratified according to certain variables and then randomly assigned to one of the following three study groups: Group A (controls) chewed a placebo tablet, rinsed their teeth for 30 seconds with the resulting salivary solution, and then swallowed the material; Group B followed an identical procedure using an acidulated phosphate-fluoride (APF) tablet that contained 1 mg. F; Group C followed the same procedure as Group B except that, after at least 3 hours, the procedure was repeated with a second APF tablet that also contained 1 mg. F. The procedures were carried out each day in school under the classroom teacher's supervision for a period of six years. Interim follow-up examinations were conducted in April 1972, May 1974, September 1975 and May 1977. Final examinations were conducted in May 1979.																	



## 1. Project Description

### Objective:

To evaluate the caries-preventive effect of the daily use in school of acidulated phosphate-fluoride (APF) chewable tablets.

### Methods:

The study, a longitudinal double-blind clinical trial, was initiated in October 1969 on 1034 children attending the first and second grades of nine public schools located in Wayne County, North Carolina, an area that has negligible amounts of fluoride in its sources of drinking water. Children were stratified according to selected variables and then were randomly assigned to one of the following three study groups: Group A (controls) chewed a placebo tablet for 25 seconds, rinsed their teeth for 30 seconds with the resulting salivary solution and then swallowed the material; Group B followed an identical procedure using an APF tablet that contained 1 mg. F; Group C followed the same procedure as Group B except that, after at least 3 hours, the procedure was repeated with a second APF tablet that also contained 1 mg. F. The procedures were carried out each day in school under the classroom teacher's supervision for a period of six school years. Baseline dental examinations, using the DMF surface index, were conducted just prior to treatment initiation. Follow-up examinations were carried out during the treatment phase of the study in April 1972, May 1974 and September 1975. Examinations to evaluate post-treatment effects were conducted in May 1977 and May 1979, two years and four years, respectively, after discontinuation of treatments.

### Major Findings:

Examinations conducted in May 1977, two years after treatments had been discontinued, showed statistically significant reductions in DMFS increment for both treatment groups (B and C), compared with the controls (Group A). The two treatment procedures were about equal in effectiveness. Percentage reductions were approximately 25% for early erupting teeth (incisors and first molars), 45% for late erupting teeth (cuspids, bicuspid and second molars) and 32% for all teeth combined. These percentage figures are of the same magnitude as those found in September 1975, shortly after treatments were terminated, indicating that benefits derived during the treatment period are retained thereafter for at least a two year period. Analysis of data from the May 1979 examinations has not yet been completed. Findings thus far have clearly demonstrated that administration of fluoride tablets in school should be recommended in nonfluoridated areas as an effective and feasible public health measure for the prevention of dental caries.

Significance:

Approximately 50% of the U.S. population resides either in areas that have no central water supplies or in areas that do have central water supplies but have not implemented community water fluoridation. Because these persons are deprived of the benefits afforded by community water fluoridation, other methods of caries prevention must be developed and utilized. One method that offers considerable potential is administration of fluoride tablets to children in school. The present study has proved important in demonstrating both the efficacy and the feasibility of the procedure. By continuing to do dental examinations after treatments were terminated, valuable information with regard to retained benefits are being gained.

Proposed Course:

The examinations conducted in May 1979 were the final examinations and marked the completion of the study. A final report will be prepared and submitted for publication.

2. Publications

Driscoll, W.S., Heifetz, S.B. and Brunelle, J.A.: Treatment and Post-Treatment Effects of Chewable Fluoride Tablets on Dental Caries: Findings After Seven and One Half Years. J. Amer. Dent. Assoc. 99: 817-821, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00070 08 CPR
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PERIOD COVERED October 1, 1979 to September 30, 1980	CT 0500045
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TITLE OF PROJECT (80 characters or less) Combined self-applied fluorides for caries prevention in a non-fluoridated area
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NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
Horowitz, Herschel S.	Chief, CPS	NCP	CPR NIDR
Heifetz, Stanley B.	Clinical Investigator	NCP	CPR NIDR
Meyers, Rhea	Clinical Investigator	NCP	CPR NIDR
Driscoll, William S.	Clinical Investigator	NCP	CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP	CPR NIDR

COOPERATING UNITS (if any) Nelson County, Virginia, Public School System
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LAB/BRANCH Caries Prevention and Research
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SECTION Community Programs
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INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
<input checked="" type="checkbox"/> (a1) MINORS	<input type="checkbox"/> (a2) INTERVIEWS	

SUMMARY OF WORK (200 words or less - underline keywords)  
 Baseline dental examinations were conducted in October 1972, on approximately 2200 children (grades 1-12). All participants in grades K-6 chew daily in school under supervision an acidulated phosphate-fluoride (APF) tablet containing 1 mg. F, and swish and swallow the resulting solution. Once a week in school the same children also swish 10 milliliters of a 0.2 percent sodium fluoride solution for 60 seconds and then empty the contents of the mouth into a cup. A fluoride-containing dentifrice and toothbrushes are distributed to the same children for use at home. These combined preventive procedures will continue in the elementary schools for a minimum of ten years. Follow-up dental examinations are carried out biennially. Final examinations will be made in the fall of 1982 when all senior high school students will have participated continuously in the elementary school program since entering first grade. Children in the 7th and 8th grades of Nelson County's junior high school began to participate in the program in the fall of 1978 and 1979 respectively. High school grades will be brought into the program on an incremental basis in future years.



1. Project Description

Objective:

The purpose of the study is to determine the total effectiveness of a combination of some of the most feasible methods of self-administering fluorides in a non-fluoride area.

Methods:

In October 1972, a self-administered dental health program was started in Nelson County, Va., a fluoride-deficient community. Children in the County's 7 elementary 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. Baseline DMFS examinations were made of 2135 children in the County's elementary (grades 1-6) junior (grades 7 and 8) and senior high schools (grades 9-12). Follow-up examinations are 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.

Major Findings:

The most recent follow-up examinations that have been analyzed are those done in 1978. Those findings showed that continuous participants in grades 1-7 (ages 6-12) had 45 percent fewer DMF surfaces in 1978 than their cohorts in 1972. These children in 1978 had 85 percent fewer DMF mesiodistal surfaces than comparable children in 1972. Children in 1978 who had participated in the preventive program at any time had 37 percent fewer DMFS than their cohorts.

Significance:

Self-administered procedures, unlike traditional, professionally administered fluoride applications, can be implemented extensively with few demands on dental manpower, school personnel, facilities and financial resources. From the standpoint of optimizing dental health programs in areas where community water fluoridation is not possible, there is a compelling need to determine the impact of various combinations of feasible, self-administered methods of fluoride delivery.

Proposed Course:

Children in the 9th grade of Nelson County's high school will begin to participate in the mouthrinsing phase of the program in the fall of 1980. Extending the program into the high school will necessitate the scheduling of treatments through the spring of 1984 and the final examinations in the fall of 1985.

2. Publications

Interim findings after 2 and 4 years have been presented at scientific meetings and have been published.

Horowitz, H.S., Heifetz, S.B., Meyers, R.J., Driscoll, W.S., and Li, S. A program of self-administered fluorides in a rural school system. Community Dentistry and Oral Epid. No. 4, 1980. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00081 07 CPR										
PERIOD COVERED October 1, 1979 to September 30, 1980												
TITLE OF PROJECT (80 characters or less) Development of an anticaries vaccine												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:33%;">Bowen, William H.</td> <td style="width:33%;">Chief, CPR Branch</td> <td style="width:10%;">NCP</td> <td style="width:10%;">CPR</td> <td style="width:14%;">NIDR</td> </tr> <tr> <td>Gomez, Irma M.</td> <td>Laboratory Technician</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR	Gomez, Irma M.	Laboratory Technician	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR								
Gomez, Irma M.	Laboratory Technician	NCP	CPR	NIDR								
COOPERATING UNITS (if any)												
LAB/BRANCH Caries Prevention and Research												
SECTION Etiology												
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland												
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:										
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) <p>A colony of primates, <u>Macaca fascicularis</u>, infected with <u>Strep. mutans</u> and being fed a cariogenic diet, is being used to develop a vaccine against dental caries. Animals vaccinated with glucosyl transferase preparations continue to show protection and to harbor serum antibodies which react with GTF and other potential immunogens.</p> <p>A group of recently imported young animals was found to have high levels of serum antibody reactive with <u>S. mutans</u> although <u>S. mutans</u> was not detected in any animals using either FA or cultural techniques. The organism believed responsible for inducing antibody reactive with <u>S. mutans</u> has been isolated.</p>												



## 1. Project Description

### Objective

The purpose of this phase of the study is to determine whether monkeys can be vaccinated against dental caries by using whole bacteria administered by gastric intubation or in their drinking water. Results from earlier studies indicated that monkeys form antibodies readily when vaccinated with S. mutans.

### Methods

Twenty-one recently captured monkeys were screened for the presence of S. mutans, and for serum and salivary antibodies re-active with S. mutans. Because anti-S. mutans antibody was found, the fecal flora was examined and found to contain a microorganism which reacted with antibody in monkey serum. The animals were treated with antibiotics until the organism was eliminated from the feces and antibody levels in serum had declined. The monkeys were then vaccinated by gastric intubation with a heavy suspension of S. mutans cells which had been grown in a dialysed medium. A second group of animals was vaccinated by placing S. mutans cells in their drinking water.

### Major Findings

Caries results from these animals are not yet available. However, the isolation of a microorganism from the feces of these animals capable of inducing antibody reactive with S. mutans opens up several possibilities including the use of this microorganism as an immunogen.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00112 07 CPR
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Preclinical screening of anticaries agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Shern, Roald J.                                      Laboratory Scientist                                      NCP CPR NIDR Kingman, Albert                                      Statistician    NCP CPR NIDR		
COOPERATING UNITS (if any) American Dental Association Health Foundation, National Bureau of Standards, Gaithersburg, MD    Drs. W.E. Brown and L.C. Chow		
LAB/BRANCH Caries Prevention and Research		
SECTION Preventive Methods Development		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The principal objectives of this project are to identify antiplaque and anti-caries agents suitable for short-term clinical investigation and to develop methods for assessing these agents. Screening is conducted both <u>in vitro</u> and in animals. The <u>in vitro</u> studies include measurement of the <u>minimal inhibitory concentration</u> and <u>modification</u> of enamel dissolution rate conferred by the test agent. The animal studies measure the effects of an agent on dental caries and plaque. Two recently developed antiseptics, a piperazine derivative and a bispyridine, provided protection against dental caries and plaque similar to that provided by chlorhexidine. Methods used for plaque assessment should consider the agent being tested and the objectives of the study. The fluorescence microscopy method is particularly suitable for studies in which both plaque and caries is being measured.		

1. Project Description

Objective:

The objective of our project is to identify anticaries agents suitable for clinical testing and to improve screening methods.

Methods:

The methods of evaluation are based on the postulated properties of the agents and the therapeutic objectives sought. Most methods have been described in the NCP booklet, "Procedures for Screening Antiplaque Compounds."

In the animal studies staining, plaque, caries and fluoride uptake of rat teeth is measured following regimens of oral rinsing with test agents.

Major Findings:

Cationic antiseptics including a bisbiguanide, a piperazine and a bispyridine have been screened and found very effective against dental caries and plaque when used at low levels once or twice daily as an oral rinse.

Various plaque disclosants and scoring criteria were compared. Erythrosin, fast green and fluorescein are effective plaque disclosants. In contrast to erythrosin and fast green, fluorescein has the advantage of not staining carious lesions. The fluorescence microscopy method uses fluorescein as a disclosant and has proven to be useful in screening antiplaque and anti-caries agents. It was reliable and sensitive for measuring small amounts of dental plaque. Correlations between plaque and caries score varied emphasizing the need to consider the qualitative aspects of dental plaque.

Proposed Course:

Additional testing of the antiseptics will be influenced by the nature of proposed clinical investigations. Because dental plaques vary in their pathogenicity, plaque scores will be compared with the qualitative aspects of dental plaque such as the number of colony forming units of pathogenic bacteria per unit of plaque mass.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00113 07 CPR CT 0606075															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Short-term clinical trials of antiplaque agents																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">Shern, Roald J.</td> <td style="width: 33%;">Laboratory Scientist</td> <td style="width: 33%;">NCP CPR NIDR</td> </tr> <tr> <td>Brunelle, Janet A.</td> <td>Chief, B Section</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Leake, William C.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Kennedy, John B.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> </table>			Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR	Brunelle, Janet A.	Chief, B Section	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR	Leake, William C.	Laboratory Technician	NCP CPR NIDR	Kennedy, John B.	Laboratory Technician	NCP CPR NIDR
Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR															
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Leake, William C.	Laboratory Technician	NCP CPR NIDR															
Kennedy, John B.	Laboratory Technician	NCP CPR NIDR															
COOPERATING UNITS (if any) Department of Periodontology, School of Dentistry, U. of PA, Philadelphia, PA Drs. S.L. Yankell, P.A. Green and N. Stoller																	
LAB/BRANCH Caries Prevention and Research																	
SECTION Preventive Methods Development																	
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords)  The objectives of this project are: (1) to identify, adapt and pretest methods of measuring the bacterial and chemical composition of dental plaque and saliva and (2) to conduct short-term clinical studies of agents which might be capable of restricting dental plaque and caries. A fluoride rinse and two types of fluoride tablets provided different time courses for fluoride in the oral fluid.																	

1. Project Description

Objectives:

The objectives are twofold: to identify, adapt and pretest methods of measurement of oral responses, and to conduct short-term clinical studies of agents which might be capable of restricting dental plaque and caries.

Methods:

Most methods are described in the NCP screening booklet "Procedures for the screening of antiplaque compounds." The methods selected vary according to the objectives of the study and the agent being evaluated. In a recent study the levels of fluoride in whole saliva were assessed using the method of Gron (1968) modified for measuring microliter samples.

Major Findings:

Fluoride tablets which are allowed to dissolve slowly in the mouth confer much higher fluoride levels than those provided by tablets that are chewed, swished and swallowed. However, slow-dissolving tablets fail to yield homogeneous fluoride levels within the mouth.

Significance:

Short-term clinical testing provides assurance that agents are safe and potentially effective prior to commitment to long-term testing in large groups of subjects. This project is to conduct these short-term trials and to develop and rigorously evaluate techniques for measuring parameters associated with safety and effectiveness.

Proposed Course:

A short-term study of an antiplaque agent will be done pending receipt of an IND number by the sponsor of the agent.

2. Publication

Yankell, S.L., Paskow, G.W., Rann, R.M., Green, P.A., and Shern, R.J. Evaluation of tiodonium chloride as an antiplaque and anticaries agent. V. Effects on plaque microbiology and plaque and saliva pH. Journal of Pharmacology and Therapeutics in Dentistry, 4:73-80, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00147-06 CPR									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less)  Lectins in the study of plaque and caries development											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">Mirth, Dale B.</td> <td style="width: 33%;">Laboratory Scientist</td> <td style="width: 33%;">NCP CPR NIDR</td> </tr> <tr> <td>Adderly, Donna D.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> </table>			Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR	Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR									
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR									
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR									
COOPERATING UNITS (if any)											
LAB/BRANCH Caries Prevention and Research											
SECTION Preventive Methods Development											
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Lectins, which are proteins capable of interacting with certain macromolecules and/or cell types via specific sugar moieties, are being used to investigate the interactions between saliva and/or bacteria in order to better elucidate the role these interactions play in plaque and caries development. Findings to date support the conclusion that 4 lectins, wheat germ agglutinin, concanavalin A, fucose binding protein and soybean agglutinin, can reversibly bind to and inactivate by complexation and/or precipitation the aggregating factor in saliva that is responsible for inducing the aggregation of <u>Streptococcus mutans</u> cells. These results provide evidence that the salivary aggregating factor contains <u>N-acetyl-D-glucosamine (GlcNAc)</u> , D-mannose and/or D-glucose, L-fucose and <u>N-acetylgalactosamine (GalNAc)</u> and/or D-galactose (D-Gal). Lectins specific for <u>D-Gal</u> , <u>D-GlcNAc</u> , and <u>D-GalNAc</u> have been the most effective for the direct aggregation of oral bacteria.											



1. Project Description:

Objective:

Lectins, which are naturally occurring proteins capable of interacting with certain macromolecules and/or cell types via specific sugar moieties, are being used to investigate the interactions between saliva and oral bacteria in order to elucidate the nature of the substances responsible for these interactions and the role they play in plaque formation and caries development. Lectins are also being assayed for direct effects on oral bacteria.

Methods:

The ability of saliva to induce the aggregation of various strains of Streptococcus mutans and the effect of lectins on this process was determined using a spectrophotometric assay procedure. Aggregation was detected by measuring the decrease in absorbance at 700 nm over 2 hours of a suspension of S. mutans mixed with saliva samples that had been treated with various concentration of lectin and comparing this to the change in A<sub>700</sub> of control suspensions of S. mutans.

Standard immunization techniques were used to immunize rabbits with lectin-induced precipitates from saliva in order to produce antibodies to the salivary aggregating factor for S. mutans.

The ability of lectins to aggregate various oral bacteria was determined using standard microtiter techniques.

Major Findings:

Several lectins have been found that can aggregate various strains of oral bacteria. The amount of aggregation was influenced by the media in which the cells were grown.

Lectins specific for D-galactose, N-acetyl-D-glucosamine, and N-acetyl-D-galactosamine displayed the greatest aggregating activity. The lectins Ricinus communis-60 and -120, Abrus precatorius, and wheat germ agglutinin produced the greatest amount of aggregation. The lectin-induced aggregation could be inhibited by the sugars for which the lectins were specific, indicating that sugar specific binding was involved in the aggregation process.

Significance:

This investigation has shown that lectins are useful for elucidating the structure of salivary aggregating factors and cell surfaces. Earlier findings that at least 4 lectins can precipitate the salivary aggregating factor for S. mutans and the observation that the lectin-aggregating factor interactions can be reversed by the sugars for which the lectins are specific, suggest that these lectins could be useful for purifying salivary aggregating factors by precipitation and/or affinity chromatography. The development of a purified anti-aggregating factor antiserum could lead to a convenient methods of assaying saliva for aggregating activity by rocket immunoelectrophoresis or similar techniques. The finding that lectins can aggregate oral bacteria suggests that lectins will be useful in elucidating the cell surface of these microorganisms and may lead to the development of a therapeutically useful lectin.

These studies will help to elucidate the role of saliva-induced bacterial aggregation in plaque and caries preventive measures.

Proposed Course:

The effect of lectins on in vitro and/or in vivo plaque development will be investigated.

2. Publications:

Mirth, D.B., Miller, C.J., Kingman, A., and Bowen, W.H.: Binding of salivary aggregating factors for Streptococcus mutans by concanavalin A and fucose-binding protein. Caries Research. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00154 06 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Biochemical product and energy requirements of plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Kemp, Christopher	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Emilson, Claes-Goran	Laboratory Scientist (vis.)	NCP CPR NIDR
Sharer, Sue Ann	Laboratory Assistant	NCP CPR NIDR
Barnes, Peter A.	Laboratory Aide	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of antiseptics on dental plaque samples obtained from monkeys has been evaluated using conventional microbiologic as well as adenine nucleotide analysis. Consistent interpetations of the data were obtained from both methods. When the viable counts were normalized for the mass of the sample, a persistent reduction in viable count was noted after use of Octenidine. The persistent effect was supported by calculation of adenylate energy charge following analysis of the samples for three adenine nucleotides.



1. Project Description

Objective:

The objective for this project is to develop essential assays for measures of metabolism in mixed bacterial systems. Though specifically designed for the analysis of dental plaque associated with caries, these assays potentially have wide use in studying other diseases and in enhancing basic understanding of microbial ecology.

Methods:

Conventional microbiological plate counts were used in this study as well as the adenine nucleotide analyses. The adenine nucleotide analyses were first used in this laboratory to estimate the viable mass in dental plaque and have been described in previous reports. In addition, we have explored the possibility of using adenylate energy charge (AEC) because this ratio of adenine nucleotides is more stable in the presence of external nutrients for cellular storage products than is cellular content of ATP. Adenylate energy charge is defined as  $AEC = \frac{ATP + 1/2 ADP}{ATP + ADP + AMP}$ . We found that when a culture of bacterial cells was deprived of nutrients or heat treated the drop in AEC coincided with the reduction in viable count of the suspension.

In collaboration with Dr. Emilson, we tested the use of these assays in vivo. Dental plaque samples were obtained from monkeys which have been treated with a variety of antiplaque agents. The monkeys were maintained on a high sugar diet and treated with different antiplaque agents over a period of six months. A plaque index was obtained and organisms have been enumerated by conventional microbiological methods. The ratio of actinomyces and streptococci were determined using fluorescent antibody analysis. The plaque samples were analyzed for the content of the individual adenine nucleotides and for the AEC value.

The effects of chlorhexidine, was compared to that of a bis-pyridine compound supplied by Cooper Laboratories and Octenidine supplied by Sterling Winthrop. Plaque index and viability of the samples were compared for samples obtained before and after application of each test agent. Viability of the samples were assessed using conventional bacteriological plate counts as well as extractable ATP. In addition, a comprehensive adenine nucleotide analysis was performed on each sample and the energy charge calculated from these data.

Major Findings:

A comparison of the bacterial counts before and after treatment revealed no more information than did plaque scores and showed that plaque had been removed by all three agents. When the data normalized, however, it is the viability of the samples increased following treatment with chlorhexidine and the Cooper substance and decreased after treatment with Octenidine. All agents removed accumulated plaque associated with some fraction of dead bacteria. New plaque growth, therefore, had a large fraction of viable cells. The viability of the samples was reduced following treatment with Octenidine indicating a persistent effect on the growth of new microorganisms following removal of the plaque mass. These effects were consistent when analyzed by either specific viable count or by specific ATP content showing that the simpler chemical analysis was a reliable measure of the effect. The effects were confirmed by energy charge calculations following extraction and analysis of three adenine nucleotides.

Adenylate energy charge (AEC) values for the plaque samples were difficult to obtain because of sample size limitations and the number of controls needed to monitor the analysis. However, the results obtained by AEC, ATP analysis and viable counts were the same. The energy charge data proved valuable in confirming the effects observed from specific viable counts and ATP analysis.

Significance:

Determination of the relative effectiveness of alternative antimicrobial and antiplaque agents is an important objective of the National Caries Program. Currently one can measure the fraction of cells in plaque that remain alive only by tedious plating and colony counting procedures. Development of techniques to measure the effect of agents on viable cell mass would be a major step forward for the Program.

Proposed Course:

In collaboration with Dr. Emilson we will continue assessing the relative value under in vivo condtions of alternative chemical methods of assaying important dental plaque parameters.

2. Publications

Robrish, S.A., Kemp, C.W., Chopp, D.E., and Bowen, W.H. Viable and total cell masses in dental plaque as measured by bioluminescence methods. Clin. Chem. 25:1649, 1979.

Kemp, C.W., Robrish, S.A., and Bowen, W.H. An interactive computer program for the computation of adenylate energy charge. Clin. Chem., 25:1653, 1979.

Kemp, C.W. Adenylate energy charge; a method for the determination of viable cell mass in dental plaque samples. J. Dent. Res Special Issue D, Nov. 1979.

Robrish, S.A., Emilson, C.G., Kemp, C.W., Eberlein, D. and Bowen, W.H. The Effects of Three Antiseptics on the Dental Plaque From Monkeys as Measured by Viable Counts and Adenine Nucleotide Analysis. In preparation. 1980.

Kemp, C.W., Robrish, S.A., Eberlein, D., Emilson, C.G. and Bowen, W.H. A Comparison of Two Methods of Dental Plaque Collection. In preparation. 1980.

Emilson, C.G., Bowen, W.H., Robrish, S.A., and Kemp, C.W. Effect of the Antibacterial Agents Octenidine and Chlorhexidine on the Plaque Flora in Primates and Effect of the Antibacterial Agent Octenidine on the Plaque Flora in Primates. Presented at Animal Caries Models Workshop, Sturbridge, MA. April 20-23, 1980. Proceedings in press 1980.

Emilson, C.G., Bowen, W.H., Ciardi, J.E., Robrish, S.A., Kemp, C.W., and Kingman, A. Effect of Antimicrobial Agents on Dental Plaque and Microflora in Rats and Primates. Submitted for publication 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00190 05 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Extracellular macromolecules and virulence of cariogenic streptococci

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR	NCP CPR NIDR
Reilly, J. Allen	Laboratory Technician	NCP CPR NIDR
Lee, Terry	Laboratory Technician	NCP DPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research Branch

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Glucosyltransferases (GTF) and lipoteichoic acids (LTA) produced by S. mutans are implicated in the formation of cariogenic dental plaque. Caries vaccine studies have indicated that protection against S. mutans is associated with antibody to GTF, but vaccines also contain, at least LTA and/or dextranase. Immunization experiments using gnotobiotic rats suggest more protection with a vaccine of LTA/dextranase deficient in GTF than with one enriched for GTF. For further immunization studies, GTF has been purified free of LTA from a mutant of 6715 that apparently lacks dextranase activity. GTF, sucrase, and dextranase activities and LTA have been measured in monkey dental plaque. Highly purified LTA and antibodies produced against it are being used to study the role of LTA in S. mutans dental plaque formation. These studies are designed to gain insight into the biochemical and immunological aspects of dental caries which may lead to a rational approach for the control of this disease.

## 1. Project Description

### Objectives:

Several extracellular macromolecules synthesized by Streptococcus mutans have been implicated in cariogenic dental plaque formation. Among these are the glucosyltransferases (GTF), fructosyltransferases (FTF), dextranases and lipoteichoic acids (LTA). The present study proposed: 1) To determine the mechanisms by which these macromolecules are involved in the formation of cariogenic dental plaque; 2) To characterize them both biochemically and immunologically; and, 3) To test their effectiveness as vaccines for protection against infection by Streptococcus mutans.

### Methods Employed:

Glucosyltransferase and fructosyltransferase activities are assayed by measuring the incorporation of  $^{14}\text{C}$ -glucosyl or  $^{14}\text{C}$ -fructosyl labeled sucrose into ethanol insoluble polysaccharides. Dextranase activity is measured by the release of reducing sugar from dextran T2000. The amount of LTA is determined using specific antisera to LTA in conjunction with the passive hemagglutination and rocket immunoelectrophoresis methods. Antibody activities to the above enzymes are measured as the amount of functional inhibition of enzyme activity. Antibody activity to LTA is measured as the maximum titer that causes agglutination of LTA-coated RBC's.

### Major Findings:

Our previous studies in rats and monkeys have shown a correlation between immunization with extracellular GTF preparations from S. mutans strain 6715 and decreased S. mutans colonization and/or dental caries. However, it can not be concluded that GTF is the protective immunogen because the vaccines were found to contain, at least, lipoteichoic acid and/or dextranase. In an immunization study with gnotobiotic rats we also found a greater decrease in colonization of S. mutans after administration of LTA/dextranase enriched preparations than with preparations enriched with GTF. Recently we found a mutant of 6715 (see previous report) that appeared to make only water-soluble glucans and not to have extracellular dextranase activity. However, rabbit antiserum formed to the extracellular enzyme preparation inhibited, by greater than 90%, both water-insoluble glucan formation and dextranase activity by strain 6715. This latter result suggested either that inactive dextranase was present in the vaccine or that antibody to GTF inhibits dextranase activity closely associated with GTF in a molecular complex. To obtain a GTF preparation free of dextranase and LTA, to test as an anticaries vaccine, the extracellular GTF has been purified to high specific activity by molecular sieving, ethanol precipitation, affinity chromatography and hydrophobic chromatography. Characterization of the purified preparation is in progress.

Dental plaque fluid from monkeys have been examined for bacterial products implicated in plaque and caries formation. Dextranase, sucrase and GTF activities have been measured. LTA was detected by a modified rocket immunoelectrophoresis method (see Last year's report).

Another mutant of strain 6715 defective in glucan synthesis was found to produce an increased amount of extracellular dextranase. The purification and characterization of this dextranase will be reported under project Z01 DE 00302 01 CPR. Three different animal isolates of S. mutans 6715 were found to make different amounts of insoluble glucan. Dextranase activity in these strains was inversely proportional to the total GTF activity.

LTA has been highly purified from S. mutans strain BHT. The preparation produced rabbit antibody of high titer only when both methylated bovine albumin and complete Freund's adjuvant were present in the vaccine. The high degree of immunological cross reactivity seen among the LTAs of oral streptococci, lactobacilli and staphylococci make LTA an unlikely candidate for a specific vaccine to protect against S. mutans infection. However a specific antibody to LTA might help to determine the importance of LTA in the colonization of S. mutans in dental plaque.

Antibody response to GTF and LTA was measured in eight humans before and after swallowing large doses of S. mutans formalin killed cells in enteric coated capsules. Serums (20) and salivas (22) were collected from each subject over a 12 month period and tested for antibody to GTF by functional inhibition of enzyme activity and to LTA by passive HA titer. Dramatic effects were not observed. In a few human subjects the results suggested a correlation between functional inhibition of GTF activity or LTA titer and immunization with S. mutans. Statistical evaluation of these results are in progress.

#### Proposed Course:

Further studies on the effect of immunization of monkeys and gnotobiotic and conventional rats with purified macromolecules will be performed. Various routes of administration of immunogens will be evaluated.

Studies with mutant strains of cariogenic streptococci deficient in the ability to synthesize extracellular polymers implicated in virulence will be continued in order to elucidate the roles of these polymers in the formation of cariogenic dental plaque.

GTF, LTA, dextranase will be measured in monkey and human dental plaque samples to determine if a relationship exists between the amount of these S. mutans products and extent of dental caries.



Significance to Program:

A more thorough understanding of the mechanisms by which GTF, FTF, LTA and dextranase are involved in the formation of cariogenic dental plaque will lead to the discovery of more effective means of prevention. The development of a safe and effective vaccine against dental caries, comprised of well-defined purified molecules would be well accepted by the population in general and would be instrumental in the control and prevention of this disease.

2. Publications

Ciardi, J.E., Reilly, J.A. and Bowen, W.H. Inhibition of sodium fluoride of  $^3\text{H}$ -Glycerol incorporation into lipoteichoic acid in Streptococcus mutans. Caries Research 14:24-31, 1980.

Rolla, G., Oppermann, R.V., Bowen, W.H., Ciardi, J.E. and Knox, K.W. High Amounts of lipoteichoic acid in sucrose-induced plaque in vivo. Caries Research 14:235-238, 1980.

Ciardi, J.E., Rosenthal, A.B., and Bowen, W.H. Rapid quantitative determination of the effect of antiplaque agents and antisera on the growth, acid production and adherence of Streptococcus mutans. In Press.

Emilson, C.G., Bowen, W.H., Ciardi, J.W., Robrish, S.A. and Kemp, C.W. Effect of antimicrobial agents on dental plaque and microflora in rats and primates. In J. Tanzer (ed). Symposium and Workshop in Animal Models in Cariology. Information Retrieval, Inc., Washington, D. C. In Press.

## PERIOD COVERED

October 1, 1979 to September 30, 1980

## TITLE OF PROJECT (80 characters or less)

Adherence and coherence of cariogenic streptococci in dental plaque

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ciardi, Joseph E.	Laboratory Scientist	NCP	CPR	NIDR
Olsson, Jan	Laboratory Scientist(vis.)	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR
Reilly, John Allen	Laboratory Technician	NCP	CPR	NIDR
Lee, Terry	Laboratory Technician	NCP	CPR	NIDR
Haller, Robert	COSTEP (Dental)	NCP	CPR	NIDR

## COOPERATING UNITS (if any)

Dr. Gunnar Rolla, University of Oslo, Norway  
Dr. Claes-Goran Emilson, University of Goteborg, Sweden

## LAB/BRANCH

Caries Prevention and Research

## SECTION

Etiology

## INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

## TOTAL MAN-YEARS:

## PROFESSIONAL:

## OTHER:

## CHECK APPROPRIATE BOX(ES)

 (a) HUMAN SUBJECTS (b) HUMAN TISSUES (c) NEITHER (a1) MINORS  (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms by which oral streptococci interact with teeth, saliva and with each other to form cariogenic dental plaque are under investigation. Significant differences have been observed in the aggregation and adsorption to hydroxylapatite (HA) surfaces of S. mutans serotypes b, c, and d cells with salivas obtained from humans, monkeys and rats. The different salivas all inhibited sucrose-mediated adherence to glass of each of the three cell types in a similar manner. A method using a HA coated glass rod has been developed to measure the substantiveness of potential cariostatic agents on a saliva pellicle formed on the hydroxylapatite; the effects of each test agent on growth, acid production and sucrose-mediated adherence of S. mutans have been determined. HA adsorption patterns in the presence of sucrose of S. mutans mutants defective in glucan synthesis did not correlate with their decreased sucrose-mediated adherence to glass surfaces. Interactions of LTA, hydroxylapatite, sucrose and oral streptococci suggest a role for LTA in bacterial colonization to teeth.

## 1. Project Description

### Objectives:

This project is designed to elucidate the mechanisms by which oral streptococci adhere to teeth and to each other in the formation of cariogenic dental plaque in order to find effective means to control dental caries. The information obtained from in vitro studies on adherence of bacteria to various smooth surfaces and the effects of oral secretions and cariostatic agents on adherence will eventually lead to the formulation of relevant in vivo experiments.

### Methods Employed:

Methods used to measure the adherence of oral bacteria to solid surfaces and to each other are: (1) Sucrose-mediated adherence of radiolabeled bacteria to the walls of glass rods and test tubes in the presence and absence of saliva. (2) The adsorption of radiolabeled bacteria and bacterial products to hydroxylapatite in the presence and absence of saliva. (3) The rate of agglutination by sucrose of bacterial cells determined by changes in absorbance at 400nm. (4) The rate of aggregation by saliva of bacterial cells determined by changes in absorbance at 700nm.

### Major Findings:

Rats and monkeys have been used as models to study dental caries caused by S. mutans. The present study was carried out to assess and compare the influence of human, monkey and rat saliva on the aggregation of and the adsorption to hydroxylapatite (HA) of S. mutans serotypes b (BHT), c (Ingbritt) and d (OMZ-65) cells. Preliminary results with human and rat salivas were reported last year. Significant differences have now been found among human, monkey and rat salivas in their abilities to aggregate and support adsorption to hydroxylapatite of S. mutans serotypes b, c, and d cells. All saliva samples decreased cell adsorption to HA. Human and rat saliva, but not monkey saliva favored adsorption of serotype c cells. Only human and rat salivas caused aggregation of c cells; type b and d cells were not aggregated. (Ciardi, J.E., McAllister, P.F., and Bowen, W.H. Interaction of Human, Monkey, and Rat Saliva with S. mutans, J. Dent Res. 59A: Abs 678, p. 437, 1980). In experiments which measured growth and sucrose mediated adherence of bacterial cells to glass surfaces approximately 40%, 85% and 93% of the total cell yields of serotype b, c, and d cells adhered, respectively. Inhibition of adherence of each cell type was very similar on the addition of up to 48% of human, monkey or rat saliva in the assay system. In most instances S. mutans growth was stimulated by the presence of saliva.

The method, to quantitate the effects of cariostatic agents on growth, acid production and sucrose-mediated adherence of S. mutans has been modified to allow the substantiveness of a test agent in saliva pellicle to be assessed. The glass rod is uniformly coated with hydroxylapatite and the



entire system treated with test saliva to produce a pellicle on the hydroxylapatite. Experiments are in progress to test the retention and antibacterial/antiplaque activity of several biological and chemical agents possessing a cariostatic potential. The results of adsorption of non-growing radiolabeled bacterial cells to untreated and saliva-treated hydroxylapatite coated rods are very similar to results using non-supported hydroxylapatite powder or beads.

A technique has been developed to assess the role of cell-associated glucosyltransferase on the adsorption of S. mutans cells to hydroxylapatite beads in the presence and absence of sucrose. Cell-associated enzyme activity was determined by measuring the amount of radiolabeled glucans formed by washed, non-growing cells after incubation with  $^{14}\text{C}$ -glucosyl-labeled sucrose. For hydroxylapatite adsorption studies the bacteria were made radioactive by growing them in media containing  $^3\text{H}$ -thymidine and glucose as carbohydrate source. The  $^3\text{H}$ -labeled bacteria were percolated upward through a moving suspension of hydroxylapatite beads. In some experiments sucrose was added to the cell suspension under conditions that do not cause agglutination. S. mutans 6715 and three mutants defective in glucan synthesis and with decreased ability to adhere to glass surfaces in the presence of sucrose were tested in the present study. Previous results which showed that the mutant cells could be rendered adherent to glass by pretreating them with an extracellular GTF preparation from the parent strain suggested that the mutants were deficient in cell associated GTF. However, experiments with  $^{14}\text{C}$  glucosyl-labeled sucrose and washed cells suggest that the mutants all possess substantial amounts of cell-associated GTF; two of the mutants synthesized more  $^{14}\text{C}$ -glucan than did the parent strain. HA adsorption experiments using the method described above show that in the absence of sucrose, all three mutants adsorbed to HA to a higher degree than parent cells. In the presence of sucrose, adsorption of parent and two mutant cell types were enhanced; however, adsorption of the third mutant was decreased.

Lipoteichoic acid has been implicated in the attachment and colonization of oral streptococci on tooth surfaces. Previous results showed that human dental plaque, which contains large amounts of polysaccharide formed in the presence of sucrose also contained significant concentrations of LTA. Our recent results of experiments on the interactions among LTA, hydroxylapatite and oral streptococci support a role for LTA in bacterial adherence to tooth surfaces. However, sucrose-mediated adherence of S. mutans to glass surfaces which is completely inhibited by antibody to glucosyltransferase is not significantly affected by antibody to LTA.

#### Significance:

A more thorough understanding of the mechanism by which oral streptococci adhere to solid surfaces, to each other and to other oral bacteria and the influence of oral secretions on these interactions will lead to the discovery and use of more effective inhibitors of dental plaque formation. Accurate and reproducible methods for assaying adherence and coherence of cariogenic streptococci will prove useful in testing potential inhibitors of plaque formation.

Proposed Course:

The results of the in vitro assay systems employed in the present studies have contributed much to our knowledge of the interactions between oral bacteria, solid surfaces and animal saliva. Experiments using these systems will be carried out to examine more closely the interactions between specific components from animal saliva or from oral bacteria and bacterial cells. Analysis of dental plaque and in vitro experiments which result in "artificial plaque" formation will be carried out to determine whether lipoteichoic acid-glucan complexes render dental plaque more cariogenic. Our in vitro assay methods that measure growth, adherence and acid production by cariogenic bacteria will continue to be used to test substances with a cariostatic potential.

2. Publications

Ciardi, J.E., Reilly, J.A. and Bowen, W.H. Inhibition of Sodium Fluoride of <sup>3</sup>H-Glycerol Incorporation into Lipoteichoic Acid in Streptococcus mutans. Caries Research 14: 24-31 (1980).

Rolla, G., Oppermann, R.V., Bowen, W.H., Ciardi, J.E. and Knox, K.W., High Amounts of Lipoteichoic Acid in Sucrose-Induced Plaque in vivo. Caries Research 14: 235-238. (1980).

Ciardi, J.E., Rosenthal, A.B. and Bowen, W.H. Rapid Quantitative Determination of the Effect of Antiplaque Agents and Antisera on the Growth, Acid production and Adherence of Streptococcus mutans. J. Dent. Research. In press.

Emilson, C.G., Bowen, W.H., Ciardi, J.E., Robrish, S.A. and Kemp, C.W., Effect of Antimicrobial Agents on Dental Plaque and Microflora in Rats and Primates. In J. Tanzer (ed). Symposium and Workshop in Animal Models in Cariology. Information Retrieval, Inc., Washington, D. C. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00206-04 CPR

PERIOD COVERED

October 1, 1979 to September 30, 1980

CT-0600118

TITLE OF PROJECT (80 characters or less)

Effect of daily and weekly rinsing with sodium fluoride solutions in a non-fluoridated area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Heifetz, Stanley B.  
Meyers, Rhea  
Kingman, Albert

Clinical Investigator  
Clinical Investigator  
Statistician

NCP CPR NIDR  
NCP CPR NIDR  
NCP CPR NIDR

COOPERATING UNITS (if any)

Biddeford School Department, Biddeford, Maine

LAB/BRANCH

Caries Prevention and Research

SECTION

Community Programs Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS

(b) HUMAN TISSUES

(c) NEITHER

(a1) MINORS  (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1976, a sodium fluoride (NaF) mouthrinse study was started in Biddeford, Maine, a non-fluoride area. Baseline dental examinations (DMFS Index) were made of 825 children in grades 5-7 attending seven schools in the community. Participants were randomly divided into three groups. Under teacher supervision, they rinsed either weekly with a 0.2% NaF solution or a 0.1% sodium chloride solution (Placebo) or daily with a 0.05% NaF solution. Treatments were carried out for three school years. Follow-up dental examinations were scheduled annually to compare the anti-caries effectiveness of the two fluoride mouthrinse procedures. The third and last year of treatments and final examinations were carried out in 1979.



## 1. Project Description

### Objective:

To compare the effectiveness of weekly rinsing with 0.2% sodium fluoride (NaF) solution and daily rinsing with a 0.05% NaF solution in a non-fluoride area.

### Methods:

In 1976, parental consent to participate in the F mouthrinse study was obtained for 825 students in grades 5-7 attending seven schools in Biddeford, Maine, a non-fluoride area. Baseline caries prevalence was registered and participants were randomly divided into three comparable study groups: Group 1 rinses weekly with a placebo solution containing 0.1% sodium chloride; Group 2 rinses weekly with a 0.2% NaF solution; and Group 3 rinses daily with a 0.05% NaF solution. The mouthrinse procedures are carried out in school under teachers' supervision. Treatments will extend over a period of three school years. Follow-up dental examinations are conducted annually for a period of three years.

### Major Findings:

Although findings after one and two years of study showed lower incremental caries rates for daily as compared with weekly fluoride rinsing, the differences were not statistically significant.

### Significance:

Studies have shown that both fluoride mouthrinse procedures can reduce the incidence of dental decay. However, there is insufficient evidence to determine if one regimen of fluoride mouthrinsing is clearly more effective than the other. Information on the comparative benefits of the two procedures will be helpful to school and health officials interested in implementing a school mouthrinse program.

### Proposed Course:

The last year of treatments and three-year follow-up examinations were carried out in 1979. The data are currently being tabulated and analyzed.

## 2. Publications

Interim one-and two-year reports have been presented at scientific meetings. A final report suitable for publication, will be prepared.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01-DE-00220-04 CPR																				
PERIOD COVERED October 1, 1979 to September 30, 1980		CT 0060121																				
TITLE OF PROJECT (80 characters or less) Comparison of daily and weekly rinsing with sodium fluoride in a fluoridated community																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0"> <tr> <td>Driscoll, William S.</td> <td>Clinical Investigator</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Swango, Philip A.</td> <td>Project Scientist</td> <td>NCP</td> <td>CRGC</td> <td>NIDR</td> </tr> <tr> <td>Horowitz, Alice M.</td> <td>Public Health Educator</td> <td>NCP</td> <td>OAD</td> <td>NIDR</td> </tr> <tr> <td>Kingman, Albert</td> <td>Statistician</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR	Swango, Philip A.	Project Scientist	NCP	CRGC	NIDR	Horowitz, Alice M.	Public Health Educator	NCP	OAD	NIDR	Kingman, Albert	Statistician	NCP	CPR	NIDR
Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR																		
Swango, Philip A.	Project Scientist	NCP	CRGC	NIDR																		
Horowitz, Alice M.	Public Health Educator	NCP	OAD	NIDR																		
Kingman, Albert	Statistician	NCP	CPR	NIDR																		
COOPERATING UNITS (if any) Des Moines Independent Community School District, Iowa																						
LAB/BRANCH Caries Prevention and Research																						
SECTION Community Programs																						
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																						
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) <p>The study was initiated in September 1977 with 1000 children in the seventh grade of nine junior high schools located in Des Moines, Iowa, a community that has optimal amounts of fluoride in its supply of drinking water. The children were randomly assigned to one of the following three study groups: Group I (controls) rinsed their mouths once every week in school for 60 seconds with a placebo solution; Group II followed an identical procedure using a 0.2% neutral sodium fluoride solution (0.09%F). Group III rinsed their mouths once every day in school for 60 seconds using a 0.05% neutral sodium fluoride solution (0.023%F). The procedures were carried out under the classroom teacher's supervision for a period of three years. Baseline dental examinations, using the DMF surface index, were conducted in November 1977. An interim, follow-up examination was conducted in April 1979 and the final examination was conducted in May 1980.</p>																						

## 1. Project Description.

### Objective:

To compare the caries-inhibiting effect of weekly rinsing with a 0.2 percent sodium fluoride solution (0.09%F) and daily rinsing with a 0.05 percent sodium fluoride solution (0.023%F) in children residing in an optimally fluoridated community.

### Methods :

The study, a longitudinal double-blind clinical trial, was initiated in September 1977, with 1000 children in the seventh grade of nine junior high schools located in Des Moines, Iowa, a community that has optimal amounts of fluoride in its supply of drinking water. The children were randomly assigned to one of the following three study groups: Group I (controls) rinsed their mouths once every week in school for 60 seconds with a placebo solution; Group II followed an identical procedure using a 0.2% neutral sodium fluoride solution (0.09%F); Group III rinsed their mouths once every day in school for 60 seconds using a 0.05% neutral sodium fluoride solution (0.023%F). The procedures were carried out for a period of three years under the supervision of the classroom teachers. Baseline dental examinations, using the DMF surface index, were conducted in November 1977. An 18-month follow-up examination was conducted in April 1979 and a final examination, after 30 months, was conducted in May 1980.

### Major Findings:

Findings after 18 months showed that both weekly and daily fluoride mouthrinsing provided significant caries-preventive benefits in addition to those already accrued from consuming optimally fluoridated drinking water. Percentage reductions in mean DMFS increment were about 31 percent for the weekly group and 40 percent for the daily group, compared with the controls. The difference in effectiveness between the weekly procedure and the daily procedure was not statistically significant. Both procedures appeared to be effective in reducing dental caries incidence in all types of tooth surfaces, although the small increments occurring during the 18-month period in buccolingual and mesiodistal surfaces provided only a limited opportunity to demonstrate a cariostatic effect in those surfaces. Analysis of data from the final examination has not yet been completed.

### Significance:

Although the practice of water fluoridation makes a sizable inroad into the widespread problem of dental decay that exists in most communities, complementary public health measures must be developed to prevent the dental decay that remains despite fluoridation. Rinsing in school with a fluoride solution is a logical choice for investigation, because it is simple to carry out and is inexpensive. Information gained from this study will be helpful in determining whether fluoride mouthrinsing programs should be recommended and promoted for wide scale use in optimally fluoridated communities.



Proposed Course:

The examinations conducted in May 1980 were the final examinations and marked the completion of the study. A report of 18-month findings has been submitted for publication. A final report will be prepared and submitted for publication after the data analysis has been completed.

2. Publications

Driscoll, W.S., Swango, P.A., Horowitz, A.M. and Kingman, A.:  
Caries-Preventive Effects of Daily and Weekly Oral Rinsing with Sodium  
Fluoride Solutions in an Optimally Fluoridated Community: Findings  
after Eighteen Months. J. Amer. Dent. Assoc. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00222 04 CPR									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less) Specific and non-specific immune factors in plaque fluid and saliva											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">Cole, Michael F.</td> <td style="width: 33%;">Laboratory Scientist (vis.)</td> <td style="width: 33%;">NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Hsu, Su-Cheng D.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> </table>			Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR	Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR									
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR									
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR									
COOPERATING UNITS (if any) National Institute of Aging - Dr. B. Baum											
LAB/BRANCH Caries Prevention and Research											
SECTION Preventive Methods Development											
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)  <p>The free aqueous phase was obtained from individual samples of dental plaque and the plaque matrix was then eluted with a chaotropic buffer in an attempt to remove bound protein. The fluid and the chaotropic phases were assayed for secretory immunoglobulin A (SIgA), IgG, IgM, the third component of complement (C'3), lysozyme, lactoperoxidase and lactoferrin. The presence of these specific and non-specific immune factors in the free and bound phases suggest they are important in host defense at the plaque-enamel interface.</p>											

## 1. Project Description

### Objective:

The purposes of this study are to measure specific and non-specific defense mechanisms in dental plaque and saliva and to determine their role in host defense at the enamel plaque interface.

### Methods:

Supragingival plaque free of visible blood was centrifuged at high speed to obtain the free fluid phase. The plaque was then washed with neutral pH buffer until no protein remained. The plaque was then treated with a chaotropic buffer in order to elute proteins bound to the matrix. The proteins were measured by single radial diffusion, rocket immunoelectrophoresis, solid phase fluoroimmunoassay and spectrophotometry.

### Major Findings:

The levels of the specific and non-specific immune factors found in plaque fluid suggest that predominantly saliva and, to a lesser extent, gingival exudate contribute to the protein pool. Significant levels of these immune factors were bound to the plaque matrix suggesting that they participate in host defense at the enamel plaque interface.

### Significance:

Despite extensive studies of the composition of saliva in caries active and caries free subjects, it has not been possible to correlate the concentration or activity of any salivary protein with resistance or susceptibility to dental caries. This observation may suggest that the ability of host defense factors to control dental caries depends more on their concentration and ability to retain biological activity in dental plaque than in their concentration and activity in saliva.

The study of the qualitative and quantitative composition of plaque fluid in populations with high and low caries experience could help in understanding the mechanisms by which the host protects the tooth surface.

### Proposed Course:

1. Design in vitro systems to study the interactions of these proteins.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00225 04 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Cost analysis of implementing school-based community mouthrinse programs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Brunelle, Janet Ann Miller, Ann J.	Chief, B Section Project Scientist	NCP NCP	CPR CPR	NIDR NIDR
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COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Biometry

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on costs of administering the program, student participation and caries experience were collected from seventeen communities throughout the U.S. and Guam which conducted three-year demonstrations of school-based mouthrinse programs in grades K-6/8. An additional three years of data was collected from five programs which later extended the regimen into the Junior and Senior High Schools. Analysis of implementation costs, acceptance of community and cost-effectiveness will be performed during and after the total period.

## 1. Project Description

### Objective

The objective of the project is to obtain detailed data on the cost of implementing a school-based mouthrinse program in a variety of community settings.

### Methods

Data on costs of personnel, consummable materials, permanent equipment and building overhead were collected from 17 sites in the United States and Guam where school-based mouthrinsing programs were being conducted in the elementary grades and from 5 sites where these programs had been extended into the secondary levels, grades 7/9 through 12. Participation statistics from consent forms, attendance and drop-out records were also collected and evaluated.

### Findings

Participation in the elementary classes was high throughout the four school years. Of the total eligible population, an average of 83% gave consent to participate in the mouthrinse programs. Some communities increased their percent participation as programs continued. The highest levels of participation were, as expected, in the lower grades with the rates declining into the upper grades. This pattern held in the high school programs where overall participation rates ranged from 23% to 77% at the five study sites.

The real costs for supplies and equipment only, averaged less than 50¢ per child per year for the seventeen sites. Personnel costs ranged from zero to almost nine dollars with an average of approximately three dollars for all programs. The differences in administrative levels used to supervise, deliver or dispense materials account for the wide range in personnel costs. The high school costs for personnel will probably be slightly higher than for elementary grades as most of the five sites hired extra personnel for these programs. Cost-effectiveness for the elementary grades ranged from 40¢ to \$6.00 per child per year per surface saved.

### Significance

The demonstration programs show that the procedure is readily accepted by school systems, parents and children in the elementary grades. Measures of cost-benefit indicate that the procedure is highly cost-effective under a variety of administrative systems and the nominal fee for materials makes it affordable to almost any community which desires to implement such a program.

### Proposed Course

The cost-effectiveness of the program in the upper grade levels will be determined.

2. Publications

Brunelle, J.A. and Miller, A.J. Cost Analysis of School-Based Mouth-rinse Programs in 17 Communities in the U.S. and Guam. In IADR Program and Abstracts of Papers: J. Dent. Res. 58:388, 1979.

Miller, A.J. and Brunelle, J.A. Fluoride Rinses. In text Pediatric Dentistry: Scientific Foundations and Clinical Practice. Stewart, R.E., Troutman, K.C., Barber, T.K. and Wei, S.H. (Eds.) C.V. Mosby Co., St. Louis, Mo. Pending Publication, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00228 04 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Association of Streptococcus mutans with dental caries reduction in a school population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Thomson, Lynn A.	Laboratory Scientist	NCP	CPR	NIDR
Little, Wayne A.	Laboratory Technician	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR

COOPERATING UNITS (if any)  
Biddeford School Department, Maine

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)  
 (a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER  
 (a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)  
 Dental plaque is being studied to determine whether changes in microorganisms, specifically Strep. mutans, occur during fluoride preventive procedures. The school population consists of children using fluoride rinses either daily or weekly, as well as some children using a placebo solution. Dental plaque samples are obtained periodically and analyzed using cultural methods and fluorescent antibody reagents specific for bacteria under study. Plaque samples were obtained at the following times: Fall, 1976, Spring, 1977, Fall, 1977, and Fall, 1979. Saliva samples were also obtained during the 1979 examination. Alterations in plaque composition during the study period will be compared with the changes in the caries incidence.

## 1. Project Description

### Objectives:

The objectives of the project are to:

- a. determine any changes that occur in the Strep. mutans population in the plaque of children using fluoride rinses in a school program;
- b. associate changes in the Strep. mutans populations with the reduction in dental caries;
- c. evaluate the effect of transportation and storage methods on the viability of bacteria in the plaque specimens;
- d. examine the apparent absence of Strep. mutans d or g organisms in the dental plaque samples of this school population.

### Methods:

Thirty students for the plaque study were selected randomly by the Biometry Section, NCP. Three plaque samples were obtained from each child. These samples included one specimen for fluoride determination and two for bacterial examination at specific tooth sites. All samples were immediately frozen and stored at -70°C until removed for analysis.

### Major Findings

Using cultural methods, 63.3% of the Maine children were determined to harbor S. mutans. When FA methods were employed, the number with detectable levels of S. mutans increased to 70.17%. The S. mutans colonies comprised about one-third of the colony forming units on the MS agar plates. In comparison, the FA/phase contrast results indicated that S. mutans averaged 1.9% of the total cell count. Serotype c was the most frequently detected serotype of S. mutans (89.5% culturally and 76.1% with FA). Serotypes a, b, d and g were not detected in this population. Serotype e and f were encountered in 21.1% and 10.6% of the specimens which were positive for S. mutans. Strep. sanguis, Actinomyces israelii and Actinomyces naeslundii, Actinomyces viscosus were found to be present in over 98% of all children studied and were observed to account for 9.6%, 10.4% and 38.4% (respectively) of all cells visible in the plaque specimens.

Specimens obtained during the Fall, 1979 examination were analyzed using more sensitive FA and cultural methods to confirm the absence of serotype d/g strain in this population. Of the original 30 students, eighteen were examined. Although the sensitivity of the methods was increased 1,000

fold over routine methods, serotype, d/g cells were detected in only two specimens from one of the students.

The effect of dry ice transportation and -70°F storage of plaque and saliva samples was evaluated for material obtained during the fall, 1979 examination. Results to date suggest that conventional transport medium (RTF-Loesche) does not maintain adequate plaque bacterial viability. Viability was significantly lower in plaque examined culturally after freezing and storage than it was in plaque cultured within minutes after the sample was obtained. Undiluted saliva samples were observed to tolerate freezing with less loss of viability than occurred with dental plaque. FA enumeration of S. mutans has not been observed to be significantly affected by these transport and storage procedures.

#### Significance:

Data on the prevalence of these species in this school-age population provides valuable information on the frequency of these bacteria and their variation during preventive measures.

#### Proposed Course:

The prevalence of certain plaque bacteria in this population will be compared with other populations experiencing different caries attack rates.

#### 2. Publications

Thomson, L.A., Little, W.A., Bowen, W.H., Sierra, L.I.  
Aguirrer, M., and Gillespie, G. Prevalence of Streptococcus mutans  
Serotypes, Actinomyces, and Other Bacteria in the Plaque of Children.  
J. Dent. Research: In Press, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00229 04 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Plaque variations in populations ingesting different levels of water fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Stiles, Horace M.	Chief, E. Section	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR
Brunelle, Janet A.	Chief, B. Section	NCP	CPR	NIDR
Dinsmore, Edwin W.	Laboratory Technician	NCP	CPR	NIDR

COOPERATING UNITS (if any)

-AB/BRANCH Caries Prevention and Research

SECTION Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINDRS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three communities having 4.2, 2.3 and less than 0.1 ppm fluoride in the drinking water were chosen as study sites. Children 12-18 years of age who had been lifelong residents in the communities comprised the study populations. Plaque and saliva samples, collected from each participant, are being analyzed for microbial and fluoride content. DMF surfaces also were recorded for each participant. Data will consist of comparisons of various parameters among the three groups.

1. Project Description

Objectives:

The purpose of this study is to examine associations among DMFS, plaque fluoride concentration and certain microflora of dental plaque from populations with different concentrations of naturally-occurring fluoride in the drinking water. The microbiologic phase of the study is still in progress.

Methods:

Three population groups residing in Texas were selected for study, each with a different concentration of fluoride naturally occurring in the drinking water. The sites were designated as low (L), mid-range (M) and high (H) in fluoride, with water concentration of fluoride less than 0.1, or 2.3 or 4.2 ppm. The water fluoride concentrations had been at these levels for at least 18 years. The recommended optional fluoride concentration for the geographic region is 0.7-0.9 ppm.

Major Findings:

- a. Mean age for the 3 groups was essentially the same, 14.6, 14.3 and 14.0 years. As expected the caries experience of the group consuming less than 0.1 ppm fluoride was higher than in those groups consuming 2.3 or 4.2 ppm fluoride. This difference was significant ( $P < 0.001$ ).
- b. No significant difference in caries scores was seen between the participants consuming 2.3 or 4.2 ppm fluoride in their drinking water.
- c. The mean plaque dry weight for the three groups was essentially the same 0.91, 0.92 and 1.19 mgms.
- d. The fluoride concentration in the dry plaque specimens was significantly different in each group.
- e. Positive correlations were found between age of subject and plaque fluoride concentration. However, these correlations were significant only for groups I and III, the low ( 0.1 ppm F) and high (4.2 ppm F) water fluoride groups.
- f. Results from the microbiological studies of plaque and saliva from the participants are presently being processed.

Significance to Program:

A major problem for the Program is to distinguish the various mechanisms through which fluoride prevents caries and evaluate their significance. Fluoride in saliva and drinking water is concentrated by dental plaque. This project should provide important data on the fluoride levels reached in plaque and on associated changes in the numbers and species of cariogenic microorganisms.

Proposed Course:

The study will continue to examine the microbiology of plaque and saliva from these three populations. The salivary fluoride is also being investigated.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00231 04 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Radiation caries in primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Edgar, William M.	Laboratory Scientist (Vis.)	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR
Cole, Michael F.	Laboratory Scientist (Vis.)	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Plaque fluid and saliva were collected from 4 irradiated monkeys (*Macaca mulatta*) and from 8 groups of non-irradiated controls all of which were receiving a cariogenic diet. Plaque was also collected from non-irradiated group which was fed a non-cariogenic diet. The investigation will provide information on the biochemical composition of plaque fluid and saliva associated with the development of caries and provide enhanced insight into the pathogenesis of dental caries. Composition of the plaque fluid was influenced by ingestion of sucrose.

## 1. Project Description

### Objective

The purpose of this investigation is to identify those constituents in plaque fluid and saliva most likely to be associated with rampant caries by analyzing samples from animals with contrasting levels of caries experience.

Four *Macaca mulatta* monkeys were irradiated as previously described (250 rads each side twice weekly for week 2 weeks). Normal controls were 8 *Macaca fascicularis* being fed the same high sugar diet as controls. In addition a group of *Macaca fascicularis* on stock diet were studied.

Inorganic analyses were carried by atomic absorption ion specific electrodes or chemical methods.

The effect of an exposure to sucrose was also determined.

### Major Findings

Plaque fluid from the irradiated animals had significantly more phosphate than did the other groups. Calcium content did not differ significantly and ranged from about 3.8 (mmol/l) to 5.84.

Plaque from the irradiated animals had a much greater capacity to lower the pH of sugar solutions than did the controls. Calcium and magnesium increased in plaque fluid in irradiated animals immediately following exposure to sucrose. There was more iodine in the saliva of irradiated animals than in that from controls.

### Significance

This study shows that specific changes occur in the composition of plaque fluid which appear to be associated with a carious attack. The observed changes are consistent with the observation made by others that magnesium salts are the first removed from enamel during caries attack.

### Proposed Course

The study is continuing and the model will be further developed to facilitate screening of anticaries compounds.





1. Project Description

Objectives:

The goal of this project is to develop a method which will be useful in predicting the cariogenicity of foods, in measuring the bioavailability of fluoride, and in measuring other biological phenomena in the mouth.

Methods:

Equipment needed for making the various intraoral measurements is not commercially available and must be fabricated. This project which was initiated several years ago remains in the pretest phase. However, substantial progress has been made in developing a workable wire telemetry apparatus and conventional and transistor sensors (CHEMFET) have been tested for intraoral use.

Major Findings:

The CHEMFET and the pH microelectrode responded accurately and reliably in in vitro tests. Both the CHEMFET and the radiotelemetry device failed after prolonged contact with moisture. The signal from the wire telemetry device that used a conventional glass pH electrode was degraded by inadequate shielding and other deficiencies. However, wire telemetry using a glass electrode shows enough promise to warrant further testing.

Significance:

Telemetry of pH, F<sup>-</sup> and other ions would be useful to government, industry and universities. Physiologists could gain much useful information concerning interactions in the mouth of bacteria, host, and diet. As an example, government and industry could study the cariogenicity of various foods and snack items using telemetry and coordinated animal studies. This information would be useful for developing less cariogenic diets.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00236 03 CPR									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less)  Purification of rat immunoglobulins											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" data-bbox="126 439 1305 540"> <tr> <td>Cole, Michael F.</td> <td>Laboratory Scientist (vis.)</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Hsu, Su-Cheng D.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Monell-Torrens, Esteban</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> </table>			Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR	Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR	Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR									
Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR									
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR									
COOPERATING UNITS (if any)											
LAB/BRANCH Caries Prevention and Research											
SECTION Preventive Methods Development											
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Immunoglobulins M and G (IgM and IgG) were purified from rat serum and secretory IgA from rat colostrum by molecular sieve, ion exchange and affinity chromatography. Purification of IgM was complicated by contamination with $\alpha_2$ macroglobulin. Antisera to these purified immunoglobulins were raised in New Zealand white rabbits. The antisera were rendered monospecific by immunoabsorption on affinity supports. Antisera have been coupled to fluorescein isothiocyanate and alkaline phosphatase.											

1. Project Description

Objective:

The purpose of this study was to purify immunoglobulin A, G and M in order to raise monospecific antisera to these proteins.

Methods:

Rat serum was collected and the immunoglobulins precipitated with 20 volumes of 2.5% boric acid. The precipitate was resuspended in 0.1 M Tri HCl pH 8.0 containing 0.01 M glycine and 0.15 M NaCl and chromatographed on Sephadex G200. The exclusion peak containing IgM was concentrated and chromatographed on Sepharose 6B. Immunoglobulin M was found in the second peak and was free of detectable  $\alpha_2$  macroglobulin.

The supernatant from the precipitated serum was dialyzed and chromatographed on QAE Sephadex A-50 to purify IgG. Colostrum was obtained from rat dams following stimulation with oxytocin and was precipitated with 50%  $(\text{NH}_4)_2 \text{SO}_4$ . the precipitate was redissolved, dialyzed and chromatographed on Sephadex G200. the exclusion peak was rechromatographed on Sepharose 6B to yield pure SIgA.

Recently the immunocytoma bearing rat strain LOU/WSL has been obtained from Dr. Herve Bazin, Experimental Immunology Unit, University of Louvain B-1200, Brussels, Belgium. The following tumors are maintained in rats as solid subcutaneous or intraperitoneal ascitic tumors or the neoplastic cells have been frozen in liquid nitrogen:

IR 202 IgM  
IR 22 IsA  
IR 677 IgG<sub>1</sub>

Single cells suspension of the solid tumors are made by mincing the tissue and passing it through stainless steel screens. The erythrocytes are lysed and the numbers of viable cells enumerated by Trypan Blue exclusion. The cell density is adjusted to  $1 \times 10^7$  cells/ml and 1 ml of the cell suspension in RPMI 1640 tissue culture medium injected intraperitoneally. The ascitic fluid is collected by aspiration after 7-10 days and serves as a rich source of monoclonal immunoglobulin.

Purified rat IgG, IgA and IgM were emulsified 1:1 in complete Freund's adjuvant (CFA) and each emulsified protein was injected subcutaneously in 20-30 sites of 3 New Zealand white rabbits. One month after primary immunization, the immunization was repeated with incomplete Freund's adjuvant (IFA). After two weeks the rabbits were injected intramuscularly with antigen



in IFA. Blood for titering was obtained from the ear vein and serum assayed by immunoelectrophoresis (IEP). High titer bleedings were pooled and cross-reacting antibodies and light-chain activity removed by affinity chromatography. The following affinity supports were used:

- (1) Neonatal/germfree rat serum
- (2) Rat IgG
- (3) Rat IgM

IgG functions of the absorbed antisera were then coupled to alkaline phosphatase and fluorescein isothiocyanate.

Significance:

Antiglobulin reagents, i.e., antibodies directed against immunoglobulin isotypes are indispensable for the assay of class specific antibody in secretions and serum. without these well-characterized reagents it is impossible to measure class specific antibody. These reagents can be utilized in antiglobulin augmentation assays, enzyme linked immunoabsorbant assays, fluoroimmunoassays and radioimmunoassay.

The production of monospecific antiglobulin reagents will allow the detection of antibody forming cells and the quantitation of secretory antibodies in the rat caries model.

Proposed Course:

Devise sensitive assays to measure mucosal antibodies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00237 03 CPR																		
PERIOD COVERED October 1, 1979 to September 30, 1980																				
TITLE OF PROJECT (80 characters or less) Immunoglobulins and antibodies in plaque fluid and saliva in two populations with contrasting levels of caries																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 40%;">Cole, Michael F.</td> <td style="width: 40%;">Laboratory Scientist (vis.)</td> <td style="width: 20%;">NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Hsu, Su-Cheng D.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Kingman, Albert</td> <td>Statistician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Brunelle, Janet</td> <td>Chief, B Section</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Rodgers, Patricia</td> <td>Statistical Assistant</td> <td>NCP CPR NIDR</td> </tr> </table>			Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR	Hsu, Su-Cheng D.	Laboratory Technician	NCP CPR NIDR	Kingman, Albert	Statistician	NCP CPR NIDR	Brunelle, Janet	Chief, B Section	NCP CPR NIDR	Rodgers, Patricia	Statistical Assistant	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR																		
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR																		
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Kingman, Albert	Statistician	NCP CPR NIDR																		
Brunelle, Janet	Chief, B Section	NCP CPR NIDR																		
Rodgers, Patricia	Statistical Assistant	NCP CPR NIDR																		
COOPERATING UNITS (if any) Univ. of Antiqua, Medellin, Colombia, S.A. Drs. Sierra, Espinal and Aguirra PAHO, Wash., D.C. Dr. G. Gillespie																				
LAB/BRANCH Caries Prevention and Research																				
SECTION Preventive Methods Development																				
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD																				
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords)  Plaque fluid and saliva was collected from 25 children aged 7-12 years from two communities with contrasting levels of caries (DMFS 1 v DMFS 39). The samples were assayed for SIgA, IgG, IgM, the third component of complement (C'3), lactoferrin, lactoperoxidase, lysozyme, albumin and total protein. With the exception of lactoperoxidase and IgG the levels of the specific proteins were significantly higher in plaque fluid than in saliva. However, no significant differences were detected between the communities in either plaque fluid or saliva.																				

1. Project Description

Objective:

The purposes of this study are to measure immunoglobulins, antibodies and non-specific immune factors in saliva and plaque fluid and to determine their relationship to caries activity.

Methods:

Supragingival plaque, free of visible blood, was collected from each subject and centrifuged at 38,000.g to obtain the free fluid phase. The plaque solid was then washed with PBS pH 7.0 until no free protein remained. Bound protein was then released from the plaque solid with successive washes of 3.5M KSCN. Whole saliva was also obtained from each subject. Secretory IgA (SIgA), IgG, IgM, the third component of complement (C'3), lactoferrin, lysozyme and albumin were determined by a solid phase immunofluorescence assay. Lactoperoxidase activity was determined colorimetrically and total protein by reaction with o-phthaldehyde.

Major Findings:

With the exception of lactoperoxidase and IgG the levels of the specific proteins were significantly higher in plaque fluid than in saliva. However, no significant differences were detected in the concentrations of the specific proteins in plaque fluid and saliva between the communities.

Significance:

Study of the levels of specific and non-specific immune factors in saliva and plaque fluid from subjects with high and low caries experience could aid in understanding the role of host defense factors in protection against dental caries.

Proposed Course:

1. Expand the numbers of subjects in the study populations in order to better understand the role of host defense in the etiology of dental caries.
2. Examine the physical nature of the proteins in plaque fluid.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00240 03 CPR
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Cariogenicity of foodstuffs		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
Bowen, William H. Monell-Torrens, Estaban Amsbaugh, Suzanne M. Cole, Michael F. Gomez, Irma M. Emilson, Claes-Göran Edgar, William M.	Chief, CPR Branch Laboratory Technician Laboratory Technician Laboratory Scientist (Vis.) Laboratory Technician Laboratory Scientist (Vis.) Laboratory Scientist (Vis.)	NCP    CPR    NIDR NCP    CPR    NIDR NCP    CPR    NIDR NCP    CPR    NIDR NCP    CPR    NIDR NCP    CPR    NIDR NCP    CPR    NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH Caries Prevention and Research		
SECTION Etiology		
INSTITUTE AND LOCATION NIH, NIDR, Bethesda, Maryland		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>There continues to be a growing interest in identifying those foods which are most likely to produce dental caries when frequently ingested. This study has resulted in the development of an animal model which permits the assessment of the cariogenicity of foods in a simple unequivocal and reproducible manner.</p>		

## 1. Project Description

### Objective

The purpose of this investigation was to determine whether a technique which entailed animals receiving their essential nutrition by gastric intubation and test foods by mouth through a König-Hofer machine could permit the ranking of the cariogenic potential of foods. In addition it was hoped to determine the effect of foods on the implantation of S. mutans and also to determine the effect of frequency of eating on the population of S. mutans.

### Methods

A total of 242 Osborne Mendel rats was used in this study. Animals received their essential nutrition twice daily by means of gastric intubation. Test foods were fed to the animals 17 times daily at hourly intervals. As a result any lesions which develop can be ascribed solely to the interaction of the test food with microorganisms on the tooth surface. Caries and S. mutans were estimated in the usual manner.

### Major Findings

There is a clear association between the frequency of eating and incidence of caries. The number of S. mutans present in the mouth is also related to the frequency of eating. Because of variation in absolute caries scores from one experiment to another it is necessary to express results as a ratio of those achieved with 17 meals of sucrose. Assuming a score of 1 for sucrose, cream-filled chocolate cookies gave an index of 1.4, a fortified breakfast cereal 1.06, potato chips 0.84, chocolate bar 0.72, and plain starch 0.45. The ratio is termed the cariogenic potential index CPI. Reproducibility was excellent.

### Significance

These results show that it is possible to distinguish the cariogenicity of foods in rats in a simple manner and that this information will be useful in generating dietary information for the public.

### Proposed Course

More foods are being investigated and in addition the influence of different intervals between feeds is being determined.

### Publications

Bowen, W.H.; Amsbaugh, S.M.; Monell-Torrens, S.; Brunelle, J.; Kuzmiak-Jones, H.; Cole, M.F. A method to assess cariogenic potential of foodstuffs. JADA 100:677-681, May 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00243 03 CPR

PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Growth energetics and interaction of plaque microorganisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Robrish, Stanley A.	Laboratory Scientist	NCP CPR NIDR
Kemp, Christopher	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Sharer, Sue Ann	Laboratory Assistant	NCP CPR NIDR
Barnes, Peter A.	Laboratory Aide	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, Bethesda, MD 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER  
 (a1) MINDRS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Streptococcus sanguis and S. mutans were grown in the continuous mode both singly and in coculture. The results showed that S. sanguis had a higher affinity for glucose used as a limiting energy source than S. mutans. S. mutans, however, appeared to produce an inhibitor to S. sanguis. When S. mutans is inoculated first in the continuous culture, it remains the dominate organisms after subsequent inoculation of S. sanguis.

We have continued to accumulate data on the chemical composition of oral bacteria which have been grown in highly controlled conditions of pH and nutrient composition.



## 1. Project Description

### Objective:

The objectives of this project are: (a) to obtain pure cultures of bacteria grown under high controlled conditions to be used as antigens for a variety of immunologic experiments and (b) to investigate the interactions of Streptococcus sanguis grown in mixed culture using continuous culture methods.

### Methods:

Bacteria grown under controlled conditions for experiments testing the immune response of S. mutans have been supplied to the Branch. It is important for these studies that the cells be grown under highly defined conditions so that the antigenic content of the cells may be reproduced. The pH of the medium and gas atmosphere are controlled. The growth medium for the cells is made free of high molecular weight components which may behave as antigens. In addition to usual growth assays the turbidity of the culture is monitored continuously using fiber optic probe. Cells from a large batch of a strain of S. mutans grown under these conditions have been used as antigenic material to stimulate secretory immunoglobulin in human volunteers. Organisms prepared similar also have been supplied to Dr. Cole for in vitro immunological experiments, and to Dr. Bowen for in vivo studies using monkeys. The growth of S. mutans and S. sanguis in mixed culture also is being studied. The organisms are being grown under energy limitation at several growth rates in a continuous culture apparatus. The experiments which are planned are to grow each of the organisms separately and together in limiting energy conditions to determine if there is any competition between the organisms for the same substrate.

### Findings:

Pure cultures of both S. mutans and S. sanguis were grown in a continuous mode with carbon limitation. The saturation constants for glucose were calculated and predictions made for the growth of the organisms together in the continuous culture. When the organisms were grown together, S. sanguis did not dominate at low dilution rates when S. mutans was inoculated first. When S. sanguis was grown in the same medium following growth of S. mutans the lag phase of growth was markedly extended indicating that an inhibitor had been produced by S. mutans in growth on that medium.

### Proposed Course

Further investigations into the nature of the inhibitory substance and the interactions between streptococci and other plaque bacteria is proposed.

Significance:

Preparation of these antigenic materials will allow initial testing of the possibility of immunizing persons against caries. The mixed culture studies will lead to a better understanding of the complex ecological interactions present in dental plaque. This may aid in our understanding of the factors involved in the disease process.

2. Publications

Kemp, C.W., Robrish, S.A., Sharer, S.A., Barnes, P. and Bowen, W.H. Interaction of Streptococcus mutans 6715-15 and Streptococcus sanguis 10558 in mixed continuous culture. In preparation. 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00244 04 CPR									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less) The evaluation of media for the isolation of <u>Actinomyces</u> from dental plaque											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>Little, Wayne A.</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Thomson, Lynn A., Jr.</td> <td>Laboratory Scientist</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> </table>			Little, Wayne A.	Laboratory Technician	NCP CPR NIDR	Thomson, Lynn A., Jr.	Laboratory Scientist	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Little, Wayne A.	Laboratory Technician	NCP CPR NIDR									
Thomson, Lynn A., Jr.	Laboratory Scientist	NCP CPR NIDR									
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR									
COOPERATING UNITS (if any)											
LAB/BRANCH Caries Prevention and Research											
SECTION Etiology											
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)  The relative recoveries of stock strains of <u>Actinomyces viscosus</u> , <u>A. naeslundii</u> and other plaque bacteria were evaluated on CNAC-20, CNAC-20F (Fluoride), FC and GMC media. Colony counts were compared with counts from blood agar media. Preliminary studies have begun on the recovery of <u>Actinomyces</u> from human and animal plaque.											



1. Project Description

Objective:

The objective of this project is to evaluate recently introduced media selective for Actinomyces and determine their usefulness for both human and animal studies.

Methods:

Stock strains of Actinomyces and other plaque bacteria isolated from both human and animal sources were plated in triplicate onto Trypticase Soy-Blood CNAC-20, CNAC20F, FC and GMC Agar. In addition, several samples of human and rat plaque were examined by the same methods.

Major Findings:

The FC medium described by Beighton and Coleman allowed the greatest recoveries of both stock strains and plaque isolates of Actinomyces. A. viscosus strains of animal origin and human isolates of A. naeslundii serotype 3 were completely inhibited by CNAC-20, CNAC-20F and GMC media. The four media examined were not inhibitory to certain strains of Streptococcus sanguis, Rothia dentocariosa, Bacterionema matruchotii and Propionibacterium sp.

Significance:

Little is known about the ecology of A. viscosus and A. naeslundii and their relationship to human dental disease. Part of the problem is that the identification of Actinomyces is difficult and does not lend itself to processing numerous samples from clinical studies. The availability of well characterized selective media has the potential for greatly facilitating the identification of these two species.

Proposed Course:

Jordan's Actinomyces media (CFAT) will be examined and compared to the other media. A recently proposed reference method for antimicrobial testing of anaerobes will be used to determine the antimicrobial susceptibility profiles of Actinomyces and related plaque bacteria in order to improve the selectivity of available media.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00256-02 CPR																				
PERIOD COVERED October 1, 1979 to September 30, 1980		CT 0060130																				
TITLE OF PROJECT (80 characters or less)  The effect on dental caries of varying periods of school water fluoridation.																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" data-bbox="175 439 1209 574"> <tr> <td>Heifetz, Stanley B.</td> <td>Clinical Investigator</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Horowitz, Herschel S.</td> <td>Chief, CPS</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Meyers, Rhea</td> <td>Clinical Investigator</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Li, Shou-Hua</td> <td>Statistician (vis.)</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR	Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR	Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR	Li, Shou-Hua	Statistician (vis.)	NCP	CPR	NIDR
Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR																		
Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR																		
Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR																		
Li, Shou-Hua	Statistician (vis.)	NCP	CPR	NIDR																		
COOPERATING UNITS (if any) Division of Dental Health, Department of Health, Commonwealth of Virginia																						
LAB/BRANCH Caries Prevention and Research																						
SECTION Community Programs																						
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																						
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) Fluoride was added to the water supplies of public schools in three Virginia counties to study the anti-caries effectiveness of fluoride ingestion: throughout grades K-6 (Cumberland Co.); throughout grades K-8 (Matthews Co.); and throughout grades K-12 (Amelia Co.). All school populations live in areas that have no central water supplies and where the drinking waters contain negligible concentrations of fluoride. In May, 1979, baseline dental examinations (DMFS Index) were made of a total of approximately 4,200 students in grades K-12 in the three counties. In December 1979, schools containing the required grade levels for study fluoridated at a concentration of 4.5 times that recommended for community fluoridation in the geographic area. Follow-up examinations will be conducted after 3, 7, 9 and 13 years of study as increasingly more subjects become continuously exposed to fluoridated water at school for the desired periods of time.																						

1. Project Description

Objective:

Previous studies have shown that children who consume fluoridated water in school throughout grades 1-12 have a 40 percent reduced prevalence of dental caries. The present study will determine if similar benefits can be obtained by exposure to school fluoridation for shorter periods of time.

Methods:

In the spring of 1979 prior to the installation of fluoridation equipment, baseline dental examinations using the DMF surface index were made of approximately 4200 children (grades K-12), in the Virginia counties of Cumberland, Matthews and Amelia. Three Public Health Service investigators and two public health dentists on the staff of Virginia's Division of Dental Health comprised the examination team. In December 1979, fluoride was added to the water supply at the elementary school (grades K-6) in Cumberland, to the elementary and junior high schools (grades K-8) in Matthews, and to the elementary, junior and senior high schools in Amelia. A concentration of 4.5 times the optimum recommended for community water fluoridation in the geographic area will be maintained in the schools' water system. Results of follow-up examinations after 3, 7, 9 and 13 years of study will be compared with baseline findings to determine the benefits received from varying periods of consumption of school water fluoridation. Fluoride concentrations will be monitored by school personnel under the supervision of the Virginia Department of Dental Health.

Major Findings:

Baseline findings are currently being analyzed to determine the comparability of dental caries prevalence among children in the three selected counties.

Significance:

Currently, it is recommended that school fluoridation programs be conducted throughout grades K-12. If it were necessary to fluoridate only elementary schools (grades K-6) or even only through junior high schools (grades K-8) to obtain decay preventive benefits that approximate those conferred by exposure through senior high school (grades K-12), then the cost of implementing a nation-wide school water fluoridation program would be considerably reduced.

Proposed Course:

School fluoridation units were installed in 7 study schools located in the three Virginia counties. Fluoride at a concentration of 4.5 "times the optimum" will be maintained at the study schools until the final examinations in 1992. A comparison of results of interim and



final examinations among the three county school populations will indicate if it is necessary to expose children to fluoridated water at school throughout grades K-12 in order to produce maximum caries inhibition or if near maximum benefits can be obtained by exposure throughout only grades K-6 or K-8.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00262 02 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Study of an intraoral device designed for providing sustained low levels of fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Emilson, Claes G.	Laboratory Scientist (vis.)	NCP CPR NIDR
Kingman, Albert	Statistician	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR
Li, Shou-Hua	Statistician (vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)  
Southern Research Institute (SRI), Birmingham, Alabama Dr. D.R. Cowsar  
Hazelton Laboratories, Oral Research Section, Vienna, VA Dr. D. Dalgard

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Preventive Methods Development

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation supplements earlier studies done by SRI in collaboration with Dr. Bowen and Dr. Mirth to develop a fluoride releasing device for intra-oral use. The safety and pharmacokinetics of the device is being evaluated preliminary to possible clinical studies of its anticaries benefits. Preliminary studies in the subhuman primate and in humans suggest that the appliance will adhere to the teeth and release fluoride at the appropriate rate. Compared with pretest levels the concentration of fluoride in saliva showed a five to tenfold increase whereas the increase of fluoride in the serum and urine was less than twofold.

1. Project Description

Objectives:

The long-range objectives of this study include providing maximum anticaries benefits with minimum fluoride dosages. The current subhuman primate and clinical investigations are designed to measure the pharmacokinetics of fluoride in order to verify the performance and safety of the fluoride release devices.

Methods:

Fluoride levels were measured in the saliva, plaque, blood and urine of the subhuman primate and human before and after attachment of a fluoride releasing device. Published methods of direct measurement were used for estimating fluoride levels in urine, saliva and blood. It was necessary to develop a microdiffusion method for measuring the fluoride levels in the plaque. The evaluation and development of analytic procedures was augmented by project No. Z01-DE-00112-06.

Major Findings:

In the primate study fluoride levels in most of the biologic samples followed the expected time course. Fluoride was at increased levels while the device was in place and returned to pretest levels when the device was removed.

The findings were confirmed and extended in the clinical study. Very high levels of fluoride were noted in the oral fluids. Whereas, there was less than a twofold increase in the fluoride levels in the urine and serum.

Pretest evaluation showed that the microdiffusion method was able to detect tightly bound fluoride and complexed fluoride. Fluoride was readily measured from submilligram samples of dental plaque. Extremely high levels of ions including  $Al^{+++}$  and  $Fe^{++}$  failed to interfere with the accuracy of the fluoride analysis. Recovery of fluoride was increased when the diffusate was contained in a screw top vial and was diffused to dry NaOH.

Significance:

This controlled release delivery system appears to provide a more effective and efficient way to deliver fluoride because it provides a continuous therapeutic level of  $F^-$  to the caries site for long periods of time.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00263 02 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Cariogenicity of the different serotypes of S. mutans in gnotobiotic rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Little, Wayne	Laboratory Technician	NCP CPR NIDR
Thomson, Lynn A.	Laboratory Scientist	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)  
Gnotobiotics Unit, DRS, NIH

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of experiments has been planned utilizing gnotobiotic rats to determine the relative cariogenicity of the different serotypes of S. mutans and certain S. mutans mutants of special interest. In the first experiment two germfree isolators were used, each containing 12 Osborne-mendel rats. The rats in one isolator were orally inoculated with a serotype d strain isolated from the high caries community of Don Matias (Columbia, S.A.). Rats in the second isolator were inoculated with a serotype c strain from the low caries community of Heliconia. After 8 weeks the animals were sacrificed. Saliva and blood was collected for analysis and the heads processed for caries scoring.

1. Project Description:

Objective:

Results of studies on the prevalence of S. mutans in human populations suggest that serotype c is most prevalent and serotypes a and b are essentially absent. Serotypes d, e, f, and g are usually present but to varying degrees of prevalence.

Evidence on the relative cariogenicity of the different S. mutans serotypes is sparse. A human population study by Keene et al. demonstrated a correlation between high caries scores and the presence of biotype e in plaque. Using antibiotic suppressed SPF Sprague-Dawley rats, Hamedá et al. observed the highest caries scores with serotype d strains. They also observed significantly reduced caries activity of in serotype b reference strains compared to caries reports on the original isolates. Our long term goal is to examine fresh human plaque isolates of serotypes c, d/g, e, and f and to see if differences in cariogenicity are related to serotype or merely variations in the strains.

The second objective of this experiment involves comparing the caries activity of S. mutans isolates from Don Matias and Heliconia, Columbia, S.A. These two communities have been observed to have substantially different caries levels; furthermore, in vitro tests have demonstrated that Don Matias plaque is significantly more acidogenic than plaque from Heliconia. However, there appear to be little or no differences in the prevalence of S. mutans or the distribution of serotypes.

We propose to include strains from these communities in this series of experiments to see if S. mutans isolates from Don Matias plaque are more cariogenic than Heliconia isolates.

Methods:

Two isolators at a time are used; each containing twelve Osborne-Mendel rats (18 days old). Animals are provided with fluoride-free deionized water and diet NIH 2000 vs. The breeder animals are maintained on diet MIT 200 and fluoride-free deionized water. Animals are inoculated orally on two consecutive days with 50  $\mu$ l of broth culture containing approximately  $10^8$  CFU. Cultures will be grown for 24 hours in dialized Todd Hewitt broth and adjusted to proper cell density. Experiments will be of 8 weeks duration so that less cariogenic strains will have sufficient time to develop detectable caries levels.

Major Findings:

Preliminary data suggest that the caries activity of the two strains employed (serotype c and d) was not notably different. Additional strains have been selected and need to be evaluated.

Significance to Biomedical Research:

The results of this study could have potential significance in the selection of strains for whole cell vaccines to be used in immunization studies. Furthermore, insight may be gained into a possible relationship between DMF scores and S. mutans serotype prevalence in human populations.

Proposed Course:

A number of strains will be examined to determine if there are differences in cariogenicity among the serotypes of S. mutans. In addition, strains from two communities in Columbia, S.A. will be examined for differences in cariogenicity.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00264 02 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Fluorescent antibody methods to determine the prevalence of certain plaque bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Thomson, L. Ariel	Laboratory Scientist	NCP CPR NIDR
Little, Wayne	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)  
Center for Disease Control, Atlanta, Georgia  
Roger McKinney, Chemist; William, Harrell, Microbiologist; Sandra Bragg, Microbiologist

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The capabilities of several laboratories have been joined in collaborative research intended to develop, evaluate, and improve "reagent grade" fluorescent antibody (FA) conjugates designed to be specific for certain dental plaque bacteria considered important in caries etiology.

The conjugates produced through this collaborative effort have been used to determine the prevalence of certain organisms in plaque samples from various locations.

1. Project Description:

Objectives:

The project is intended to develop and enlist the assistance of collaborating investigators to develop methods and materials permitting the detection and enumeration of specific plaque bacteria directly in clinical specimens. The objectives include the evaluation of both FA methods and conjugates to determine procedures which can be recommended as sensitive, specific and reliable for use in determining the prevalence of selected plaque bacteria.

Methods:

Efforts to improve FA procedures for examining dental plaque have involved the following methods:

- A. Comparison of the effects of light sources on the intensity of specimen fluorescence;
- B. Adaptation and refinement of bacterial-immuno-adsorbent columns to improve the specificity of conjugates;
- C. Evaluation of immunization schedules intended to yield higher-titered antisera;
- D. Improvement of microscope slide design to conserve antisera;
- E. Reformulation of FA mounting medium and conjugate diluent.

Major Finding:

The improved specificity of FA conjugates achieved with the bacterial-immunosorbent columns recommended by Dr. Roger McKinney, has permitted direct FA examination of Streptococcus mutans in plaque smears. Following production protocols prepared during this project, the Biological Reagents Section of the Center for Disease Control has prepared "Reagent Grade Conjugates" in quantities which have permitted major population studies.

Significance to Biomedical Research:

The development of methods and FA conjugates which permit the detection and enumeration of certain oral bacteria in direct plaque smears avoids the

difficulties associated with conventional cultural methods. The extensive manipulation of specimens for cultural study and the inhibitory effect of selective media are not required in the examination of plaque samples with FA methods. These improvements in monitoring plaque organisms will permit the study of specific bacteria with the increased sensitivity and specificity associated with FA methods.

Publications:

The following publications have resulted from the collaborative studies related to this project:

Wong, M. C.; McKinney, R.M.; and Thomson, L.A.: Soluble Antigen Extract Used as Blocking Agents to Obtain Specificity in Serotyping of Streptococcus mutans. J. of Immun. Methods: 27:283-291, 1979.

Thomson, L.A.; Bowen, W.H.; Little, W.A.; Kuzmiak-Jones; and Gomez, I.M.: Simultaneous Implantation of Five Serotypes of Streptococcus mutans in Gnobiotic Rats. Caries Res. 13: 9-17, 1979.

Thomson, L.A.; Little, W.A.; Bowen, W.H.; Sierra, L.I.; Aguirrer, M.; and Gillespie, G. Prevalence of Streptococcus mutans, Serotypes, Actinomyces, and Other Bacteria in the Plaque of Children. J. Dent. Res.: In Press.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00266 02 CPR															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Comparison of the retention of two bis-GMA pit and fissure sealants																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" data-bbox="189 425 1400 535"> <tr> <td>Li, Shou-Hua</td> <td>Statistician (Vis.)</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Swango, Philip A.</td> <td>Project Scientist</td> <td>NCP</td> <td>CRGC</td> <td>NIDR</td> </tr> <tr> <td>Heifetz, Stanley B.</td> <td>Clinical Investigator</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Li, Shou-Hua	Statistician (Vis.)	NCP	CPR	NIDR	Swango, Philip A.	Project Scientist	NCP	CRGC	NIDR	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
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Swango, Philip A.	Project Scientist	NCP	CRGC	NIDR													
Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR													
COOPERATING UNITS (if any) Fairfax County Health Dept., Fairfax, Va.																	
LAB/BRANCH Caries Prevention and Research																	
SECTION Biometry																	
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) The retention of bis-GMA pit and fissure sealants polymerized chemically (Delton) or by ultraviolet light (Nuva-Seal) is being compared. Statistical comparisons of the overall differences in retention scores between the two sealants were performed using the individual subject as the unit of observation. The McNemar test was used to compare the retention rates for specific tooth types.																	

## 1. Project Description

### Objective

During the past ten years sealants have been used increasingly to prevent dental decay in the pits and fissures of the occlusal surfaces of posterior teeth. Current sealants are based on the reaction product of the Bis-phenol-A and glycidyl methacrylate (Bis-GMA) system. Early clinical studies focused on the Bis-GMA system (Nuva-Seal) which uses ultraviolet light to induce polymerization. Recent clinical studies have evaluated the use of cold-cured autopolymerized Bis-GMA system (Delton and others). Employing the half-mouth design the present study compares the retention of a chemically polymerized Delton and a widely-tested Nuva-Seal system. The half-mouth design eliminates the large inter-individual variation in retention, thus providing a very efficient experimental design to evaluate side-by-side comparison of the two systems.

### Methods

In the fall of 1976, 200 children in Fairfax County, Va. (a fluoridated community), ranging in age from 5 to 16 years, were selected for participation in the study. To participate, children had to have one or more pairs of homologous permanent posterior teeth free of decay or filling on their occlusal surfaces and free of decay on all other surfaces. One half the mouth of each participant was treated with Nuva-Seal and the other half was treated with Delton. In addition sound permanent posterior teeth that had a decayed or filled homologue were treated with the adhesive designated for use on that half of the mouth. Treated teeth were examined for sealant loss approximately every six months for a period of two years. On the 6-, 12-, 18-month examinations, teeth diagnosed as having lost any or all of the sealant were retreated. A dentist made all examinations for both dental caries and sealant loss. All sealant treatments were administered by a dental hygienist.

### Major Findings

Findings showed complete retention scores for Nuva-Seal of 86% after six months and 78% at the end of two years. For Delton, the corresponding percentages of treated teeth showing fully retained sealant was 96% and 92%. For both sealants, total retention was greatest in lower first premolars and least in lower second molars, and the greatest rate of sealant loss occurred within the first six months after placement. A comparison of overall retention scores (net gain) of Delton and Nuva-Seal revealed that Delton was significantly better than Nuva-Seal at each semi-annual examination. The McNemar test was used to compare the retention of the two sealants for each tooth type. The test showed that the retention of Delton was significantly better than that of Nuva-Seal only on upper and lower first molars.

Significance

Although studies have shown that either of the Bis-GMA sealants can have good levels of retention, there is insufficient evidence to determine if one is clearly better than the other. Information on the comparative benefits of the two types of sealants will be helpful to dentists as well in estimating acceptance of these techniques in public health use.

Proposed Course

A report of these findings are being prepared for publication.

2. Publications

Li, Shou-Hua, Gladsden, Andrew, Swango, Philip and Heifetz, Stanley: Statistical evaluation of the retention of two types of pit and fissure sealants. Abstracted, IADR Program and Abstracts of papers: J. Dent. Res. 59:889, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00268 02 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Effect of eating patterns on dental caries in rodents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Edgar, William M.	Laboratory Scientist (Vis.)	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR
Amsbaugh, Suzanne M.	Laboratory Technician	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Results of studies carried out in humans have shown that the ability of plaque to lower the pH of sugar solutions can be affected by the ingestion of cheese or peanuts immediately after sucrose ingestion. The purpose of this study was to determine whether the cariogenicity of diet 2000 could be affected by offering rats snacks of cheese or peanuts between meals of their cariogenic diet. Cheese reduced the incidence of dental caries and the numbers of Strep. mutans; peanuts were without effect. Extra snacks of the cariogenic diet enhanced caries.

1. Project DescriptionObjective

To determine whether ingestion of cheese or peanuts between meals affects the cariogenicity of a diet and to determine the effect of this regimen on the implantation of S. mutans.

Methods

Four groups of 10 Osborne Mendel rats were housed in a Konig Hofer programmed feeder and were offered the following sequence of meals:

Group A	Diet 2000	22 meals daily
Group B	Diet 2000	34 meals daily
Group C	Diet 2000	22 meals daily + 12 meals of cheese daily
Group D	Diet 2000	22 meals daily + 12 meals of peanuts daily

All animals were inoculated per os and in the drinking water at the beginning of the experiment with S. mutans 6715/15. The investigation continued for 35 days. Saliva was collected by pilocarpine stimulation; the animals were sacrificed and the submandibular salivary glands were dissected out.

Major Findings

Caries scores ( $\pm$ SD) were as follows (smooth surface/sulcal lesions):

Group A	$5.7 \pm 5.6/21.8 \pm 4$
Group B	$11.0 \pm 5.8/31.2 \pm 4.5$
Group C	$1.1 \pm 1.2/15.3 \pm 7.9$
Group D	$6.1 \pm 1.5/20.0 \pm 7.1$

S. mutans counts were 22.5, 18.4, 6.0 and  $15.5 \times 10^5$ .

Saliva flow rates were 0.44, 0.47, 0.72, and 0.74 ml per 10 min in groups A-D respectively.

Significance

The results show that a) cheese after cariogenic food may reduce caries incidence especially in smooth surfaces, and b) peanuts although not promoting caries do not reduce cariogenicity of other foods.

Proposed Course

Manuscript being submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00269 02 CPR									
PERIOD COVERED October 1, 1979 to September 31, 1980											
TITLE OF PROJECT (80 characters or less) Effect of calcium glycerophosphate and fluoride on dental caries in rodents											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
Edgar, William M. Bowen, William H. Amsbaugh, Suzanne M.	Laboratory Scientist (Vis.) Chief, CPR Branch Laboratory Technician	<table style="width:100%; border: none;"> <tr> <td style="text-align: right;">NCP</td> <td style="text-align: right;">CPR</td> <td style="text-align: right;">NIDR</td> </tr> <tr> <td style="text-align: right;">NCP</td> <td style="text-align: right;">CPR</td> <td style="text-align: right;">NIDR</td> </tr> <tr> <td style="text-align: right;">NCP</td> <td style="text-align: right;">CPR</td> <td style="text-align: right;">NIDR</td> </tr> </table>	NCP	CPR	NIDR	NCP	CPR	NIDR	NCP	CPR	NIDR
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NCP	CPR	NIDR									
COOPERATING UNITS (if any)											
LAB/BRANCH Caries Prevention and Research											
SECTION Etiology											
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES)											
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER											
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)											
<p>There has been a growing interest in identifying compounds which enhance the cariostatic effect of fluoridated drinking water. Calcium glycerophosphate has been shown to have cariostatic properties when added to portions of a cariogenic diet fed to monkeys. In the present study suboptimal levels of fluoride in the drinking water and calcium glycerophosphate combination gave greater reductions in the incidence of caries in rats than either substance separately. Statistical analysis has revealed that this interaction is significant.</p>											



1. Project Description

Objective:

This study was carried out to determine whether the cariostatic effect of fluoride in drinking water could be enhanced by the inclusion of 1% calcium glycerophosphate in the diet.

Methods:

Four groups of 18 Osborne Mendel rats were treated as follows:

A	Diet 2000	+ deionized water
B	Diet 2000	+ 2.5 ppm fluoride solution
C	Diet 2000 + 1% CaGP	+ deionized water
D	Diet 2000 + 1% CaGP	+ 2.5 ppm fluoride solution

All animals were infected with S. mutans 6715-15 at the beginning of the experiment. Both diet and drinking water were available ad libitum. The experiment continued for 35 days. Caries scores and S. mutans counts were carried out at termination of the experiment.

Major Findings:

The animals receiving the combination of calcium glycerophosphate and sodium fluoride had significantly fewer smooth surface lesions than did control groups. Differences in the number of the sulcal lesions were not significant. The treatments were without effect on the populations of Strep. mutans. Weight gains in the animals were comparable.

Significance:

It appears that a method to enhance the effect of fluoridated drinking water has been identified which could have considerable clinical benefits.

Proposed Course:

A study of the effects of a combination of CaGP and NaF in humans is being planned.

Paper has been submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00271 02 CPR															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Effect of interaction between amine derivatives and chlorhexidine on rat caries																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table data-bbox="120 445 1347 546"> <tr> <td>Emilson, Claes-Göran</td> <td>Laboratory Scientist (Vis.)</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Ciardi, Joseph E.</td> <td>Laboratory Scientist</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Emilson, Claes-Göran	Laboratory Scientist (Vis.)	NCP	CPR	NIDR	Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR	Ciardi, Joseph E.	Laboratory Scientist	NCP	CPR	NIDR
Emilson, Claes-Göran	Laboratory Scientist (Vis.)	NCP	CPR	NIDR													
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR													
Ciardi, Joseph E.	Laboratory Scientist	NCP	CPR	NIDR													
COOPERATING UNITS (if any)																	
LAB/BRANCH Caries Prevention and Research																	
SECTION Etiology																	
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords)  This study was designed to determine the effect of adding basic amino acids lysine and arginine to chlorhexidine and cetyl pyridinium chloride on the cariostatic effects of both agents. Topical application of chlorhexidine effectively prevented caries and plaque formation. Cetyl pyridinium chloride was without effect nor did the addition of the amino acids enhance the effects of either antiseptic.																	

## 1. Project Description

### Objectives

Quaternary ammonium compounds, and bisbiguanides are potent inhibitors of glucosyltransferase from S. mutans in vitro. In addition we have observed that the inclusion of lysine or arginine enhanced the inhibiting effect.

### Methods

Seven groups of 12 Osborne Mendel rats were used in each study. They were fed Diet 2000 ad libitum except for 30 minutes after each topical treatment. All animals were infected with streptomycin-resistant strain S. mutans 6715-15 and Actinomyces viscosus T-6.

The animals received 0.2 ml of the rinse once or twice daily 5 days a week for 5 weeks. The rinses contained 0.2% chlorhexidine with or without lysine (0.2m): 0.2% CPC with and without 0.5ml lysine and 0.5 ml arginine. Distilled water, amino acids solution and 1% (CH) were used as positive and negative controls.

### Major Findings

1% CH, once daily was more effective in preventing caries than was 0.2% CH once or twice daily. All other solutions were without effect. Fewer animals had stained teeth in the groups treated with CH + lysine than in other CH treated groups.

### Significance

The results show that chlorhexidine is a potent agent in the prevention of plaque and that a once-a-day treatment by 1% chlorhexidine is more effective than twice daily treatment with 0.2% solution. It is possible that the inclusion of lysine may reduce staining by chlorhexidine. CPC at the concentrations used appears to be without effect.

### Proposed Course

Agents as effective as chlorhexidine but without staining problems are being sought.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00274 02 CPR																					
PERIOD COVERED October 1, 1979 to September 30, 1980																							
TITLE OF PROJECT (80 characters or less) Role of host saliva in implantation of <u>S. mutans</u> in human dental plaque																							
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">Ciardi, Joseph E.</td> <td style="width: 33%;">Laboratory Scientist</td> <td style="width: 33%;">NCP CPR NIDR</td> </tr> <tr> <td>Olsson, Jan</td> <td>Laboratory Scientist (vis.)</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Kennedy, John</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Haller, Robert</td> <td>COSTEP (Dental)</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Reilly, John Allen</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Lee, Terry</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> </table>			Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR	Olsson, Jan	Laboratory Scientist (vis.)	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR	Kennedy, John	Laboratory Technician	NCP CPR NIDR	Haller, Robert	COSTEP (Dental)	NCP CPR NIDR	Reilly, John Allen	Laboratory Technician	NCP CPR NIDR	Lee, Terry	Laboratory Technician	NCP CPR NIDR
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Lee, Terry	Laboratory Technician	NCP CPR NIDR																					
COOPERATING UNITS (if any) Dr. Claes-Goran Emilson, University of Goteborg, Sweden																							
LAB/BRANCH Caries Prevention and Research Branch																							
SECTION Etiology																							
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD																							
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SUMMARY OF WORK (200 words or less - underline keywords)  The ability of exogenous <u>S. mutans</u> to implant and be retained in dental plaque and saliva varied considerably among eight human subjects. To determine whether a correlation might exist between host saliva and <u>S. mutans</u> implantation, the effects of the different salivas on the properties of bacterial growth, aggregation, adsorption to hydroxylapatite (HA) surfaces and sucrose mediated adherence of cells to glass were assessed. Significant differences in growth and sucrose mediated adherence were not seen with the salivas tested. The degree of aggregation and the amount of adsorption to hydroxylapatite of <u>S. mutans</u> differed for all salivas tested. Salivas from 2 of 3 subjects that resisted implantation of <u>S. mutans</u> appeared to show greatest aggregation activity and to support the adsorption of the greatest number of bacteria to hydroxylapatite. All experiments have been repeated and the results are being analyzed.																							

1. Project Description

Objectives:

The ability of exogenous S. mutans to implant and be retained in dental plaque varies considerably among human subjects. Because of differences in susceptibility to implantation by S. mutans strains Ingbritt (serotype c) and OMZ-65 (serotype d/g), test subjects were divided into three groups: 1) Highly susceptible; 2) Moderately susceptible; and 3) Not susceptible. The groups contained 3, 2, and 3 subjects, respectively. The present study attempted to determine whether a significant correlation exists between host saliva and the extent of S. mutans implantation.

Methods:

Saliva was collected over ice and stored frozen. Just before use it was thawed, clarified by centrifugation at 13,000xg, and stored over ice. pH was measured and in some experiments adjusted. Protein content of clarified saliva was estimated by measuring absorbance at 280nm and using bovine serum albumin as standard.

The effect of saliva on the following activities was measured:

1. Adsorption of radioactive S. mutans cells to hydroxylapatite.
2. The rate of aggregation of S. mutans cells determined by changes in absorbance at 700nm.
3. Growth, acid production and sucrose-mediated adherence of S. mutans. A rapid, quantitative in vitro assay has been developed in our laboratory that measures the effects of chemical and biological agents on growth and sucrose-mediated adherence of radioactive S. mutans. Acid production is measured in the same assay, by change in pH. (Ciardi, J.E., Rosenthal, A.B., and Bowen, W.H., J. Dent. Res. 58:IADR Abs. 630, 1979)

Major Findings:

Earlier studies indicated that sucrose-mediated adherence to glass surfaces of growing cells of strain Ingbritt (serotype c) was reduced by less than 15% by either pretreating the glass with pooled human saliva or adding up to 48% saliva to the assay system. Adherence of OMZ-65 (serotype d/g) cells was unaffected by saliva. In the present study, in which the salivas from

eight human subjects were compared, saliva was added to the assay system to a concentration of 12 and 25%, growth and sucrose-mediated adherence were measured. The eight saliva samples did not cause significant differences in adherence of either Ingbritt or OMZ-65 cells. In most cases saliva caused an enhancement of growth and a higher final culture pH, probably due to its buffering capacity in the growing system. All of the human salivas aggregated Ingbritt cells; OMZ-65 cells were not aggregated. The extent of aggregation of Ingbritt as determined by the sedimentation rate of the cells or by the highest dilution of saliva that caused aggregation differed among the test salivas. The results suggest that the salivas from humans (2 of 3) that resist S. mutans implantation cause greatest aggregation of Ingbritt cells and those from humans easily implanted (2 of 3) cause least aggregation. Adsorption of both Ingbritt and OMZ-65 cells to hydroxyapatite (HA) was greatly reduced by pretreatment of the HA with human saliva. However, human saliva, in general, supported adsorption of more Ingbritt than OMZ-65 cells. Adsorption patterns of either Ingbritt or OMZ-65 cells varied among the test salivas. The results suggest that the salivas from humans (2 of 3) that resist S. mutans implantation allow the greatest number of either Ingbritt or OMZ-65 cells to adsorb to HA and that those from humans easily implanted support the lowest number of bacteria adsorbed. Protein patterns after polyacrylamide electrophoresis of specific saliva samples before and after adsorption to HA were essentially the same for each saliva; however, protein patterns differed among the salivas from the eight human subjects. Dilutions of saliva as great as 1:8 used in the adsorption assay caused similar number of S. mutans to adsorb. All experiments have been repeated with fresh human whole and parotid saliva and the latest results have yet to be analyzed. Salivas from the eight human subjects were also characterized with respect to pH values, protein concentration, carbohydrate concentration, antibodies to glucosyltransferase and lipoteichoic acid, lysozyme activity and lactoperoxidase activity. All results are being statistically evaluated.

#### Significance:

In vitro assays that show a relationship between human saliva and oral implantation of specific pathogens, such as S. mutans, might be useful in helping to predict future caries activity in a human host. A more thorough understanding of the influence of host saliva, or specific factors in host saliva, on the ability of exogenous cariogenic bacteria to colonize dental plaque could lead to the formulation of more effective cariostatic agents to prevent caries.

#### Proposed Course:

The effect of host saliva on bacterial growth, acid production, aggregation and ability to adhere to surfaces has been assessed. All experiments have been repeated using fresh saliva samples and the results are being analyzed. A more thorough evaluation of the usefulness of the in vitro assays to determine specific host and microbial factors in colonization of oral bacteria will be carried out.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00277-01    CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Prevalence of dental caries and dental fluorosis in areas with optimal and above optimal concentrations of fluoride in their water supplies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR
Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR
Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR
Kingman, Albert	Statistician	NCP	CPR	NIDR

COOPERATING UNITS (if any)  
Division of Dental Health, Illinois Department of Dental Health and Eugene R. Zimmerman, Baylor College of Dentistry

LAB/BRANCH  
Caries Prevention and Research Branch

SECTION  
Community Programs

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1980, a cross-sectional survey to measure the prevalence of dental caries and dental fluorosis was conducted in several study sites served by public water supplies that contained natural fluorides at approximately the optimum recommended concentration and at two, three and four times the optimum. Only children who were continuous residents since birth at each site and who used the public water supply as their primary source of drinking water were included in the survey. About 800 children, ages 8-15 years, were examined. Dental caries was assessed with the DMF surface index and dental fluorosis was measured traditionally with Dean's Index and with a newly developed tooth surface index of fluorosis (TSIF). Fluorosis was assessed independently in each child by each index. In addition, color photographs were taken to depict varying degrees of fluorosis.

## 1. Project Description

### Objective:

To compare dental caries experience and the prevalence and severity of dental fluorosis among children who have consumed continually, since birth, drinking water containing fluorides at about two, three and four times the recommended optimum with corresponding findings for children who have consumed optimally fluoridated water.

### Methods:

In 1980, a cross-sectional survey of the prevalence of dental caries and dental fluorosis was conducted in seven communities in Illinois served by public water supplies that contained natural fluorides; the fluoride concentrations varied from the optimum level recommended for water fluoridation in the specific geographic area to two, three and four times the optimum. Only children with histories of continuous residence since birth and use of the public water supply as the major source of drinking water were included in the study. A total of about 800 children in grades 3 through 10 (ages 8-15) were examined. The DMF surface index was used to quantify dental caries experience. Dental fluorosis was traditionally assessed with the Dean's Index and a newly developed tooth surface index of fluorosis (TSIF). Each index was applied independently to the entire study population.

### Major Findings:

The data are currently being tabulated.

### Significance:

In 1975, the U.S. Environmental Protection Agency was given responsibility for enforcing drinking water standards for the United States. These standards include maximum contaminant levels of fluoride. However, there is little current data on what fluoride concentration in water is required to produce an adverse effect on tooth structure. Moreover, the fluorosis-threshold concentration of fluoride must be considered in terms of any concomitant increase in caries-preventive effect, i.e., a risk to benefit determination. The present study seeks to redefine the relation between fluoride concentration in drinking water with respect to dental fluorosis and dental caries in terms of today's conditions of fluoride ingestion. The findings may have important dental health and economic consequences for the more than 700 communities in the United States with fluoride in their water supplies at concentrations greater than twice the optimal.

### Proposed Course:

All clinical field activities in Illinois have been completed. However, because of smaller than expected sample sizes obtained at some of the study sites, it may be necessary to identify additional communities for inclusion in the study.

2. Publications

As soon as the data have been tabulated and analyzed, a report will be prepared.



PERIOD COVERED  
 October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
 Induction of secretory immunity against Streptococcus mutans in human subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Ciardi, Joseph	Laboratory Scientist	NCP CPR NIDR
Emilson, Claes-Goran	Laboratory Scientist (vis.)	NCP CPR NIDR
Mirth, Dale	Laboratory Scientist	NCP CPR NIDR
Robrlish, Stanley	Laboratory Scientist	NCP CPR NIDR
Shern, Roald	Laboratory Scientist	NCP CPR NIDR
Stiles, Horace M.	Chief, E Section	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
 Caries Prevention and Research

SECTION  
 Preventive Methods Development

INSTITUTE AND LOCATION  
 NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eight human volunteers were immunized with S. mutans strain OMZ 65 (serotype d/g) by swallowing 100mg of formalin killed bacteria in capsules for three successive days. After a period, immunization was repeated except that capsules were ingested on seven successive days. Samples of blood, whole and parotid saliva and tears were collected throughout the experiment and assayed for antibodies to OMZ 65 by a fluorescein linked immunoabsorbant assay. Antibody in the IgA class reactive with OMZ 65 was induced in whole and parotid saliva by immunization.

1. Project Description

Objective:

The purposes of this study were to determine whether local immunization via the small intestine would result in a salivary antibody response and whether such antibody would affect implantation and colonization with the homologous bacterium.

Methods:

Samples of whole and parotid saliva, blood and tears were collected from eight human volunteers to determine baseline levels of anti S. mutans antibody. Following baseline sampling, the subjects were immunized by ingesting capsules containing 100mg of formalin killed, freeze dried OMZ 65 cells for three successive days. Later the immunization was repeated except that it was conducted for seven days. Antibody activity in whole and parotid saliva, serum and tears was determined by a fluorescein linked immunoabsorbant assay.

Major Findings:

Immunization induced SIgA antibody in whole and parotid saliva and IgM and IgG antibody in serum.

Significance:

Intraintestinal immunization results in the induction of sIgA antibody in parotid and whole saliva and low levels of IgM and IgG antibody in serum. the salivary IgA antibody appears to effect a reduction in implantation and colonization with the homologous bacterium. This route of immunization may be a safe and effective method for protection against dental caries.

Proposed Course:

1. Study the duration of antibody synthesis resulting from intragastric immunization.
2. Study the effect of intragastric immunization on the incidence of dental caries in human populations.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00279 01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Effect of secretory immunity Against S. mutans on its colonization in human subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Emilson, Claes-Goran	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Robrish, Stanley	Laboratory Scientist	NCP CPR NIDR
Shern, Roald J.	Laboratory Scientist	NCP CPR NIDR
Stiles, Horace M.	Chief, E Section	NCP CPR NIDR
Thomson, Lynn A.	Laboratory Scientist	NCP CPR NIDR
Gomez, Irma	Laboratory Technician	NCP CPR NIDR
Kemp, Christopher	Laboratory Technician	NCP CPR NIDR
Ciardi, Joseph	Laboratory Scientist	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Preventive Methods Development

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

- (a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER
- (a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eight human volunteers were infected with S. mutans strains Ingbritt (serotype c) and OMZ 65 (serotype d/g) which were resistant to streptomycin; the level of implantation and duration of colonization were monitored. After both serotypes were shed from the mouth, the subjects were immunized against OMZ 65 by swallowing 100mg of formalin killed bacteria in capsules for three successive days and the subjects were reinfected with strains Ingbritt and OMZ 65. The level of implantation and duration of colonization were again monitored. After the bacteria were shed the immunization and implantation cycle was repeated except that capsules were ingested on seven successive days. The presence of antibody was accompanied by reduced implantation and colonization of OMZ 65.

1. Project Description

Objective:

The purposes of this study were to determine whether local immunization via the small intestine would result in a salivary antibody response and whether such antibody would affect implantation and colonization with the homologous bacterium.

Methods:

Samples of whole saliva and dental plaque were collected from eight human volunteers to determine baseline levels of S. mutans. Following baseline sampling, the teeth were cleaned and the subjects infected with streptomycin resistant S. mutans strains Ingbritt and OMZ 65. Colonization was monitored by plating plaque and saliva samples onto Mitis Salivarius agar containing streptomycin. After the implanted bacteria were shed the subjects were immunized by ingesting capsules containing 100mg of formalin killed, freeze dried OMZ 65 for three successive days and implantation repeated. Colonization was then repeated as before and monitored until the bacteria could no longer be detected. The immunization and implantation cycle was then repeated except that immunization was conducted for seven days.

Major Findings:

S. mutans strain OMZ 65 implanted at higher numbers and colonized for longer than strain Ingbritt.

Following immunization both strains implanted at a lower level and colonized for a shorter period of time.

Significance:

Intraintestinal immunization results in the induction of sIgA antibody in parotid and whole saliva and low levels of IgM and IgG antibody in serum. The salivary IgA antibody appears to effect a reduction in implantation and colonization with the homologous bacterium. This route of immunization may be a safe and effective method for protection against dental caries.

Proposed Course:

Study the mechanism/s by which antibody affects a reduction in implantation and colonization.





1. Project Description:

Objectives:

The purpose of this study was to develop a sensitive simple assay to determine antibacterial antibodies in secretions and serum.

Methods:

Double sided cellulose acetate discs ( 7.00mm in diameter) were coated with formalin killed Streptococcus mutans. Checkerboard titrations were performed in order to determine the optimum concentrations of bacteria for coating, and optimum dilutions of sample and fluorescein conjugated antiglobulin reagent.

The double sided cellulose acetate discs coated with bacteria on one side were incubated in 600 $\mu$ l of diluted sample for 30 minutes in 12x75mm glass tubes at ambient temperature with shaking. The discs were then removed to a second tube containing 600  $\mu$ l of wash for 10 minutes. The disc was then incubated for 20 minutes in diluted fluorescein conjugated antiglobulin reagent and finally washed for 10 minutes in a fourth tube. The double sided disc was then inserted in the stage of a committed fluorimeter. Fluorescence of the coated and uncoated sides of the disc were recorded and specific fluorescence determined by subtracting the fluorescence of the uncoated side from the fluorescence on the coated side.

Significance:

Comparison of the fluorescein linked immunoabsorbant assay (FLISA) with the enzyme linked immunoabsorbant assay (ELISA) for measurement of antibody to S. mutans in saliva and serum yielded identical results. This indicates that FLISA is a sensitive and reproducible assay which can be completed in approximately one hour.

Proposed Course:

1. Apply FLISA assay to the measurement of salivary antibodies in human populations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00281 01 CPR
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PERIOD COVERED  
October, 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
  
Analysis of oral fluids using high performance liquid chromatography (HPLC)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Preventive Methods Development

INSTITUTE AND LOCATION  
NIDR, NIH Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline !)

Recent advances in high performance liquid chromatography (HPLC) column technology have made it possible to rapidly analyze protein samples using HPLC. The present study has shown that HPLC can be used to monitor protein purification schemes, to analyze commercial proteins such as secretory - IgA (S-IgA) and lactoferrin for purity, and to obtain comparative protein profiles from saliva and plaque fluid. Preliminary results suggest that HPLC can also be used to quantitate constituents of saliva such as S-IgA.

1. Project Description

Objective:

High performance liquid chromatography is being evaluated as a means of analyzing oral fluids such as saliva and plaque and monitoring the purification of proteins of interest to caries research.

Methods:

Samples were run on a Waters Associates HPLC system using Waters' Protein-I125 columns. Buffers compatible with proteins such as Tris/sodium sulfate and sodium phosphate were used as the mobile phase.

Major Findings:

HPLC can be used to check the purity of proteins such as S-IgA and lactoferrin.

Saliva and plaque fluid can be resolved into 10 or more protein bands using HPLC, allowing comparisons of the protein profiles of different samples. However, the Protein-I125 columns do lose resolving power with repeated use.

Preliminary results suggest that HPLC can be used to quantitate S-IgA in saliva.

Significance:

Results have shown that HPLC can provide rapid analysis of protein samples, indicating that HPLC will be useful for monitoring the purification of proteins of interest to caries research such as immunoglobulins and salivary proteins. Initial results suggest that HPLC will also be useful for quantifying certain salivary proteins and for rapidly obtaining protein profiles of saliva and plaque fluids.

Proposed Course:

Columns with larger pore diameters will be investigated for improving the resolution of high molecular weight (>100,000) proteins. The feasibility of using HPLC to purify small quantities of protein will also be investigated.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00282-01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Anticaries evaluation of an intraoral fluoride-releasing device in rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Mirth, Dale B.	Laboratory Scientist	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR
Adderly, Donna D.	Laboratory Technician	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Preventive Methods Development

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous in vitro and in vivo studies have shown that an intraoral fluoride releasing device developed by the Southern Research Institute for the National Caries Program will deliver fluoride at a steady rate for up to six months. This study will evaluate the anticaries effect of the fluoride-releasing device in rats.

1. Project Description

Objective:

The anticaries effect of an intraoral device for the controlled release of fluoride will be evaluated in the rat.

Methods:

Standard NIH techniques for the evaluation of anticaries substances in rats will be used in this study. Animals will be maintained on Diet 2000 and the caries scored by the method of Keyes. The fluoride-releasing devices will be sutured inside the mouth of the rat using a nylon or stainless steel suture running through the center of the device. The anticaries effect of the fluoride-releasing device will be compared to results from placebo device treated, untreated, and fluoridated-water treated groups.

Major Findings:

Attachment studies have shown that the fluoride-releasing devices can be secured in a rat's mouth by running a thin nylon thread or stainless steel wire through the center of the device and threading the ends through the cheek of the rat from the inside out, and tying off the suture on the outside. A 50 percent device retention rate after five weeks was achieved using this method.

Significance:

This is the first study to evaluate the anticaries effectiveness of a new controlled release fluoride delivery system and to compare the efficacy of this delivery method to the anticaries efficacy of fluoride delivered through the drinking water. The results of this study will make it possible to more accurately estimate the feasibility of using the intraoral fluoride delivery system to control caries.

Proposed Course:

A caries trial will be carried out in rats using a fluoride releasing device designed to release 0.1mg of fluoride per day.

2. Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00292 01 CPR
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Analysis of the National Dental Caries Prevalence Survey		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
Brunelle, Janet Ann Miller, Ann J. Kingman, Albert Rodgers, Patricia Ann	Chief, B Section Project Scientist Statistician Statistical Assistant	NCP    CPR    NIDR NCP    CRGC    NIDR NCP    CPR    NIDR NCP    CPR    NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH Caries Prevention and Research		
SECTION Biometry		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>           A nationwide survey to assess the prevalence of dental caries throughout the United States was designed and implemented during the 1979-80 school year. A probability sample of school-aged children in grades kindergarten through twelve was selected for examination for dental caries, gingivitis, and need for dental treatment. Estimates of disease level and treatment need will be made for the continental U.S. and each of seven geographic regions by age, race and sex.         </p>		



## 1. Project Description

### Objective

A nationwide survey of school-aged children was designed to assess the prevalence of dental caries in the United States. This survey will establish current disease levels and serve as a baseline for future surveys. It has been designed so that follow-up surveys will be able to: a) detect changes in caries experience by region; b) monitor changes produced by the implementation of new caries preventive measures and c) target preventive research strategies for high risk age groups.

### Methods

A probability sample of school-aged children in grades kindergarten through twelve was designed. The continental U.S. was divided into seven geographic regions. Within each region five SMSA (Standard Metropolitan Statistical Area) and five non-SMSA's were selected. The primary sampling unit was the school district or contiguous school districts. All school districts both public and private were included in the sample. A sample of at least 1820 classrooms equally allocated among the 14 regions and grade levels was required. Visual tactile dental examinations were performed during the 1979-80 school year using the diagnostic criteria for decayed, missing and filled surfaces (DMFS), a gingival index and an index of dental treatment need. All data are being processed and analyzed by the Biometry Section. Estimates of dental caries prevalence and need for dental treatment will be made for the continental U.S. and each of seven regions by age, sex and race.

### Significance

The results of this survey will establish the disease level of dental caries in the U.S. and selected regions for school-aged children. It will also act as a baseline for future surveys to enable the National Caries Program to measure outcomes of preventive efforts.

### Proposed Course

Analysis of the data will be performed and detailed reports published.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00293 01 CPR															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Inter and intra examiner reliability levels for scoring smooth surface caries in rats																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 40%;">Kingman, Albert</td> <td style="width: 30%;">Statistician</td> <td style="width: 10%;">NCP</td> <td style="width: 10%;">CPR</td> <td style="width: 10%;">NIDR</td> </tr> <tr> <td>Shern, Roald J.</td> <td>Laboratory Scientist</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Amsbaugh, Suzanne M.</td> <td>Laboratory Technician</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Kingman, Albert	Statistician	NCP	CPR	NIDR	Shern, Roald J.	Laboratory Scientist	NCP	CPR	NIDR	Amsbaugh, Suzanne M.	Laboratory Technician	NCP	CPR	NIDR
Kingman, Albert	Statistician	NCP	CPR	NIDR													
Shern, Roald J.	Laboratory Scientist	NCP	CPR	NIDR													
Amsbaugh, Suzanne M.	Laboratory Technician	NCP	CPR	NIDR													
COOPERATING UNITS (if any)																	
LAB/BRANCH Caries Prevention and Research																	
SECTION Biometry																	
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) <p>There are many factors that can influence the outcome of a caries experiment in animals. Included in these are the age and physical health of the animal, physical conditions of the animal housing facilities, types of treatments being tested, method used in scoring dental caries, and the experience and expertise of the person scoring caries in these animals.</p> <p>In this investigation the effects of the scoring method and reproducibility of the examiner using each method were studied to see how significant a role these factors play in such animal experiments.</p> <p>The original method of Keyes and a modification of this method were compared in two separate studies at NIDR. Experienced examiners participated in each study and replicate examinations of the smooth surfaces of the animal jaws were conducted.</p> <p>In each study the results showed the examiners were able to achieve high values of reproducibility using either method in scoring dental caries in rats. Differences between groups were slightly different for the two methods, but with no consistent pattern and of no practical significance.</p>																	

## 1. Project Description

### Objective

The purpose of this study is to investigate the reliability level that could be achieved by experienced examiners when scoring smooth surface caries in rats by the Keyes method or a suggested modification. The relative role of each method in comparing treatments will be assessed. Examiner reliabilities for each method also will be compared.

### Methods

Two studies were conducted at the NIDR during 1977 and 1978 involving repeated evaluation of the rat dentitions for smooth surface caries. In the first study one examiner scored the dentitions repeatedly. In the second study two examiners scored the rat dentitions.

The intraclass correlation coefficient was used to assess examiner reliability.

### Findings

In both studies the results showed the examiners were able to achieve high reliability levels when scoring smooth surface caries. Statistically significant differences between scoring methods were detected in one study but these differences accounted for less than 1% of the total variation in the caries scores. In both studies caries scores between groups were slightly different when scored dry than when scored using the moist technique as suggested by Keyes.

### Significance

Variation in caries scores is inherent to any animal study. The magnitude of the component of variation attributable to examiner inconsistency in scoring caries in the animals was shown in both studies to be of little consequence when compared to the total variation among the caries scores.

It was also shown that experienced examiners could achieve high reliability levels when scoring smooth surface caries using either method. Therefore, either method is reasonable to use when scoring dental caries in rats.

## 2. Publications

The results of this study were presented at the Animal Models Workshop in Sturbridge, MA. on April 22, 1980. The Proceedings of this workshop will be published in late 1980 or early 1981.





1. Project Description

Objective

The purpose of this study is to develop a new method of assessing the effects of prophylactic treatments when tested in a longitudinal clinical trial. The envisioned method would enable the investigator to assess treatment effects in terms of percentage changes observed among the proportions of subjects who fall in distinct dental caries severity categories.

Methods

Each subject participating in a longitudinal trial is examined initially and at least once thereafter for the existence of dental caries by using the DMFS index. Based on the findings at each examination the subject is then classified into one of six severity categories by using the MGSI index. The subjects in each group are then cast into a two-way frequency table representing their joint category classification at the initial exam and for the subsequent examination under consideration. Markov chain methodology is then used to obtain the eventual relative frequency distribution for subjects in the distinct severity categories for each group. The resulting probability distributions are then compared to assess differences among groups. Monte Carlo studies will then be performed to compare the sensitivity of this method with the ANOVA method usually employed in this setting.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00295-01 CPR
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) The effect of fluoride pulse on <u>Streptococcus mutans</u> in continuous culture		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
Kemp, Christopher W. Robrish, Stanley A. Sharer, Sue A. Bowen, William H.	Laboratory Technician Laboratory Scientist Laboratory Assistant Chief, CPR Branch	NCP CPR NIDR NCP CPR NIDR NCP CPR NIDR NCP CPR NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH Caries Prevention and Research		
SECTION Etiology		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS	<input type="checkbox"/> (b) HUMAN TISSUES	<input type="checkbox"/> (c) NEITHER
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>A pure culture of <u>S. mutans</u> was allowed to reach a steady state in a continuous culture (chemostat). The culture was then pulsed with a mixture containing sodium fluoride and <sup>14</sup>C labeled glucose. The pulse was applied after the culture had reached a steady state at a low and high growth rate for pH values of 7.0, 6.2 and 5.4. Samples were obtained before the pulse and at timed intervals after the pulse. The samples have been analyzed for fluoride control, residual glucose lactate, and <sup>14</sup>C label incorporation. The samples will also be processed for dry weight determination, ATP and fermentation product analysis.</p>		



## 1. Project Description

### Objectives:

This project will provide data concerning the effect of a fluoride pulse on the growth and metabolism of S. mutans under varying environmental conditions. Previous investigations have studied the effects of fluoride mainly on cell preparations. This project is designed to measure the fluoride effect on cells in a steady state culture. This design will minimize any artifacts induced through the manipulation of cells.

### Methods:

S. mutans 6715-15 was grown in the chemostat in carbon source (glucose) limitation. The culture was grown at a low and high growth rate at pH values of 7.0, 6.2 and 5.4. After reaching a steady state, the culture was pulsed with a mixture containing 60-80 ppm NaF (final concentration) and  $^{14}\text{C}$  glucose (100,000 cpm final activity). Samples were obtained at 0", 1", 5", 10", 30", 60" and 120" after the pulse.

The samples collected at the two growth rates and corresponding pH values were analyzed to determine the effect of the pulse on growth and metabolism of the cells. Glucose uptake was measured using a scintillation counter to obtain a differential count from aliquots of whole cells and filtrates. Aliquots of sample filtrates will be prepared for fermentation product analysis using the gas chromatograph. Lactate production was measured by flurometric analysis. Fluoride in the culture filtrates was measured with the fluoride electrode and the data compared to theoretical washout curves to determine if significant uptake of the ion by the culture vessel existed. Dry weight measurements are being obtained using the protein method of Robrish et al. The effect of the pulse on the ATP pool of the cell population is being determined by the firefly luciferase analysis.

### Major Findings

The results from the  $^{14}\text{C}$  incorporation experiments reveal that fluoride was most effective on rapidly growing cells at a low pH. At pH 7.0 and 5.4 the fluoride effect was influenced by pH and growth rate. However, at pH 6.2 the effect was independent of growth rate.

Analysis of fluoride in the culture filtrates indicate no incorporation of fluoride by the culture vessel at both growth rates and the three pH values.

Significance:

This investigation will lead to a better understanding of the effect of fluoride on cell growth and metabolism. The chemostat offers a significant advance in experimental design because it is a biologically open system and, therefore, similar to the oral cavity.

Proposed Course:

Experiments of this type will be expanded to include other oral bacteria or mixtures of bacteria. This experimental design may also be used to study the effects of other antimicrobial agents on the growth and metabolism of oral bacteria.

2. Publications:

Kemp, C.W., Robrish, S.A., Sharer, S.A. and Bowen, W.H. The effect of a fluoride pulse on Streptococcus mutans in continuous culture. In preparation. 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00296-01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Effect of immunization with Actinomyces viscosus T-6 on colonization in the rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Olsson, Jan S.	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Four groups of noninfected rats, 12 in each group, were injected either intraperitoneally or in salivary glands with either formalin killed Actinomyces viscosus plus Freund's adjuvant or just Freund's adjuvant. The procedure was repeated twice in 14 day intervals. The animals were then grouped so that each cage contained one immunized animal, one sham immunized animal and a third animal which had previously been infected with Actinomyces viscosus (T-6). The rate of implantation in the non-infected animals will be monitored and the level of antibodies against T-6 in serum and saliva will be determined.



1. Project Description

Objective:

The purpose of this study is to examine the influence of immunization by two different routes on oral colonization by Actinomyces viscosus in the rat.

Methods:

Forty-eight rats were assigned to four groups, two test and two control groups so that each animal in a test group would have a littermate of the same sex and weight in the control group. The animals in the test groups were injected either intraperitoneally (IP) or in salivary glands with formalin killed Actinomyces viscosus T-6 in Freund's adjuvant three times in 14 day intervals. The control groups were injected similarly but without bacteria. Included in the experiment is also a fifth group of rats which were inoculated with Actinomyces immediately. These animals will later serve as donors of infection. After the immunization period each pair of experimental animals will be placed together with one infected animal. All animals will from now on receive Diet 2000 and will be swabbed by cotton tipped applicators for FA staining. When animals are sacrificed one lower jaw will be extracted, sonicated in saline and dilutions will be plated on blood agar. Blood and saliva will be assayed for antibodies using the ELISA technique.

Major Findings:

Experimental results are being evaluated.

Significance:

Actinomyces species have been implicated both in caries and periodontal disease. This study should provide data on the level of antibody against Actinomyces in saliva and serum after immunization and on the influence this might have on early colonization.

Proposed Course:

The study will continue as suggested above.

PROJECT NUMBER (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00297-01 CPR
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PERIOD COVERED  
 October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
 The effect of dietary factors on implantation of Actinomyces viscosus T-6 in the rat.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Olsson, Jan S.	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
 Caries Prevention and Research

SECTION  
 Etiology

INSTITUTE AND LOCATION  
 NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                     
  (b) HUMAN TISSUES                     
  (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Four groups of rats, ten rats in each group, being maintained on the feeding machine, were served 17 meals per day of either sucrose, glucose, starch or casein. All rats were given essential nutrients by gastric intubation. At the onset of the experiment the rats were inoculated with A. viscosus T-6 and the level of infection was monitored by FA and culturing techniques at least once every week. In all three carbohydrate groups the number of Actinomyces increased to about 50% of the total flora cultivable on blood agar. Whereas, in the casein group the prevalence of Actinomyces was not detectable after 14 days.

1. Project Description

Objectives:

The purpose of this study is to examine the influence of four dietary regimens on implantation of Actinomyces viscosus T-6 in the rat.

Methods;

Four groups of Osborne-Mendel rats, 10 rats in each group, from the NIH breeding colony, were served 17 meals per day by a programmed feeder of either sucrose, glucose, starch or casein. In addition, all rats were given essential nutrients by gastric intubation. At the beginning of the experiment the rats were inoculated with A. viscosus T-6. At least once every week the rats were swabbed by means of a cotton-tipped applicator. After sonication the sample was serially diluted and plated on blood agar. Portions of the sample were stained using an FA technique.

Major Findings:

Especially in the early phase of the experiment it was difficult to recover Actinomyces on the blood agar plates. This seemed to be caused by inhibition by other bacteria growing on the same plate. The inhibition of Actinomyces did not seem to occur in vivo in the rats since the FA count could reveal high numbers.

In all three carbohydrate groups the number of Actinomyces increased to about 50% of the total cultivable flora on blood sugar, whereas, in the casein group the prevalence of Actinomyces was not detectable after 14 days.

Significance to Program:

Actinomyces species are highly prevalent in most mammals and have been associated with dental caries increasingly in recent years. Studies concerning the effect of various dietary components on Actinomyces colonization have been carried out; however, we feel that more definitive results can be obtained by combining programmed feeding of test diet with gastric intubation of essential nutrients.

Proposed Course:

The study will be finished by processing and summarizing the data.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00298-01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Effect on cariogenicity and saliva composition of a suboptimal diet in rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ericson Thorild	Laboratory Scientist (vis.)	NCP CPR NIDR
Johansson, Ingegerd	Guest Worker	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Amsbaugh, Suzanne M.	Laboratory Technician	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)  
Dept. of Cariology and Laboratory of Biochemistry, Univ. of Umea, Sweden

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Some properties of saliva such as buffer capacity, secretion rate, anti-bacterial activities and effects on bacterial colonization are suggested to be of importance for the development of dental caries. Such properties would be dependant upon the biosynthesis and secretion of substances from the salivary glands. The aim of this study is to investigate the effect of malnutrition on the composition of whole saliva and on incidence of caries in rats fed sucrose. Twenty rats were fed nutritionally adequate diets by gastric intubation. Ten of these received a supplement of sucrose and 10 of starch. Another 20 rats were fed the basic diet diluted with an equal volume of water; ten were supplemented with sucrose and with starch. The supplements are distributed 17 times daily. Caries will be scored and the effect of the reduced diet on some antibacterial systems in saliva will be measured.

1. Project Description:

Objectives:

The aim of the study is to investigate in rats the effect of malnutrition on the composition of whole saliva and on the cariogenicity of sucrose.

Methods:

Four groups each of ten 21 day old Osborne Mendel rats were fed 3 ml of a liquid diet 3 times per day by gastric intubation. Groups 1 and 2 received a nutritionally adequate diet. The diet of one of the groups is supplemented with sucrose, the other one with starch. Groups 3 and 4 were fed the basic diet diluted with an equal volume of water. The diet of Group 3 is supplemented with sucrose and that of group 4 with starch. The supplements are made available to the rats in a programmed feeding machine 17 times each 24 hrs. at intervals of 30 minutes starting at 10:00 p.m. every day. Deionized water was available ad libitum. The animals were infected with S. mutans 6715-15 during three consecutive days after their arrival. The CFU of S. mutans was evaluated in oral swabbings taken on the second day after inoculation. After five weeks of intubation, whole pilocarpine isoproterenol stimulated saliva was collected under chloral hydrate anesthesia. Serum was collected from all animals and following sacrifice caries were scored. The presence of S. mutans 6715-15 will be checked on one half jaw after the termination of the experiment. The saliva will be analyzed for sIgA, LPO activity, agglutinins, transferrin, protein, amylase and lysozyme.

Major Findings:

Results are being completed. No data is available at this time.

Significance to Program:

Some properties of saliva such as immune and non-immune antibacterial factors are studied in the Program. The activity of such factors is dependent upon the biosynthesis in and secretion from the salivary glands. Several medical disorders and drugs have a strong general effect on the secretion rates of the glands but little is known about changes in saliva-composition induced through malnutrition. The present study is performed to increase our present knowledge on the effect of the nutritional quality of diet on salivary gland function in general and on caries development.

Proposed Course:

The effect of various imbalanced diets on immune and non-immune components in saliva will be studied.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00299-01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980.

TITLE OF PROJECT (80 characters or less)  
Studies on a salivary glycoprotein agglutinating serotype c strain  
of S. mutans.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Ericson, Thorild Soderstrom, Thomas Bowen, William H.	Laboratory Scientist (vis.) Guest Worker Chief, CPR Branch	NCP CPR NIDR NCP CPR NIDR NCP CPR NIDR
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COOPERATING UNITS (if any)  
Dept. Cariology and Lab. Oral Biochem., U. of Umea, Sweden, Dept. Clin.  
Immunology, U. of Goteborg, Sweden, Bureau of Biologics, FDA, Dr. Lars-Ake  
Hanson (Fogarty Scholar)

LAB/BRANCH  
Caries Prevention and Research Branch

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bacteria agglutinating glycoprotein (BAGP) from saliva is isolated from unstimulated parotid saliva collected from one donor. The isolation procedure includes 5 steps, one being affinity adsorption to the strain in a batch system. The conditions for desorption have to be carefully controlled. The resulting glycoprotein fraction is pure as judged from SDS electrophoresis in a polyacrylamide gradient under reducing conditions and by immunoelectrophoresis. Hybridoma antibodies obtained after fusion of spleen lymphocytes from NIH general purpose mice (N:GP(S)) and of SP2 mice myeloma cells, inhibit the agglutinating activity of the BAGP in the assay system. The monoclonal antibody is presently isolated from the tissue culture supernatant and from ascites fluid from nude mice injected intraperitoneally with hybridoma cells.

## Project Description

### Objectives:

The purpose of the study is to purify and characterize a salivary high molecular weight component responsible for agglutination of a human S. mutans serotype c strain.

### Methods:

Unstimulated parotid secretion is collected from one donor and absorbed with a serotype c strain of S. mutans. The microorganisms are washed with phosphate buffer. The washings are saved and centrifuged for 18 hrs at 100,000 g. The bottom fraction is chromatographed on a BioGel A5 column. The active fractions are pooled and again concentrated by high force centrifugation. Antibodies against this fraction are produced in rabbits. Hybridomas are produced by fusion of spleen lymphocytes from NIH general purpose mice (N:GP(S)) and myeloma cells from SP2 mice. Monoclonal antibodies are isolated from tissue culture supernatants of hybridoma cells and from ascites liquid of male mice.

### Major Findings:

a) The bacteria agglutinating glycoprotein (BAGP) has a molecular weight of approximately 450 K daltons as judged by SDS polyacrylamide electrophoresis in the presence of 1% mercaptoethanol.

b) The BAGP is rich in fucose and has a carbohydrate content of 30%.

c) The BAGP fraction contains no lactoferrin lysozyme or sIgA as judged from immunodiffusion and IEP.

d) Several kinds of hybridomas produce antibodies towards the BAGP. Predominantly IgG is produced but some give IgM and possibly some IgA.

e) Some hybridoma antibodies inhibit agglutination induced by the BAGP from the donor but not by parotid saliva from another individual.

f) Some monoclonal antibodies react with other glycoproteins, like Tamm Horsfall (TH) protein indicating the presence of a determinant common for several glycoproteins.

g) Monoclonal antibodies inhibiting agglutination of the S. mutans strain do not react with TH protein.

Significance to Program:

A deeper understanding of the relative role of different salivary antibacterial systems requires the possibility of quantitation of individual factors. Also, the understanding of mechanisms involved in the actions of the systems is important. To achieve both these goals purified active substances are required as well as sensitive detection measures including immunological techniques. Purified BAGP and monoclonal antibodies to it are now available to the Program.

Proposed Course:

Further characterization and testing of the effect of the BAGP on bacterial colonization and plaque morphology.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00300 01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Microbial composition of dental plaque from recently imported primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Emilson, Claes-Göran	Laboratory Scientist (Vis.)	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR
Thomson, L. Ariel	Laboratory Scientist	NCP	CPR	NIDR
Little, Wayne	Laboratory Technician	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although primates Macaca fascicularis are being used increasingly in dental research, little information is available on the composition of the microbial flora of recently captured animals. Plaque was collected from young animals and examined using highly specific fluorescent antisera. The following organisms were sought: S. mutans a-g; S. sanguis; S. salivarius; A. viscosus + naeslundii; A. odontolyticus; A. israelii; L. helveticus; L. casei; L. fermentum; L. salivarius; L. acidophilus; and B. melaninogenicus.

1. Project Description

Objective

The purpose of this study was to determine the prevalence of some common oral microorganisms in plaque from recently captured monkeys using fluorescent antibody techniques.

Methods

Animals were deprived of food for 12 hours before collection of plaque. Plaque was collected with a dental scaler from the premolars and first molar in two opposite quadrants and was dispensed by ultrasonication. Suspensions were placed on epoxy-coated slides and stained using FA conjugates specific for S. mutans (all serotypes), S. sanguis, S. salivarius, A. odontolyticus, A. israeli and 6 serogroups of Lactobacilli. For A. viscosus and A. naeslundii a polyvalent conjugate reacting with both species was used. B. melaninogenicus was detected using a commercially available antiserum. The proportion of each species was determined in randomly chosen fields containing 100 to 300 cells.

Major Findings

S. mutans was not found in any animals. S. sanguis comprised 11.8% of the total count. Actinomyces accounted 20.7% of the total count; A. viscosus and A. naeslundii were present in all animals and comprised 17.5% of the microscopic count. B. melaninogenicus comprised 2.4% and was detectable in all animals. Lactobacilli were found in a few animals only.

Significance

These observations show that animals coming from the wild do not harbor S. mutans and that the plaque flora is comparable to that found in humans.

Proposed Course

Manuscript in preparation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00301 01 CPR															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less)  Non-cariogenic sweeteners																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" data-bbox="126 473 1288 574"> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Amsbaugh, Suzanne M.</td> <td>Laboratory Technician</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> <tr> <td>Monell-Torrens, Stephen</td> <td>Laboratory Technician</td> <td>NCP</td> <td>CPR</td> <td>NIDR</td> </tr> </table>			Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR	Amsbaugh, Suzanne M.	Laboratory Technician	NCP	CPR	NIDR	Monell-Torrens, Stephen	Laboratory Technician	NCP	CPR	NIDR
Bowen, William H.	Chief, CPR Branch	NCP	CPR	NIDR													
Amsbaugh, Suzanne M.	Laboratory Technician	NCP	CPR	NIDR													
Monell-Torrens, Stephen	Laboratory Technician	NCP	CPR	NIDR													
COOPERATING UNITS (if any)																	
LAB/BRANCH Caries Prevention and Research																	
SECTION Etiology																	
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) There is a growing demand for a safe effective non-cariogenic sweetener which can be used in a large range of snack foods. The program in collaboration with industry has examined the effect of Talin, Stevioside and Aspartame on the incidence of caries in rats.																	



## 1. Project Description

### Objective

The purpose of this study was to determine the effect of the naturally occurring sweeteners, Talin and Stevioside and the synthetic sweetener Aspartame on the incidence of caries in rats. Results of other laboratories suggest that all of these materials would be safe for human use. Aspartame is a dipeptide of aspartic acid and phenylalanine. Stevioside is extracted from the leaves of *Stevia nebaudiana* and is found at a concentration of 7% in dried leaves. Talin is found in the fruit of *Thaumatococcus danielli* from which it is readily extracted.

### Methods

Nine groups of 12 rats were used in this study as follows:

- Diet 2000 control (56% sucrose)
- Diet 2114 (56% starch)--control
- Diet 2114 (56% starch) + Aspartame
- Diet 2114 (56% starch) + Stevioside
- Diet 2114 (56% starch) + Talin
  
- Diet 2142 (42% starch + 14% sucrose)--control
- Diet 2142 (42% starch + 14% sucrose) + Aspartame
- Diet 2142 (42% starch + 14% sucrose) + Stevioside
- Diet 2142 (42% starch + 14% sucrose) + Talin

All groups were infected with *S. mutans*. Experiments continued for 35 days.

### Major Findings

None of the agents tested were cariogenic. There is some evidence which suggests that some of the agents may have cariostatic properties.

No effects on the population of *Strep. mutans* was observed.

### Significance

These results show that several compounds are well developed which may be suitable substitutes for sugar.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00302 01 CPR												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (80 characters or less) The role of oral bacterial dextranases in dental plaque formation														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">Ciardi, Joseph E.</td> <td style="width: 33%;">Laboratory Scientist</td> <td style="width: 33%;">NCP CPR NIDR</td> </tr> <tr> <td>Futakami, Katsuyuki</td> <td>Laboratory Scientist (guest)</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Lee, Terry</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> </table>			Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR	Futakami, Katsuyuki	Laboratory Scientist (guest)	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR	Lee, Terry	Laboratory Technician	NCP CPR NIDR
Ciardi, Joseph E.	Laboratory Scientist	NCP CPR NIDR												
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Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR												
Lee, Terry	Laboratory Technician	NCP CPR NIDR												
COOPERATING UNITS (if any)														
LAB/BRANCH Caries Prevention and Research														
SECTION Etiology														
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD														
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  Dextranases synthesized by many different dental plaque bacteria appear to function in the formation of fermentable carbohydrates from $\alpha$ 1,6 glucans. <u>S. mutans</u> dextranase has also been implicated in the formation of glucosyltransferase-mediated insoluble glucans which appear to be necessary for optimal colonization of tooth surfaces by <u>S. mutans</u> . The purpose of the present investigation is to determine the importance of dextranases in dental plaque and in a vaccine to protect against <u>S. mutans</u> infection. Initial studies involve the purification and characterization of dextranase from a high producing mutant of <u>S. mutans</u> strain 6715, and the comparison of the <u>S. mutans</u> enzyme with dextranases present in monkey dental plaque formed under various dietary conditions. The effect of purified <u>S. mutans</u> dextranase on glucan formation and sucrose-mediated adherence by mutants of strain 6715, defective in the synthesis of dextranase will also be determined.														

1. Project Description

Objectives:

Dextranases synthesized by many different dental plaque bacteria appear to function in the hydrolysis of alpha-1, 6 glucans to fermentable carbohydrate. S. mutans dextranase has also been implicated in the formation of glucosyltransferase-mediated insoluble glucans which appear to be necessary for optimal colonization of S. mutans on teeth. The purpose of the present investigation is to acquire a more thorough understanding of how bacterial dextranases function in dental plaque and to determine their usefulness as an antiplaque agent and in a vaccine to protect against colonization of oral pathogens.

Methods:

Dextranase activity is measured either as the amount of reducing sugar released from Dextran T-2000 by the chemical methods described by Park-Johnson and Somogyi-Nelson, or as the amount of ethanol soluble glucans formed from <sup>14</sup>C-labeled water soluble; but ethanol insoluble, glucans made by S. mutans.

Dextranase will be purified, free of glucosyltransferase, from culture fluids of a mutant of S. mutans strain 6715 which produces large amounts of this enzyme. Standard methods available for purification of proteins are used. Glucosyltransferase activity was assayed by measuring the incorporation of the glucosyl moiety of (U-glucosyl-<sup>14</sup>C) sucrose into ethanol insoluble glucan. The effect of dextranase on the sucrose-mediated adherence of S. mutans to surfaces is determined by the number of radiolabeled bacteria adhering to glass or hydroxylapatite coated glass surfaces.

Major Findings:

Eleven strains of S. mutans including strains of serotypes a to e and four mutants of strain 6715 were tested for extracellular dextranase activity. The enzyme activity from several strains was also compared in culture supernatant fluids after growth in complex, semi-defined, and chemically defined media. Addition of Tween 80 to the growth medium enhanced the level of enzyme activity measured. A mutant of strain 6715 (d/g), which is defective in glucan synthesis, was the best producer of extracellular dextranase. Highest levels of enzyme activity were found in stationary phase culture fluids after growth in complex medium containing Tween 80 and limited carbohydrate. The enzyme had a pH optimum between pH 5.7 and 6.0 when tested in acetate, citrate, or citrate-phosphate buffers. It showed no requirement for metal ion and was inhibited by Hg<sup>++</sup>, Fe<sup>+++</sup>, Ag<sup>+</sup> and SDS. EDTA at a concentration of 1mM had no effect on dextranase activity.



Preliminary experiments to purify dextranase from culture supernatants from the 6715 mutant have included such methods as concentration and ultrafiltration using Amicon membranes, ammonium sulfate fractionation and gel-filtration chromatography. Dextranase was separated from insoluble glucan synthesizing enzymes and the major sucrase activity by  $(\text{NH}_4)_2\text{SO}_4$  fractionation; the dextranase activity was present in the 70-100%  $(\text{NH}_4)_2\text{SO}_4$  fraction.

Gel filtration on BioGel A-0.5 agarose further separated sucrase activity from the dextranase peak. The estimated molecular size of the dextranase was between 100,000 and 200,000 daltons.

Significant amounts of dextranase activity were assayed in dental plaque fluid samples obtained from 32 different monkeys. Levels of enzyme activity did not appear to be associated with caries activity in the animal subjects. The pH profiles showed that the major dextranase had a pH optimum of 6.8; a second dextranase activity had an optimum pH between 5.0 and 5.7. Addition of S. mutans dextranase (pH optimum of 5.7) to the plaque fluid did not appreciably change the activity at pH 6.8 but did substantially increase the dextranase measured at pH 5.7. The microbial source of the major dextranase peak is under investigation.

#### Significance:

A more thorough understanding of how bacterial dextranses function in dental plaque might determine whether these enzymes, under proper conditions, could be used as antiplaque agents and if immunizations with purified dextranses could restrict the colonization of oral pathogens such as S. mutans.

#### Proposed Course:

Extracellular dextranase from S. mutans will be further purified and characterized and comparison of the S. mutans enzyme with dextranses present in dental plaque will be made. The effect of glucosyltransferase-free S. mutans dextranase on glucan formation and sucrose-mediated adherence of mutants of strain 6715 found defective in the synthesis of dextranase will also be determined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00303 01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Local Immunity to Escherichia coli in the Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Cole, Michael F.	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Ericsson, Thorild	Laboratory Scientist (vis.)	NCP CPR NIDR

COOPERATING UNITS (if any)  
Bureau of Biologics - Drs. L. Hansen, T. Söderström, R. Schneerson, J. Robbins

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Preventive Methods Development

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS                       (b) HUMAN TISSUES                       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two groups of rats were either infected with viable E. coli K13 or immunized with formalin killed E. coli K13 by intragastric immunization. Half the rats in each group were subsequently immunized subcutaneously with K13 polysaccharide-BSA conjugate and the antibody response in serum and saliva determined.

Intragastric immunization followed by parenteral boosting appears to be a successful means for reproducibly stimulating a local salivary immune response.

1. Project Description

Objectives:

1. To determine whether infection of the gastro-intestinal tract with E. coli results in the induction of local and/or serum antibody.
2. To study the kinetics of the antibody response to infection.
3. To determine whether subcutaneous immunization with K13/antigen-Bovine serum albumin conjugates can induce SIGA and/or serum antibodies.
4. To determine whether subcutaneous immunization with K13 polysaccharide antigen-bovine serum albumin conjugates can protect against E. coli infection.
5. To study the kinetics of the antibody response to immunization.

Methods:

Forty, 28 day old Osborne-Mendel rats were divided into four groups of 10. Groups 1 and 2 were infected with  $1 \times 10^5$  viable CFU of E. coli K13 in 0.1ml of 0.01M PBS pH 7.0 by intragastric intubation on day 1 and 7 of the experiment. Groups 3 and 4 were immunized with  $1 \times 10^8$  CUF of formalin killed E. coli K13 by the same route daily throughout the experiment. Groups 1 and 3 were also immunized subcutaneously with 2.5 ug of K13 polysaccharide-BSA conjugate on day 28 of the experiment. The experiment was terminated on day 42 when saliva and blood were collected and rectal swabs taken to monitor colonization. Antibody in serum and saliva was assayed by an enzyme-linked immunoabsorbant assay.

Major Findings:

Immunization with E. coli K13 polysaccharide-BSA conjugate successfully induced an immune response in saliva and serum. The rats were successfully colonized with  $1 \times 10^5$  CFU of E. coli but this level of colonization did not appear to induce antibody at least in serum.

Significance:

The combination of primary local immunization with a secondary parenteral immunization appears to reproducibly stimulate a local immune response in external secretions.

Proposed Course:

- (1) Repeat experiment with different E. coli strain.
- (2) Study effect of varying concentration of antigen and viable bacteria.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00304 01 CPR									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less) Production of <u>Strep. mutans</u> serotype <u>c</u> antisera											
NAMES, LABORATORY, AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" style="width: 100%;"> <tr> <td style="width: 33%;">Thomson, Lynn A</td> <td style="width: 33%;">Laboratory Scientist</td> <td style="width: 33%;">NCP CPR NIDR</td> </tr> <tr> <td>Little, Wayne</td> <td>Laboratory Technician</td> <td>NCP CPR NIDR</td> </tr> <tr> <td>Bowen, William H.</td> <td>Chief, CPR Branch</td> <td>NCP CPR NIDR</td> </tr> </table>			Thomson, Lynn A	Laboratory Scientist	NCP CPR NIDR	Little, Wayne	Laboratory Technician	NCP CPR NIDR	Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
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Little, Wayne	Laboratory Technician	NCP CPR NIDR									
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR									
COOPERATING UNITS (if any)											
LAB/BRANCH Caries Prevention and Research											
SECTION Etiology											
INSTITUTE AND LOCATION NIDR, NIH, Bethesda MD											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)  <p>In producing rabbit antisera to <u>S. mutans</u> serotype <u>c</u> whole cells, investigators have suggested a variety of procedures and immunization schedules. Prior experiments suggest that the I.V. route was the only method that produced high titers, but a high mortality rate was associated with the booster injections. To overcome these deficiencies, several experiments were conducted to study variations of a multiple site immunization schedule suggested by investigators at Hazelton Laboratories. Hughes had also reported that rabbits immunized with different serotype <u>c</u> strains exhibited different levels of anaphylactic response. The strains least associated with fatal anaphylactic responses were studied as antigens to produce antisera and reduce loss during booster injections. Vaccine concentrations during booster series were also varied to assess the dose effect on toxicity and the production of high titered antisera.</p>											

## 1. Project Description

### Objective:

The objective of this project is to determine improved procedures for producing high titered rabbit antisera to serotype c S. mutans whole cells. A principal goal will be to reduce the mortality of rabbits during the booster injections.

### Methods:

A series of experiments will be conducted to investigate the effect of different immunization routes, various immunizing strains and different vaccine concentrations. The conditions will be standardized for experiments in order to assure only one dependent variable with each series. The multiple site studies employed aluminum hydroxide, complete and incomplete adjuvants. These bacterial suspensions were homogenized with micro-emulsifying needles and a Mulsijet R.

### Major Findings:

Investigation of several modifications of the multiple site immunization schedule proposed by Hazelton Laboratories have indicated that although this route of immunization gives rise to high titers for other serotypes of S. mutans (eg. serotypes a or d), titers sufficiently high to prepare a direct FA conjugate were not obtained for serotype c. Work at CDC and our laboratory suggests that adding an I.V. booster series will give titer increases, but also leads to increased rabbit mortality.

Efforts to reduce rabbit mortality during the booster phase included the use of several strains of serotype c cells which were reported to cause less anaphylactic response. These strains did not reduce rabbit loss. Additional homogenization of the cell suspension, the growth of the cells in a special non-clumping medium, and the preliminary use of an antihistamine drug (Phenergan) was investigated and found to not reduce mortality.

Results from a subsequent experiment suggest that a reduction of the I.V. booster dose from 40 International Turbidity Units (IU) to 15 IU reduced rabbit loss without reduction in the titers obtained with the I.V. route.

Rabbit antibody titers were monitored during these experiments using Indirect Fluorescent Antibody titering (IFA), Direct Whole Serum conjugate titering (DFA), and microagglutination procedures. In anticipating direct conjugate titers, the whole serum titering gave the best predictability.

Significance:

Serotype c is the most prevalent serotype of S. mutans in humans and should be carefully studied as to its involvement in the initiation of human caries. Reagent grade antisera for this serotype is required to complete meaningful diagnostic and epidemiologic studies. Improved methods for producing high titered serotype c antisera will expedite these studies.

Proposed Course:

Complete additional experiments to confirm the reduced mortality rates with 15 IU and determine the optimal immunization schedule for this vaccine dose.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 DE 00305-01 CPR
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PERIOD COVERED  
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)  
Effect of gastric intubation on an established Actinomyces viscosus microflora in the rat.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Olsson, Jan S.	Laboratory Scientist (vis.)	NCP CPR NIDR
Bowen, William H.	Chief, CPR Branch	NCP CPR NIDR
Monell-Torrens, Esteban	Laboratory Technician	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH  
Caries Prevention and Research

SECTION  
Etiology

INSTITUTE AND LOCATION  
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) HUMAN SUBJECTS       (b) HUMAN TISSUES       (c) NEITHER

(a1) MINORS     (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fifteen rats, fed Diet 2000 ad libitum, were inoculated with Actinomyces viscosus T-6. Once the rats harbored a manifest flora of Actinomyces they were fed by gastric intubation and were not allowed to eat Diet 2000. The level of infection was monitored once every week by oral swabbings which were primarily stained with FA. During a period of two weeks the prevalence of Actinomyces decreased rather slowly and it was decided that four animals should be given headcups to prevent them from eating feces which could eventually affect the oral flora. Also, at the same time, six other animals were given casein ad libitum, which in a previous study had proved not to support implantation of Actinomyces in the rat. The rest of the rats were continually fed just by gastric intubation. In all rats in the experiment the Actinomyces flora decreased below detectable levels, which occurred within different periods of time depending on the dietary regimen applied. However, characteristically high numbers of Actinomyces were rapidly recovered once the rats were again fed a carbohydrate containing diet ad libitum.

1. Project Description

Objectives:

The purpose of this study is to examine to what extent an existing oral microflora of Actinomyces viscosus in the rat is dependent on constant addition of nutrients.

Methods:

Fifteen Osborne Mendel rats were inoculated with Actinomyces viscosus T-6 and fed Diet 2000 ad libitum. After 14 days, when all rats harbored Actinomyces, Diet 2000 was withdrawn and instead the rats were fed by gastric intubation. After 14 days the rats were furnished with headcups to prevent coprophagy. After four days the headcups were removed and the animals were put on Diet 2000 ad libitum and no longer fed by tube. Six animals were, in addition to gastric intubation of essential nutrients, given casein. After 14 days these six animals and those of the 15 animals which had been fed solely by tube were given a carbohydrate-rich diet ad libitum. The level of Actinomyces infection was checked once every week using FA. At the termination of the experiment saliva, serum and fecal samples were collected.

Major Findings:

All 15 rats rapidly were colonized by Actinomyces viscosus T-6. When the animals were subjected to tube feeding and were not allowed to eat anything the level of Actinomyces infection decreased slowly. However, when rats were prevented from eating their own feces there was a rapid clearance of the Actinomyces flora from the mouth and in three days the numbers fell below detectable level. These rats rapidly developed severe diarrhea and, in fact, one animal died. Once the rats received Diet 2000 the numbers of Actinomyces rapidly increased above detectable levels. At first it appeared as if the clearance of Actinomyces had increased in the animals given casein, but soon these animals had the same level of infection as the animals which were being fed solely by tube. When it was no longer possible to detect Actinomyces by FA the rats received a carbohydrate-rich diet and within a few days high numbers of Actinomyces were again found with FA.

Significance:

This experiment is a sequel to a series of experiments dealing with implantation of Actinomyces viscosus in the rat. In a previous study

implantation of this bacterium in the rat proved to be fostered by dietary carbohydrate whereas, when the rats were given e.g., casein the bacteria could not be traced. These findings raised further questions: 1) is casein either inhibitory or does it simply not support implantation of Actinomyces like various carbohydrates; 2) to what extent does an already established Actinomyces flora depend on addition of nutrients through the mouth.

Proposed Course:

Data analysis and publication





# Section III

## CONTRACT ACTIVITIES

The following reports describe NCP contracted projects active during FY 1980 that are not collaborative extensions of specific direct operations. The Biometry Section computes data and provides statistical services for many of these projects.





CONTRACT		N01-DE-42434
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 5/1/74 - 6/30/81	
TITLE OF PROJECT: Experiments in Anti-Caries Immunizations in Sub-Human Primates.		
CONTRACTOR INSTITUTION AND UNIT: Hazleton Labs. PRINCIPAL INVESTIGATOR(S): Dan Dalgard ACTIVITY SITE: Vienna, Virginia		
PROJECT SCIENTIST: William Bowen ORGANIZATIONAL POSITION: NIDR, NCP, CP&R Branch		
RELEVANCE TO NCP OBJECTIVES: Moderate protection against animal caries has been achieved by stimulating antibodies against streptococcal glucosyl transferase. There are reasons to believe that other bacterial fractions might be even more potent antigens and that the protective effect of salivary immunoglobulins may be less bacterial-specific than first believed. Both possibilities would greatly enhance the feasibility of this approach to caries prevention and should be tested. The primate is the model of choice for such experiments since its oral microflora, dentition and immune response is very similar to humans and caries can be readily induced by "human-type" high sucrose diet.		
ACTIVITY: Provide personnel, facilities and materials necessary to NIDR staff to conduct experiments in anti-caries immunization with monkeys. This includes initial provision of 50 disease-free <i>Macaca fascicularis</i> , (the colony size later was increased), appropriate housing for the animals and facilities and technical assistance to NIDR to enable examination and vaccination of and collection of biological samples from the monkeys. At intervals during the project, measure the incidence of caries in the animals and specified blood, saliva, and plaque constituents.		
RESULTS: The monkeys have developed caries as planned and otherwise remain in good health. Groups of animals have been vaccinated with potentially important immunogens at several anatomic sites to study host secretory immune responses. Administration of antigens intraductally does not appear to confer protection against caries. On the other hand administration of glucosyl transferase intramucosally with $Al(OH)_3$ gel appears to provide substantial protection. Caries incidence appears to be associated with levels of <i>S. mutans</i> but not lactobacilli. A consistent pattern was not observed between the levels of carbohydrate in dental plaque and caries scores.		

CONTRACT		N01-DE-42446
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/28/74 - 6/30/80	
TITLE OF PROJECT: Development of a Fluoride Releasing System for Prolonged Oral Use.		
CONTRACTOR INSTITUTION AND UNIT: Southern Research Institute PRINCIPAL INVESTIGATOR(S): Donald Cowsar and Danny Lewis ACTIVITY SITE: Birmingham, Alabama		
PROJECT SCIENTIST: Dale Mirth ORGANIZATIONAL POSITION: NIDR, NCP, PMD Section		
<p>RELEVANCE TO NCP OBJECTIVES: Fluoridation of drinking water continues to be the simplest and least expensive way to deliver fluoride for the prevention of caries. For areas in which a municipal water supply does not exist or in which there is opposition to municipal fluoridation, other vehicles (school water, tablets and salt) have been used to provide fluoride. It is believed that for these areas controlled release of fluoride from a repository within the mouth might be superior to school water, tablet and salt fluoridation since it would provide fluoride continuously, would not entail daily actions by the participant, and might prevent caries at lower levels of fluoride. Devices that continuously release controlled levels of fluoride are needed for testing the feasibility of this approach in animal models and in humans.</p>		
<p>ACTIVITY: A biocompatible system for use in the oral environment is to be developed and tested which will release fluoride at a constant predetermined rate for at least six months. These devices are to be evaluated in dogs and other animals, an IND filed with the FDA and short-term human clinical trials carried out, provided there is clearance from the FDA.</p>		
<p>RESULTS: The contractor has developed and delivered devices made of biocompatible copolymers of hydroxyethyl methacrylate and methylmethacrylate. The devices release fluoride intraorally at a constant predetermined rate for several weeks. Acute and chronic toxicity tests have been completed in several species with no indication of adverse reactions. An IND was prepared by the contractor and filed with the FDA by NCP and a one-month (Phase I-Phase II) clinical study was carried out in October 1979. During the month-long study, fluoride levels in plaque and saliva remained elevated and serum levels unchanged with respect to levels prior to placement of the devices.</p>		

CONTRACT		N01-DE- 52449 CT 0600094
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 4/1/75 - 6/30/80	
TITLE OF PROJECT: Clinical Field Trial to Assess the Cost Effectiveness of Various Caries Preventive Agents.		
CONTRACTOR INSTITUTION AND UNIT: University of Connecticut PRINCIPAL INVESTIGATOR(S): Neville Doherty ACTIVITY SITE: University of Connecticut Health Center, Farmington, CT.		
PROJECT SCIENTIST: Philip Swango ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: The NCP has initiated several studies to determine the maximum caries-prevention effect which can be achieved with currently available methods and agents. These studies are designed to demonstrate the total effect of such procedures as topically applied fluoride and adhesive sealants when used in combination in school-aged children. Before specific community preventive programs can be recommended, however, it is important to assess the relative cost-effectiveness of each of these procedures, since it is possible that their combined use results in only minor increases in protective benefits compared to the use of a single agent or method.		
ACTIVITY: A multi-group clinical trial is being carried out to determine the cost and clinical effectiveness of topical fluoride (semi-annual professional application of APF), pit and fissure sealant (bis-GMA) and fluoride mouthrinse (supervised weekly mouthrinse with dilute NaF) when used singly and in combination. There are approximately 200 children in each of the eight groups. Caries incidence is recorded on NIH forms and submitted to NCP Biometry Section for processing. The cost of each procedure and combination of procedures is computed and related to the degree of caries prevention that is achieved.		
RESULTS: Differences in caries increment between groups were small and in some cases contrary to expectations. The most notable treatment effect for a single regimen was for fluoride rinsing. Cost figures confirm the expected ranking of treatment by cost (sealants > topical gels > rinsing), and show a ratio of 4:1 between the most costly combination and the least costly single regimen. Considering all regimens in aggregate, about 90% of cost was for labor, either direct or indirect.		



CONTRACT		N01-DE-52456
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/20/75 - 10/19/80	
TITLE OF PROJECT: Possible Role of Salivary Immunoglobulin A-Deficiency in Humans as Related to Dental Caries.		
CONTRACTOR INSTITUTION AND UNIT: University of Alabama, Birmingham PRINCIPAL INVESTIGATOR(S): Jerry McGhee ACTIVITY SITE: Birmingham, Alabama		
PROJECT SCIENTIST: Andrew Vargosko (previously Norman Ikari) ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: Partial immunization against caries and increased salivary IgA has been achieved in animal models. To establish evidence that immunization is feasible in man several groups have looked for correlations between caries experience in man and salivary immunoglobulins. Thus Lehner, <i>et al</i> found higher IgA levels in whole saliva of caries-free subjects than in caries-active patients. Zengo, <i>et al</i> also noted higher IgA levels in submandibular but not parotid gland secretions. To obtain further evidence on this possible relationship one could examine the caries level in patients who lack IgA in their serum and external secretions. These patients appear particularly susceptible to diseases involving the oral membranes.</p>		
<p>ACTIVITY: A population of patients with salivary IgA deficiency is being studied. Complete medical histories have been obtained on each patient and complete oral/dental examinations carried out. Periodically saliva and plaque samples are obtained and analyzed for immunoglobulins and enzymes that act in conjunction with immunoglobulins. The numbers of various bacterial species in plaque also are determined.</p>		
<p>RESULTS: Information obtained so far indicates that salivary IgA-deficiency does not necessarily correlate with increased caries activity. This is most likely due to some compensation with salivary IgM and elevation of at least 2 of the 3 innate immune factors, e.g., lactoferrin, lactoperoxidase and lysozyme in these patients. In the absence of any secretory antibody to <u>Streptococcus mutans</u>, there appears to be some increase in caries incidence. Plaque microbial composition shows no significant changes from control subjects. <u>In vitro</u> studies show salivary IgA will block <u>S. mutans</u> from hydroxyapatite. Saliva from IgA deficient patients will not affect <u>S. mutans</u>. However, patients with compensating IgM antibody will block <u>S. mutans</u>.</p>		

CONTRACT		N01-DE- 52459
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/30/75 - 4/30/80	
TITLE OF PROJECT: Community Caries Prevention Demonstration Project		
CONTRACTOR INSTITUTION AND UNIT: University of Connecticut Health Center PRINCIPAL INVESTIGATOR(S): Douglas Macko and Howard Bailit ACTIVITY SITE: Ansonia and Derby, Connecticut		
PROJECT SCIENTIST: Ann Miller ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.</p> <p>School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.</p>		
<p>ACTIVITY: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 3,100 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. Utilize combinations of teachers aides, parents/or students to mix and dispense the rinse solution. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.</p> <p>REVISED ACTIVITY: Commencing in September 1977, include approximately 3,700 children in grades 7-12. Provide information on caries prevalence, cost and participation for these grades separate from the initial contract. As a result of this modification conduct the program in grades K-12 through April 1980.</p>		
<p>RESULTS: The average participation for all sites at the end of four school years for grades K-8 was 82.7% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades ranged from 22% to 77% in the final year. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to \$9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The demonstration clearly showed the procedure to be highly cost-effective in a variety of community settings.</p>		



CONTRACT		N01-DE- 52461
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/30/75 - 4/30/80	
TITLE OF PROJECT: Community Caries Prevention Demonstration Project		
CONTRACTOR INSTITUTION AND UNIT: Forsyth Dental Center PRINCIPAL INVESTIGATOR(S): Paul DePaola ACTIVITY SITE: Arlington, Massachusetts		
PROJECT SCIENTIST: Ann Miller ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.</p> <p>School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.</p>		
<p>ACTIVITY: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. Carry out the program by volunteers under a paid coordinator. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.</p> <p>REVISED ACTIVITY: Commencing in September 1977, include approximately 4,000 children in grades 7-12. Provide information on caries prevalence, cost and participation for these grades separate from the initial contract. As a result of this modification conduct the program in grades K-12 through April 1980.</p>		
<p>RESULTS: The average participation for all sites at the end of four school years for grades K-8 was 82.7% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades ranged from 22% to 77% in the final year. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to \$9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The demonstration clearly showed the procedure to be highly cost-effective in a variety of community settings.</p>		



CONTRACT

N01-DE- 52470

REPORT PERIOD: FY 80

TOTAL PERIOD OF SUPPORT: 6/30/75 - 4/30/80

TITLE OF PROJECT: Community Caries Prevention Demonstration Project

CONTRACTOR INSTITUTION AND UNIT: University of California, San Francisco  
PRINCIPAL INVESTIGATOR(S): Steven Silverstein  
ACTIVITY SITE: Livermore, CA

PROJECT SCIENTIST: Ann Miller  
ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch

RELEVANCE TO NCP OBJECTIVES: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

ACTIVITY: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,200 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. Utilize teachers or part-time aides to dispense the rinse for the lower grades and paid community aides or trained student aides to mix and dispense the solution for upper grades. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

REVISED ACTIVITY: Commencing in September 1977, include approximately 4,175 children in grades 9-12. Provide information on caries prevalence, cost and participation for these grades separate from the initial contract. As a result of this modification conduct the program in grades K-12 through April 1980,

RESULTS: The average participation for all sites at the end of four school years for grades K-8 was 82.7% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades ranged from 22% to 77% in the final year. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to \$9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The demonstration clearly showed the procedure to be highly cost-effective in a variety of community settings.

CONTRACT		N01-DE- 52473
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/30/75 - 4/30/80	
TITLE OF PROJECT: Community Caries Prevention Demonstration Project		
CONTRACTOR INSTITUTION AND UNIT: Research for Health in Erie County, Inc. PRINCIPAL INVESTIGATOR(S): Donald Bissell ACTIVITY SITE: Angola, Derby, and North Collins, NY		
PROJECT SCIENTIST: Ann Miller ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.</p> <p>School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.</p>		
<p>ACTIVITY: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. Utilize teachers and school nurses to dispense the rinse solution. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.</p> <p>REVISED ACTIVITY: Commencing in September 1977, include approximately 1,800 children in grades 9-12. Provide information on caries prevalence, cost and participation for these grades separate from the initial contract. As a result of this modification conduct the program in grades K-12 through April 1980.</p>		
<p>RESULTS: The average participation for all sites at the end of four school years for grades K-8 was 82.7% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades ranged from 22% to 77% in the final year. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to \$9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The demonstration clearly showed the procedure to be highly cost-effective in a variety of community settings.</p>		



CONTRACT		N01-DE- 52474
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/30/75 - 4/30/80	
TITLE OF PROJECT: Community Caries Prevention Demonstration Project		
CONTRACTOR INSTITUTION AND UNIT: Geauga County Health Department PRINCIPAL INVESTIGATOR(S): Carol Sherman and Frank Kellogg ACTIVITY SITE: Geauga County, Ohio		
PROJECT SCIENTIST: Ann Miller ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.</p> <p>School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.</p>		
<p>ACTIVITY: Commencing in September 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 7,600 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In the Amish schools carry out the entire program with teachers. In the other schools conduct the program with school nurses assisted by teachers and students. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.</p> <p>REVISED ACTIVITY: Commencing in September 1977, include approximately 4,000 children in grades 7-12. Provide information on caries prevalence, cost and participation for these grades separate from the initial contract. As a result of this modification conduct the program in grades K-12 through April 1980.</p>		
<p>RESULTS: The average participation for all sites at the end of four school years for grades K-8 was 82.7% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades ranged from 22% to 77% in the final year. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to \$9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The demonstration clearly showed the procedure to be highly cost-effective in a variety of community settings.</p>		



Contract (no intramural research component)		NO1-DE- 52476 CT 0600108
Period Covered: FY 80	Contract Period: 6/27/75-3/31/80	
Title of Project: Development and Evaluation of a Diagnostic System for the Early Detection of Dental Caries		
Contractor: Tufts University School of Dental Medicine, Boston Principal Investigator(s): George White Activity Site: Boston, MA		
P.O.: Ralph Frew Organizational Position: NIDR, NCP, OD		
<p>Relevance to NCP Objectives: Due to the etiology of caries, clinical trials typically require 3 years and several hundred children per group for a significant difference to be measureable between treatment and control groups with meaningful sensitivity and confidence. If caries could be detected earlier, the length of time for a clinical trial could be reduced and NCP comparison of alternative agents or modes of delivery would be markedly expedited. Capability to detect caries at a reversible stage could allow (1) the consideration of clinical research in which caries challenge is experimentally increased, (2) reversal of incipient lesions to be a cost-attractive alternative to prevention programs or operative therapy and (3) study of caries etiology at earlier stages.</p>		
<p>Activity: An instrument to detect caries by conductivity measurement (and primarily for use with pits and fissures of molar teeth) is being developed and evaluated. The contractor has designed an instrument, evaluated its safety and ease of use and determined the sensitivity and reproducibility of measurements made with it under laboratory conditions. Currently the accuracy of the instrument in identifying early lesions is being determined in a longitudinal clinical trial.</p>		
<p>Results: Sixty-two children, aged 10 to 14, were examined for occlusal carious lesions by: (1) routine clinical examination or (2) electrical resistance measurement, and results were compared to histological findings. An excellent correlation (<math>r=0.965</math>) was found between the instrumental diagnosis and histological findings. In 36 instances the instrument indicated the presence of carious occlusal lesions that could not be detected clinically with the dental explorer, but were confirmed by histologic findings. The instrument was found to be more sensitive than, and as specific as, the conventional examination for occlusal caries detection. Moisture contamination which may result in an increase in false positive diagnoses, has required modification of the instrument.</p>		

CONTRACT

NO1-DE- 52484

REPORT PERIOD: FY 80

TOTAL PERIOD OF SUPPORT: 6/27/75 - 6/26/81

TITLE OF PROJECT: Clinical Screening of Antiplaque and Antibacterial Agents.

CONTRACTOR INSTITUTION AND UNIT: University of Pennsylvania, Philadelphia  
PRINCIPAL INVESTIGATOR(S): Samuel Yankell  
ACTIVITY SITE: Philadelphia

PROJECT SCIENTIST: Roald Shern  
ORGANIZATIONAL POSITION: NIDR, NCP, CP&R Branch

RELEVANCE TO NCP OBJECTIVES: A promising approach to prevent caries is to use antimicrobial or antiplaque agents that decrease bacterial colonization or erosion of the tooth surface. To identify such agents and to ensure that they are evaluated accurately and comparably, the NCP is providing a service to screen promising compounds in vitro, in animals, and in short- and long-term clinical trials. Contracts and NCP direct operations are used jointly in separate phases of this screening service.

ACTIVITY: This "task-order" contract provides short-term clinical evaluation as specifically requested by NCP of IND certified agents. The trials are up to 16 weeks in length and involve not more than 50 male subjects. Agents are closely monitored for untoward effects and compared in their effects on oral microflora and several indices of plaque suppression.

RESULTS: Short-term clinical studies have been carried out to determine effects on plaque of conventional and new topical agents:

- a) fluoride gel (SnF<sub>2</sub> and/or APF)--no significant effect
- b) calcium glycerophosphate rinses--no significant effect
- c) DAPA-1 rinses--significant effects on plaque
- d) SnF<sub>2</sub> rinses--significant effects on plaque

New criteria have been established for describing plaque responses.

CONTRACT		N01-DE-62488
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 5/1/76 - 10/31/79	
TITLE OF PROJECT: Immunologic Cross-Reactivity of Antigens from Oral Streptococci with Mammalian Tissue and Tissue Components.		
CONTRACTOR INSTITUTION AND UNIT: Research Foundation, SUNY PRINCIPAL INVESTIGATOR(S): Russell Nisengard ACTIVITY SITE: SUNY, Buffalo, NY		
PROJECT SCIENTIST: David Klein (previously Norman Ikari) ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Microbial antigens proposed for use as immunogens may resemble host tissue antigens. Since host response to the bacterial substance may damage host tissue, the possibility of cross-reactivity must be carefully examined and eliminated. In 1972, rabbit antiserum to strains of <u>S. mutans</u> BHT and FA1 was noted to react with human myocardial tissue and skeletal muscle. To establish the dimensions of this phenomenon the Program is screening the oral microorganisms that would be used as sources of immunogens for cross-reactivity with human tissue. For convenience screening is carried out through two contracts, N01-62488 (oral streptococci) and N01-52481 (oral non-streptococci).		
ACTIVITY: Representative strains and serotypes of <u>S. mutans</u> , <u>sanguis</u> and <u>salivarius</u> are collected. Antisera to these microorganisms are prepared, labelled with fluorescent dye and tested for binding to human heart (normal and rheumatic), kidney, skeletal muscle, liver and brain tissue. Crude antigen preparations are fractionated and the antigens responsible for cross-reactivity are defined and characterized.		
RESULTS: Contract N01-52481 (oral streptococci) expired on 12/31/76. Rabbit antibody to <u>Lactobacillus casei</u> and <u>Propionibacterium acnes</u> bound to monkey brain and kidney tissue. The significance of this cross-reactivity is not known. Contract N01-62488 (oral streptococci) and three sub-contract collaborators have found that many details of the methodology affect the final reading of the immunofluorescent reaction of rabbit anti-streptococcal sera to mammalian tissues. The original (1973) report of binding of anti-- <u>S. mutans</u> rabbit sera to heart and skeletal muscle remains valid but is now subject to cautious interpretation because of some normal rabbit serum binding and lack of correlation between intensity of binding and anti-bacterial titer. Much further study of this phenomenon is required.		



CONTRACT		N01-DE- 62491
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/28/76 - 3/31/82	
TITLE OF PROJECT: Research Study on the Use of Mutants of Cariogenic Streptococci to Prevent Dental Caries.		
CONTRACTOR INSTITUTION AND UNIT: University of Alabama in Birmingham PRINCIPAL INVESTIGATOR(S): Theodore Shiota ACTIVITY SITE: Birmingham, Alabama		
PROJECT SCIENTIST: Andrew Vargosko (previously Norman Ikari) ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Glucan and fructan production by <u>S. mutans</u> is commonly considered to be a critical factor in the accumulation of plaque and bacterial fermentation acids that cause caries. Conceivably one might prevent caries by modifying the amount or nature of these microbial polymers. To achieve this a possible approach is to substitute <u>S. mutans</u> , with mutant polymer synthesis, for the indigenous strain of <u>S. mutans</u> . It is already known that some mutants of <u>S. mutans</u> do have modified capability to synthesize glucan and fructan <u>in vitro</u> and diminished caries virulence in rat models. If this approach is to be feasible, non-cariogenic mutants would have to be found that can supplant the wild type and neither revert to nor be supplanted by the wild type.		
ACTIVITY: A variety of techniques are used to prepare and isolate mutants of <u>S. mutans</u> with altered agglutination and plaque and colony forming ability. Cariogenicity of the mutants, ability to withstand being supplanted by the wild type, and stability to reversion are determined in rat models.		
RESULTS: A large number of genetic mutants of <u>S. mutans</u> has been obtained that are deficient in one or more <u>in vitro</u> characteristics associated with cariogenicity. Several have caused less caries in gnotobiotic rats and have been transmitted from dams to pups. However, reversion to the parental type is occurring. Mutants which are defective in glucosyltransferase (GTF) activity can be restored to full virulence <u>in vivo</u> by addition of cell free <u>S. mutans</u> components. A mutant defective in serotype antigen, has been found to exhibit characteristics which are considerably more virulent than the parent type, probably the result of altering normal cell wall polysaccharides.		

CONTRACT		N01-DE-72404 CT 0600125
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 8/15/77 - 2/14/81	
TITLE OF PROJECT: Clinical Investigation of the Effect of Concentration and Frequency of the Use of a Sodium Fluoride Mouthrinse on Dental Caries Formation		
CONTRACTOR INSTITUTION AND UNIT: University of Florida at Gainesville PRINCIPAL INVESTIGATOR(S): Stanley Lotzkar and A. J. Conti ACTIVITY SITE: Polk County, Florida		
PROJECT SCIENTIST: Philip Swango ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: The use of fluoride mouthrinsing in school-based programs for the prevention of dental caries is gaining widespread acceptance in the U. S. It would be of benefit to these programs if the NCP could recommend the most effective combination of frequency of rinsing and concentration of F solution. Unfortunately, the variations in the procedures that have been tested and the diversity of results do not support clear-cut recommendations. To obtain the data that is desired it is necessary to carry out a clinical trial in which the major combinations of frequency and concentration are compared side-by-side.		
ACTIVITY: A three-year longitudinal clinical trial with approximately 1700 children is being carried out to compare the levels of caries prevention that are obtained with the major variations of rinse frequency and concentration used in school-based NaF mouthrinse programs. There are five groups in the study: a placebo rinse group, a daily and a weekly .05% NaF rinse group, and a daily and a weekly 0.2% NaF rinse group. Caries experience, measured at baseline, is redetermined annually.		
RESULTS: Final dental examinations have been conducted and data analysis is in progress. Interim results (after 2 years) showed trends that frequency of rinsing may be a more important factor than fluoride concentration.		

CONTRACT

N01-DE-72407  
CT 0600126

REPORT PERIOD: FY 80

TOTAL PERIOD OF SUPPORT: 9/30/77 - 1/31/81

TITLE OF PROJECT: Evaluation of Effects of Tooth Cleaning Prior to Weekly Rinsing with NaF.

CONTRACTOR INSTITUTION AND UNIT: Eastman Dental Center, Rochester, New York  
PRINCIPAL INVESTIGATOR(S): Dennis Leverett  
ACTIVITY SITE: Rochester, New York

PROJECT SCIENTIST: Philip Swango  
ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch

RELEVANCE TO NCP OBJECTIVES: Supervised weekly mouthrinsing with a 0.2% solution of NaF (900 ppm) is increasingly being recommended to communities that wish to implement programs for dental caries prevention. It has been suggested that the removal of plaque and other debris from the surface of the teeth prior to rinsing could enhance the effectiveness of the procedure. This should be investigated. In school programs, however, it is desirable that rinsing procedures occupy a minimal amount of classroom time. Thus any advantages of prior cleaning would have to be significant before it could be recommended as a routine adjunct to rinsing.

ACTIVITY: A three-year longitudinal clinical trial is being carried out to establish whether cleaning the teeth prior to customary weekly fluoride rinsing prevents significantly more caries than fluoride rinsing by itself. A population of approximately 900 school children 12-13 years of age is involved in the study. All of the children rinse weekly with 0.2% NaF solution. The children in one group do not clean their teeth prior to the weekly fluoride rinse. Children in the two other groups clean their teeth by brushing or by brushing and flossing. Caries scores, determined at baseline, are remeasured periodically and reported on NIDR data forms.

RESULTS: First year data have been collected and analyzed. Statistically significant differences in caries scores were not observed between study groups after one year. However, mesial and distal surfaces showed a trend toward lower caries activity in the two groups that had prior toothcleaning. Second and third-year findings are being analyzed.



CONTRACT		N01-DE-72408
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/1/77 - 8/31/81	
TITLE OF PROJECT: Search for Cross Reacting Antigens to Acidogenic Bacteria of the Human Oral Flora.		
CONTRACTOR INSTITUTION AND UNIT: Research Foundation, SUNY PRINCIPAL INVESTIGATOR(S): Murray Stinson ACTIVITY SITE: SUNY, Buffalo, NY		
PROJECT SCIENTIST: David Klein (previously Norman Ikari) ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: A major NCP strategy is to develop caries immunization for public health use. There would be risks however in using immunogens from acidogenic streptococci, lactobacilli and Actinomycetes because these classes of microorganisms have known pathogenicity. The possibility exists, however, to substitute immunogens from non-pathogenic microorganisms if there is sufficient similarity in antigenic properties. Thus, for example, in other areas of medicine, overt immunization with certain serological types of <u>E. coli</u> and species of staphylococci and bacilli have been found to give rise to antibodies which cross-react with the disease pathogens, pneumococci, meningococci and Hemophilus influenzae "b".</p>		
<p>ACTIVITY: Antigens from non-pathogens are being sought that are similar to antigens from oral strains of acidogenic streptococci, lactobacilli and Actinomyces. Bacterial colonies are made into air-dried films, exposed to rabbit antisera and stained by a goat anti-rabbit IgA conjugate. Presumptive identification is made of those bacteria yielding significant cross-reactivity in indirect immunofluorescence screening. Rabbit antisera is raised to these cross-reactors. The ability of these antisera to inhibit adherence of acidogenic bacteria is measured. Preliminary characterization of the cross-reactive sites in the bacteria is performed by fractionation and extraction of the bacterial cells and further testing of these fractions for cross-reactivity and adherence-inhibitory capability of their antisera.</p>		
<p>RESULTS: A total of 51 bacteria isolated from various sources have been found to cross-react with three or more antisera specific for acidogenic bacteria. Six of these isolates have been reciprocally tested against the acidogenic bacteria. Some loss of reactivity was noted. An assay for bacterial adherence currently being tested shows promise in measuring effects of salivary constituents as well as effects of antisera.</p>		

CONTRACT

N01-DE-72409

REPORT PERIOD: FY 80

TOTAL PERIOD OF SUPPORT: 9/30/77 - 12/31/80

TITLE OF PROJECT: Effect of Strontium, Lithium and Fluorine on the in vitro Formation and Metabolism of Dental Plaque and on Plaque Formation and Development in the Rat.

CONTRACTOR INSTITUTION AND UNIT: Eastman Dental Center  
PRINCIPAL INVESTIGATOR(S): Martin Curzon  
ACTIVITY SITE: Rochester, New York

PROJECT SCIENTIST: Albert Kingman (formerly G. Roussos)  
ORGANIZATIONAL POSITION: NIDR, NCP, CPR Branch

RELEVANCE TO NCP OBJECTIVES: Epidemiological studies in the 1930's that revealed a relationship between caries incidence and environmental fluoride after many years of research and testing have led to current wide-spread use of this element for caries prevention. Continuing field studies suggest that caries incidence is related to additional trace elements. Repeatedly these investigations have implicated lithium and strontium in an inverse relationship to caries and pointed to selenium in a caries promoting role. At present in vitro and animal models are needed with which these effects can be confirmed and defined with respect to their strength. High priority certainly should be given to exploring models based on plaque formation and activity and on enamel development and properties. It is reasonable that trace elements would interact in these models so this possibility also should be examined.

ACTIVITY: The contract is to search for model systems with which Sr, Li and F effects on caries can be studied. The in vitro models that are being tested are major salivary and microbial phenomena involved in caries etiology. The in vivo models involve rats exposed to the trace elements prenatally or at various periods in tooth development. Various levels and combinations of the trace elements are being screened in the two types of systems.

RESULTS: In each of the models there appears to be definite effects of lithium when tested at low concentration. Maximal caries reduction and weight gains in rats occurred at 20  $\mu\text{g/L}$ . Effects on bacterial aggregation, sorption and extracellular polysaccharide production occurred at lithium concentrations of 100  $\mu\text{g/L}$ . Additional studies are currently underway to confirm these unusual patterns of concentration effects.



CONTRACT		N01-DE-72410
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/30/77 - 10/31/79	
TITLE OF PROJECT: Application of Fluorescent Antibody Methods to the Clinical Determination of the Etiologic Role of Oral Microorganisms in Human Dental Caries.		
CONTRACTOR INSTITUTION AND UNIT: Research Foundation, SUNY PRINCIPAL INVESTIGATOR(S): Ernst Beutner ACTIVITY SITE: Buffalo, New York		
PROJECT SCIENTIST: Stanley Robrish (previously O'Brien) ORGANIZATIONAL POSITION: NIDR, NCP, CPR Branch		
RELEVANCE TO NCP OBJECTIVES: Though there is direct evidence that <u>S. mutans</u> causes caries in the rat the evidence for the involvement of this microorganism in human caries is limited to cross-sectional epidemiological studies that show correlations in large populations between the level of caries and the microorganism. This evidence could be made stronger by longitudinal studies of the sequence of the appearance of caries and <u>S. mutans</u> at specific sites on teeth. Such a study has been made technically feasible by the development by NCP of reagents that facilitate enumeration of plaque microorganisms and media and techniques that facilitate sampling and manipulation of minute amounts of plaque.		
ACTIVITY: Develop FA methodology for enumerating <u>S. mutans</u> , serotypes (a-g) and other selected microorganisms in dental plaque and assess the feasibility of determining the etiologic role of these microorganisms in caries through longitudinal epidemiologic studies with this methodology.		
<ol style="list-style-type: none"> <li>1) Prepare and test FA reagents to specified microorganisms.</li> <li>2) Proceed with developing direct or indirect FA methodologies depending on an evaluation of the antibody conjugates used in the indirect method.</li> <li>3) Test the FA reagents for specificity in identifying <u>S. sanguis</u>, <u>S. mutans</u>, <u>Actinomyces</u> and <u>Neisseria</u>.</li> <li>4) Test the reagents, methodology and system for use in epidemiologic studies.</li> </ol>		
REVISED ACTIVITY (1978): Prepare sensitive, specific immunofluorescent reagents for specified strains of <u>S. sanguis</u> and <u>Neisseria</u> .		
<ol style="list-style-type: none"> <li>1) Prepare antisera to the strains in rabbits.</li> <li>2) Employ the antisera in preparing direct and indirect (along with goat anti-rabbit antiserum) immunofluorescent reagents.</li> <li>3) Render the reagents free of cross-reactivity to selected strains of other plaque bacteria and test the final reagents on plaque samples.</li> </ol>		
RESULTS:		
<ol style="list-style-type: none"> <li>1) Antisera to <u>S. sanguis</u> and <u>Neisseria</u> were prepared, freed of cross reactivity to 5 other species, conjugated with fluorescein and tested using direct and indirect methods.</li> <li>2) Studies with plaque showed that neither the <u>S. sanguis</u> nor <u>Neisseria</u> reagents was completely specific in either direct or indirect methods.</li> <li>3) The indirect method did not provide significant improvements in sensitivity or specificity.</li> </ol>		



CONTRACT		N01-DE-72411 CT 0600119
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/30/77 - 9/29/81	
TITLE OF PROJECT: Acidulated Phosphate-Fluoride Used Daily as a Tablet or Solution.		
CONTRACTOR INSTITUTION AND UNIT: Applied Management Sciences PRINCIPAL INVESTIGATOR(S): Douglas Skinner ACTIVITY SITE: Pittsylvania County, Va.		
PROJECT SCIENTIST: Philip Swango ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Though less effective than with full-time use, fluoride supplements given only in school can impart important caries-preventive benefits and allow large numbers of children to be treated with minimal demands on dental manpower equipment and funds. The NCP is actively promoting these programs and needs information on which of the several technics and fluoride formulations is best. Among these are fluoride tablets that are chewed and then swallowed and fluoride solutions that are swished around the mouth and swallowed. Both are available as neutral NaF or as acidulated phosphate fluoride.		
ACTIVITY: A three-year longitudinal clinical trial is being carried out to determine the effect on dental caries of neutral sodium fluoride (NaF) and acidulated phosphate fluoride (APF) when each is used as a tablet or as a solution. The study population of approximately 1600 school children, 12-14 years of age, is divided into five groups. One group receives a placebo tablet. The other groups receive 1 mg F per day as NaF or APF either in a tablet that is chewed and swallowed or solution that is swished and swallowed. Caries experience is recorded on NCP data forms and submitted to NCP Biometry Section for processing.		
RESULTS: First and second year data have been collected and are being analyzed. Results are not yet available.		

CONTRACT		N01-DE- 82416 CT 0600127
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/30/78 - 9/29/81	
TITLE OF PROJECT: Effect of Mouthrinsing with Stannous Fluoride or Sodium Fluoride.		
CONTRACTOR INSTITUTION AND UNIT: Univ. of Texas Health Science Center, Houston PRINCIPAL INVESTIGATOR(S): Lawrence Friedman ACTIVITY SITE: Houston, Texas		
PROJECT SCIENTIST: Philip Swango ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Daily mouthrinsing with dilute solutions of either sodium fluoride or stannous fluoride has been found effective against caries. Stannous fluoride has been reported to have an unattractive taste, to stain teeth and to be unstable in solution. On the other hand it has been reported to remove plaque from teeth. If use of a stannous fluoride mouthrinse can produce meaningful reductions in plaque and gingivitis scores with minimal tooth staining and is equivalent to sodium fluoride in cariostatic properties it may be the preferred agent in regimens that involve daily use.		
ACTIVITY: The clinical trial involves approximately 500 students, 11-13 years of age, living in Houston, Texas, a low-fluoride area. The students rinse daily during three school years with a dilute solution of either stannous or sodium fluoride. Caries, dental plaque, gingivitis and extrinsic staining evaluated at baseline are redetermined at appropriate intervals. Data is recorded on NIH forms and processed by the NCP Biometry Section.		
RESULTS: First year findings have been analyzed. Differences in plaque and gingivitis scores between the two groups after three and six months were small and non-significant. Staining scores show a tendency to increase for the SnF <sub>2</sub> group but the difference between groups was not significant. Early findings for caries have not revealed important differences between the two agents.		

CONTRACT		N01-DE- 82417 CT 0600113
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/30/78 - 9/29/81	
TITLE OF PROJECT: Effect of Mouthrinsing with Stannous Fluoride or Sodium Fluoride.		
CONTRACTOR INSTITUTION AND UNIT: Eastman Dental Center PRINCIPAL INVESTIGATOR(S): Dennis Leverett and William McHugh ACTIVITY SITE: Seneca and Tompkins Counties, NY		
PROJECT SCIENTIST: Philip Swango ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Daily mouthrinsing with dilute solutions of either sodium fluoride or stannous fluoride has been found effective against caries. Stannous fluoride has been reported to have an unattractive taste, to stain teeth and to be unstable in solution. On the other hand it has been reported to remove plaque from teeth. If use of a stannous fluoride mouth-rinse can produce meaningful reductions in plaque and gingivitis scores with minimal tooth staining and is equivalent to sodium fluoride in cariostatic properties it may be the preferred agent in regimens that involve daily use.		
ACTIVITY: The clinical trial involves approximately 500 students, 11-14 years of age, living in Seneca and Tompkins Counties, NY, a low-fluoride area. The students rinse daily during three school years with a dilute solution of either stannous or sodium fluoride. Caries, dental plaque, gingivitis and extrinsic staining, evaluated at baseline, are redetermined at appropriate intervals. Data is recorded on NIH forms and processed by the NCP Biometry Section.		
RESULTS: First year findings for plaque, gingivitis, and staining have been analyzed. Results showed only small differences in plaque and gingivitis scores between groups, with the trend favoring an effect for SnF <sub>2</sub> . The trend was significant for plaque but not for gingivitis. Staining scores for SnF <sub>2</sub> were nearly four times as high as the NaF scores (p<0.001) but the degree of staining was considered low in both groups. Caries findings are being analyzed.		



CONTRACT		NO1-DE-92419 CT 0600129
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 1/15/79 - 6/30/82	
TITLE OF PROJECT: Effect of Prior Toothcleaning upon the Efficacy of Semi-Annual Topical Fluoride Treatment.		
CONTRACTOR INSTITUTION AND UNIT: Research Foundation of SUNY PRINCIPAL INVESTIGATOR(S): Louis Ripa and Gary Leske ACTIVITY SITE: Stony Brook, New York		
PROJECT SCIENTIST: Philip Swango ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Several clinical studies have clearly shown that appreciable caries prevention can be obtained by treating the teeth semi-annually with an APF gel containing approximately 1% fluoride. Customarily topical treatment is preceded by a thorough prophylactic cleaning of the teeth surfaces. It has been suggested, however, that tooth cleaning may not be necessary or indeed be detrimental--that plaque would hold fluoride in proximity to the enamel and serve to increase effective treatment time. The value of prior prophylaxis should be determined so that the Program can properly advise on this technique.		
ACTIVITY: A 3-year longitudinal clinical trial with school children is being carried out to determine whether cleaning the teeth prior to semi-annual professionally applied topical fluoride prevents significantly more caries than topical treatment without prior cleaning. An appropriate study population of approximately 1500 school children, 11-13 years of age, is divided into 3 groups. Children in all groups receive periodic instruction in the proper dental hygiene and receive a semi-annual professional topical application of acidulated phosphate fluoride gel. Children in one group do not clean their teeth prior to the topical application. Those in the other groups either brush and floss their own teeth or receive a thorough dental prophylaxis prior to the topical treatment. Caries experience is recorded on NIH data forms and submitted to NCP Biometry Section.		
RESULTS: The clinical trial is underway. First year results will be available approximately October, 1980.		

CONTRACT

N01-DE-92421

REPORT PERIOD: FY 80

TOTAL PERIOD OF SUPPORT: 4/15/79 - 12/14/80

TITLE OF PROJECT: National Dental Caries Prevalence Survey.

CONTRACTOR INSTITUTION AND UNIT: Westat, Inc., Rockville, Md.  
PRINCIPAL INVESTIGATOR(S): Thomas McKenna  
ACTIVITY SITE: Nationwide

PROJECT SCIENTIST: Ann Miller and Janet Brunelle  
ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C and CP&R Branches

RELEVANCE TO NCP OBJECTIVES: Information on caries prevalence in children of different ages and in different areas of the U. S. would identify the populations at greatest risk, aid in deciding priority to be given to alternative caries preventive agents and delivery programs, and provide a baseline with which to measure changes in prevalence of the disease. If indications of increased or unchanged prevalence are found despite introduction of techniques that increase resistance of the host it would suggest that more attention should be given to caries promoting factors such as frequent consumption of sugary snacks.

ACTIVITY: Plan and conduct a baseline survey of the prevalence of caries among school aged children. The prevalence data will be for each of seven regions of the continental United States, be for single years of age by region, and be of sufficient precision to allow detection of a 15% change over time by single age and region.

PHASE I - Develop: (1) a sample survey, (2) plans for moving the examining team between sites and (3) plans for quality control.

PHASE II- Calibrate and standardize the examining teams and test the procedures.

PHASE III-Conduct all examinations in 1979-80 school year. Measure and record caries, gingivitis, dental treatment need and water F content.

Deliver results to NCP for processing by the Biometry section.

RESULTS: Over 40,000 sample children in grades K-12 were examined during 1979-80 school year and the data now is being analyzed. It is expected that results of the survey will be published in a series of monographs, of which the first "The Prevalence of Dental Caries in United States Children", will be available in January, 1981.

CONTRACT

NO1-DE- 92422

REPORT PERIOD: FY 80

TOTAL PERIOD OF SUPPORT: 9/27/79 - 9/26/82

TITLE OF PROJECT: Dental Plaque and Saliva from Gastric-Intubated Patients.

CONTRACTOR INSTITUTION AND UNIT: Columbia University

PRINCIPAL INVESTIGATOR(S): Solon Ellison

ACTIVITY SITE: New York

PROJECT SCIENTIST: David Klein

ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch

RELEVANCE TO NCP OBJECTIVES: Samples of dental plaque from different individuals or from laboratory animals given different dietary treatments are found to have contrasting ability to metabolize sucrose or other nutrients. Thus it is possible that plaque fermentative capacity might constitute the basis of a diagnostic measure of caries susceptibility.

In addition there are many unanswered questions concerning plaque including the range in microbial composition, differences in the matrix and physico-chemical properties, factors affecting its formation and metabolic properties.

ACTIVITY: Determine the effects of the absence of extraneous oral food supply on dental plaque composition and saliva constituents in patients who receive their entire diet by gastric intubation.

- 1) Obtain a study population of at least 25 persons who receive their diet by gastric intubation during the period of the study (at least 2 months). Obtain a matched control population.
- 2) Obtain baseline data on oral health and medical history and maintain a log of the intubated diet.
- 3) At intervals collect plaque separately from pits or fissures and smooth surfaces. Determine specified microbial and biochemical characteristics of the samples.
- 4) At intervals collect saliva samples and measure specified saliva characteristics.

RESULTS:



CONTRACT		N01-DE-02424
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 2/1/80 - 6/9/82	
TITLE OF PROJECT: Role of Dentists and Pediatricians in the Use of Caries Preventive Methods.		
CONTRACTOR INSTITUTION AND UNIT: American Dental Association PRINCIPAL INVESTIGATOR(S): Helen Gift ACTIVITY SITE: Chicago		
PROJECT SCIENTIST: Ralph Frew ORGANIZATIONAL POSITION: NIDR, NCP, OAD		
<p>RELEVANCE TO NCP OBJECTIVES: The NCP contracted with the ADA in 1973 to conduct a national survey of the knowledge, acceptance and use of caries-preventive methods among dentists and of the potential of dentists and dental professional groups to bring about the adoption of caries preventive programs. Information from the survey contributed to the decision to implement the national demonstration of community-run school-based fluoride mouthrinse programs.</p> <p>In view of potential new community-run programs it is important to obtain up-dated information on the attitudes and knowledge of practicing dentists and pediatricians concerning preventive programs.</p>		
<p>ACTIVITY: Through a national survey among dentists and pediatricians, determine the extent of knowledge about, use of, and advice to patients on caries preventive procedures and determine factors that affect these variables.</p> <ol style="list-style-type: none"> <li>1) Develop and submit from government clearance a pretest questionnaire. Compare results obtained with this questionnaire with those obtained a month later by interview. Development of the test questionnaire will be based on these results.</li> <li>2) Develop a sampling technique limited to the population of pediatricians and dentists who are professionally involved in the treatment and/or prevention of caries. The sample will be sufficiently large for results to be stratified with respect to specified independent variables.</li> <li>3) Conduct the survey using "follow-up" procedures to obtain maximum response rates.</li> <li>4) Perform specified analysis of the results.</li> </ol>		
RESULTS:		

CONTRACT		N01-DE- 02425
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 6/25/80 - 6/24/83	
TITLE OF PROJECT: Studies to Identify, Isolate, Develop and Test Naturally Occurring Non-Cariogenic Sweeteners that may be Used as Dietary Sucrose Substitutes.		
CONTRACTOR INSTITUTION AND UNIT: University of Illinois PRINCIPAL INVESTIGATOR(S): Douglas Kinghorn ACTIVITY SITE: Chicago		
PROJECT SCIENTIST: Andrew Vargosko ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Food manufacturers base their choice of available sweeteners for use in snacks, candies, desserts and soft drinks on: (1) whole-sale price per sweetness unit, price stability and availability of the sweetener; (2) market competitiveness of the food product that incorporates the sweetener; and (3) compatibility of the sweetener with food processing requirements, essential food characteristics and storage conditions. Based on these considerations the marked steady switch to use of syrups based on hydrolyzed starch and fructose-glucose mixtures, the huge sales of diet colas, willingness of a major pharmaceutical company to invest heavily in a production facility for a new sweetener, and other marketing events suggest convincingly that food manufacturers will readily convert to a non-cariogenic sweetener if its availability, cost, and other characteristics offer them a market advantage.		
ACTIVITY: Isolate, identify and physico-chemically characterize potentially non-cariogenic and commercially utilizable sweeteners: 1) including phyllo dulcin, perianthrins and osladin and <u>Stevia</u> species diterpene glycosides already isolated 2) found in 110 species of <u>Stevia</u> Evaluate the sweeteners with respect to: 1) stability and solubility, etc. 2) mutagenicity (Ames) and acute toxicity 3) sensory properties by taste panel		
RESULTS:		

CONTRACT		NO1-DE-02426
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 8/1/80 - 7/31/83	
TITLE OF PROJECT: Immunization of Rodents with Anti-Caries Vaccines containing non-Freund type Adjuvants.		
CONTRACTOR INSTITUTION AND UNIT: University of Alabama in Birmingham PRINCIPAL INVESTIGATOR(S): Suzanne Michalek ACTIVITY SITE: Birmingham		
PROJECT SCIENTIST: David Klein ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
<p>RELEVANCE TO NCP OBJECTIVES: Studies with rodents and monkeys have demonstrated increased titers of S-IgA and reduced dental caries after immunization with <u>S. mutans</u> close to the major salivary glands. The presence of Freund's complete adjuvant (FCA) in the immunization vehicle was associated with a prolonged elevation in salivary immunoglobulin titer.</p> <p>Preliminary results suggest that S-IgA responses also may be achieved by administering the <u>S. mutans</u> vaccine orally. Again there is evidence that muramyl dipeptide (MDP) adjuvants potentiate the immune response.</p> <p>At this time minimally toxic, but effective adjuvants need to be identified which are suitable for incorporation into an anti-caries vaccine.</p>		
<p>ACTIVITY: Compare potential adjuvants in formulating vaccines containing <u>S. mutans</u> antigens with respect to their ability to induce antibody, affect plaque flora, and reduce experimental dental caries.</p> <p>Phase I. Prepare reagents for measuring immunoglobulins of various types and establish standard methods of immunization and baseline data on immunoglobulin responses in rats.</p> <p>Phase II. Using adjuvants of the Al/Ca and the MDP categories measure vaccination locally with formalinized <u>S. mutans</u> and orally with live <u>S. mutans</u> with respect to serum antibody, effect on oral microbiology, localized irritation and caries experience.</p> <p>Part III. Using adjuvants found effective in Phase II, investigate vaccination with other antigens.</p> <p>Part IV. Explore oral inoculation with respect to adjuvants and responses.</p>		
RESULTS:		



CONTRACT		N01-DE- 02427
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 8/29/80 - 8/28/83	
TITLE OF PROJECT: Studies to Synthesize, Develop and Test Noncariogenic Sweeteners that may be Used as Dietary Sucrose Substitutes.		
CONTRACTOR INSTITUTION AND UNIT: Purdue University PRINCIPAL INVESTIGATOR(S): Roy Whistler ACTIVITY SITE: W. Lafayette, Ind.		
PROJECT SCIENTIST: Andrew Vargosko ORGANIZATIONAL POSITION: NIDR, NCP, CRG&C Branch		
RELEVANCE TO NCP OBJECTIVES: Food manufacturers base their choice of available sweeteners for use in snacks, candies, desserts and soft drinks on: (1) wholesale price per sweetness unit, price stability and availability of the sweetener; (2) market competitiveness of the food product that incorporates the sweetener; and (3) compatibility of the sweetener with food processing requirements, essential food characteristics and storage conditions. Based on these considerations the marked steady switch to use of syrups based on hydrolyzed starch and fructose-glucose mixtures, the huge sales of diet colas, willingness of a major pharmaceutical company to invest heavily in a production facility for a new sweetener, and other marketing events suggest convincingly that food manufacturers will readily convert to a non-cariogenic sweetener if its availability, cost, and other characteristics offer them a market advantage.		
ACTIVITY: Synthesize and physico-chemically characterize 31 specific sulfur-containing and other derivatives of simple sugars that potentially are non-cariogenic and commercially utilizable sweeteners. Evaluate the sweeteners with respect to: <ul style="list-style-type: none"> <li>1) stability and solubility, etc.</li> <li>2) mutagenicity (Ames) and acute toxicity</li> <li>3) sensory properties by taste panel</li> </ul>		
RESULTS:		

CONTRACT		N01-DE- 02428
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/22/80 - 9/21/83	
TITLE OF PROJECT: Studies to Synthesize, Develop and Test Noncariogenic Sweeteners that may be Used as Dietary Sucrose Substitutes.		
CONTRACTOR INSTITUTION AND UNIT: Research Triangle Institute PRINCIPAL INVESTIGATOR(S): Clarence Cook ACTIVITY SITE: Research Triangle Park, N. Carolina		
PROJECT SCIENTIST: William Rogers ORGANIZATIONAL POSITION: NIDR, NCP, OAD		
RELEVANCE TO NCP OBJECTIVES: Food manufacturers base their choice of available sweeteners for use in snacks, candies, desserts and soft drinks on: (1) wholesale price per sweetness unit, price stability and availability of the sweetener; (2) market competitiveness of the food product that incorporates the sweetener; and (3) compatibility of the sweetener with food processing requirements, essential food characteristics and storage conditions. Based on these considerations the market steady switch to use of syrups based on hydrolyzed starch and fructose-glucose mixtures, the huge sales of diet colas, willingness of a major pharmaceutical company to invest heavily in a production facility for a new sweetener, and other marketing events suggest convincingly that food manufacturers will readily convert to a non-cariogenic sweetener if its availability, cost, and other characteristics offer them a market advantage.		
ACTIVITY: Synthesize, identify and physico-chemically characterize potentially non-cariogenic and commercially utilizable sweeteners of the following four classes: 1) peptide sweeteners 2) sulfamates 3) acyclic saccharins and isothiourea derivatives 4) saturated analogs of the dihydrochalcone series Evaluate the sweeteners with respect to: 1) stability and solubility, etc. 2) mutagenicity (Ames) and acute toxicity 3) sensory properties by taste panel		
RESULTS:		

CONTRACT		N01-DE-02429
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/26/80 - 9/25/82	
TITLE OF PROJECT: Improved Methods of Delivering Cariostatic Agents to the Oral Cavity.		
CONTRACTOR INSTITUTION AND UNIT: Southern Research Inst. PRINCIPAL INVESTIGATOR(S): William Mayers ACTIVITY SITE: Birmingham, Alabama		
PROJECT SCIENTIST: William Bowen ORGANIZATIONAL POSITION: NIDR, NCP, CP&R Branch		
<p>RELEVANCE TO NCP OBJECTIVES: A number of natural and man-made substances have been shown to have cariostatic effects in animals. Some of these substances have been included in the food fed to experimental animals, thereby achieving maximum frequency of contact with either the tooth surface or plaque. However, a substantial number of these substances have been without cariostatic effect when tested in humans. It has been suggested that failures have in the main been due to the difficulty of maintaining the cariostatic substance in the mouth for sufficient duration for it to exercise its maximum effect.</p>		
<p>ACTIVITY: Develop techniques to gain improved access and prolonged retention time of cariostatic agents around caries-prone areas.</p> <ol style="list-style-type: none"> <li>1) Encapsulate NaF in oxidized starch as a free-flowing powder</li> <li>2) Determine F content of the microcapsules</li> <li>3) Study F release rates of unmodified and polylysine-modified microcapsules</li> <li>4) Study <u>in vitro</u> the retention of microcapsules to hydroxyapatite and other surfaces. The microcapsules will be tested with various surface charges</li> <li>5) Develop and study the retention of microcapsules coupled to anti-<u>S. mutans</u>, type d/g, antibody</li> <li>6) Prepare capsules suitable for use in humans</li> </ol>		
RESULTS:		



INTERAGENCY AGREEMENT (NO INTRAMURAL RESEARCH COMPONENT)		Y01-DE-00002
REPORT PERIOD: FY 80	IAA PERIOD: 5/15/80 - 9/30/80	
TITLE OF PROJECT: Production of Diagnostic Reagents Specific for <u>Streptococcus Mutans</u>		
AGENCY: Center for Disease Control PRINCIPAL INVESTIGATOR(S) William Harrell ACTIVITY SITE: Atlanta		
PROJECT OFFICER: Ariel Thomson ORGANIZATIONAL POSITION: NIDR, NCP, CPR Branch		
<p>RELEVANCE TO NCP OBJECTIVES: There is evidence that in the human the cariogenicity of <u>S. mutans</u> is restricted to a few of its at least 6 serotypes. This relationship can be established only by epidemiological studies involving enumeration of <u>S. mutans</u> serotypes in very large numbers of plaque samples. FA techniques have been developed to make serotype identification accurate and easy and protocols to produce the FA reagents have been worked out.</p> <p>Semi-bulk quantities of standardized FA reagent now are needed by NCP scientists to commence exploring the logistic problems of epidemiologic studies and for limited distribution to other laboratories.</p>		
<p>ACTIVITY: Produce and deliver to NIDR fluorescein and rhodamine-labeled antibody reagents specific for each of the 6 serotypes of <u>S. mutans</u> plus a polyvalent reagent. All reagents will be prepared using immunization schedules, fractionation procedures, fluorochroming methods and column immunosorption procedures specified by NCP and will be delivered in 2-ml aliquots in vials.</p> <p>Follow defined rigorous procedures to insure identity of the serotypes, purity of the vaccines, and high potency and specificity of immune sera and fluorescent conjugates.</p> <p>Deliver the diagnostic reagents within approximately 6 months.</p>		
RESULTS:		

Personal Services Contract		263-79-C-0656
REPORT PERIOD: FY 80	TOTAL PERIOD OF SUPPORT: 9/27/79 - 12/31/80	
TITLE OF PROJECT: Support Services on Predictive Analysis of Long-term Implications of Current Research in Caries Prevention.		
CONTRACTOR INSTITUTION AND UNIT: Applied Decision Analysis PRINCIPAL INVESTIGATOR(S): Allen Miller ACTIVITY SITE: Menlo Park, Calif.		
PROJECT SCIENTIST: William Rogers ORGANIZATIONAL POSITION: NIDR, NCP, OAD		
RELEVANCE TO NCP OBJECTIVES: Current decisions on the allocation of R&D resources to competing approaches to caries prevention should include estimates on various parameters of the R&D process such as cost, time and research success as well as estimates on measures of social utility such as cost, utilization and effectiveness, etc. Practical methodologies that would include the large number of R&D input variables, accommodate the imprecision of these values and compute interactions of probabilities do not exist as a consistent and comprehensive model of the overall R&D process.		
ACTIVITY: Expand the SRI model (NIH-75-C-410) to take into account research and development factors such as cost and time required to develop alternative techniques and the probability of success in achieving acceptable characteristics in the techniques. <ol style="list-style-type: none"> <li>1) Develop a model that closely simulates the repeated sequence of information gathering and decision making characteristic of the R&amp;D process.</li> <li>2) Develop an interactive computer program that solves the computations entailed in the model.</li> <li>3) Deliver the program and detailed instructions on its use to NCP.</li> </ol>		
RESULTS:		

# **Section IV**

# **GRANTS ACTIVITIES**

The following alphabetical and serial lists show the NCP research and training grants, fellowships, and career development awards that were active in FY 1980.





FY 1980  
Active Grants, Fellowships and Awards  
(serial listing)

-----Fellowships-----

F32	DE 05136	Lee, Sandra L.	Rush University	Properties and Function of Bone Phosphoprotein
F32	DE 05160	Wong, William	Temple University	Lipoteichoic Acid Excretion by Cariogenic Streptococci
F32	DE 05161	Parks, Lawrence C.	Temple University	Cell Wall in the Adherence of Cariogenic Bacteria
F32	DE 05164	Appelbaum, Benjamin	University of Pennsylvania	Molecular Basis of Plaque Formation
F32	DE 05185	Ferretti, Gerald A.	U. of Connecticut Health Center	Effect of Fluoride on Bacterial Attachment to Enamel
F32	DE 05189	McDowell, Thomas D.	Temple University	Studies of the Autolytic System of Streptococcus Mutans
F32	DE 05193	Zero, Domenick T.	Eastman Dental Center	Interactions Between Fluoride and Acquired Pellicles
F32	DE 05204	Habib, Charles M.	Tufts University	Mechanisms of Dental Caries Removal by GK 101 & GK 101E
F32	DE 05220	Thibodeau, Edward A.	University of Rochester	Fluoride and Oral Antimicrobial Systems
F32	DE 05227	Kral, Timothy A.	Temple University	Regulation of Autolysis in Streptococcus Mutans
F32	DE 05237	McGivney, Anne L.	U.S. National Institute of Health	Hybridoma Analysis of Actinomycete Coaggregation
F32	DE 5256	Hicks, M. John	University of Iowa	Effect of Calcifying Fluids on Acid-Etched Caries
F32	DE 05257	Drinkard, Carol	U. of N. C., Chapel Hill	Fluoride Effect on Proteins of Early Enamel Matrix

----Research Career Development Awards----

- K04 DE 00018 Skobe, Ziedonis Forsyth Dental Center  
Ultrastructure of Amelogenesis & Odontopathic Bacteria
- K04 DE 00024 Smith, Daniel J. Forsyth Dental Center  
Effects of Immunization on Experimental Dental Caries
- K04 DE 00050 Rawls, Henry R. La. S. U. Med. Ctr., New Orleans  
Anticaries Therapies--Polymer & Physical Chemistry
- K04 DE 00075 Ebersole, Jeffrey L. Forsyth Dental Center  
Relationship of Secretory Immunity to Dental Caries
- K04 DE 00081 Macrina, Francis L. Virginia Commonwealth University  
Plasmids of Oral Bacteria--Biomedical Implications
- K04 DE 00092 Michalek, Suzanne M. U. of Alabama in Birmingham  
Cellular Basis of Immunity to Dental Caries

----Research Grants----

- R01 DE 01554 Mandel, Irwin D. Columbia University  
Host Factors in Caries Resistance
- R01 DE 01830 Higuchi, William I. U. of Michigan at Ann Arbor  
Quantitation of Enamel Demineralization Mechanisms
- R01 DE 02901 Bleiweis, Arnold S. University of Florida  
Cell-Wall Antigens of Cariogenic Streptococci
- R01 DE 03011 Loesche, Walter J. U. of Michigan at Ann Arbor  
Effect of Chemotherapy on Plaque and Microbial Ecology
- R01 DE 03118 Smith, Eric E. University of Miami  
Inhibition of Dextranucrases of Cariogenic Organisms
- R01 DE 03180 Rosan, Burton University of Pennsylvania  
Microbiologic Studies of the Human Oral Streptococci
- R01 DE 03187 Moreno, Edgard C. Forsyth Dental Center  
Transport in Enamel and Solubility of Apatites
- R01 DE 03223 Nancollas, George H. S. U. of New York at Buffalo  
The Kinetics of Mineralization of Teeth
- R01 DE 03258 Kuramitsu, Howard K. Northwestern University  
Streptococcus Mutans: Dental Caries Mechanisms



R01	DE 03430	Kashket, Shelby	Forsyth Dental Center
		Interactions of Saliva Proteins and Bacteria	
R01	DE 03487	Shockman, Gerald D.	Temple University
		Inhibition of Human Cariogenic Streptococci	
R01	DE 03536	Shearer, Thomas R.	U. of Oregon Health Sci. Ctr.
		Selenium Metabolic Effects and Dental Caries	
R01	DE 03578	Robynt, John F.	Iowa S. U. of Science & Tech.
		Biosynthetic Study of Dental Plaque Polysaccharides	
R01	DE 03615	Perry, Dennis	Northwestern University
		Streptococcal Surface Antigens and Dental Caries	
R01	DE 03654	Schachtele, Charles F.	U. of Minnesota of Mnpls-St Paul
		Studies on the Molecular Basis of Dental Caries	
R01	DE 03708	Fuerstenau, Douglas W.	University of California, Berkeley
		Interfacial Properties of Apatite and Tooth Materials	
R01	DE 03713	Handelman, Stanley L.	Eastman Dental Center
		Effect of Fissure Sealant on Progress of Dental Caries	
R01	DE 03731	Mayer, Robert M.	Ohio State University
		Studies on the Dextran Sucrase of Streptococcus Sanguis	
R01	DE 03758	Tanzer, Jason M.	University of Connecticut Health Center
		Virulence Characterization in Streptococcus Mutans	
R01	DE 03758	Freedman, Michael L.	U. of Connecticut Health Center
		Virulence and Competition Against S Mutans	
R01	DE 03856	Shearer, Thomas R.	U. of Oregon Health Sci. Ctr.
		Fluoride-Selenium Interaction in Dental Caries	
R01	DE 03867	Shovlin, Francis E.	College of Med. & Dent. of N. J.
		Uptake of Hydroxyapatite Phosphorus by Oral Bacteria	
R01	DE 03915	Hay, Donald I.	Forsyth Dental Center
		Tooth-Saliva Interface Phenomena and Dental Caries	
R01	DE 03917	Kashket, Shelby	Forsyth Dental Center
		Fluoride and the Metabolism of Plaque Bacteria	
R01	DE 03993	Kleinberg, Israel	S. U. New York Stony Brook
		Effect of Saliva on the Metabolism of Dental Plaque	
R01	DE 04061	Evans, Richard T.	S. U. of New York at Buffalo
		Salivary Antibodies to S. Mutans: Induction and Effects	

- R01 DE 04068 Fleiss, Joseph L. Columbia University  
Statistical Methods in Dental Research
- R01 DE 04077 Kabara, Jon J. Michigan State University  
Specific Dietary Lipids as Anticariogenic Agents
- R01 DE 04116 Chang, Yung-Feng University of Maryland at Baltimore  
Amino Acids and Cell Wall Biosynthesis
- R01 DE 04123 Le Geros, Racquel Z. New York University  
Enamel Apatites Relating to Caries Susceptibility
- R01 DE 04174 Knox, Kenneth W. Institute of Dental Research, Sydney, A.  
Variations in the Surface Structure of Oral Bacteria
- R01 DE 04175 Wicken, Anthony J. University of New South Wales  
Variations in the Surface Structures of Oral Bacteria
- R01 DE 04192 Jordan, Truman H. Cornell College  
SnF<sub>2</sub> Reaction with Apatite and Other Ca/PO<sub>4</sub> Systems
- R01 DE 04217 McGhee, Jerry R. U. of Alabama in Birmingham  
Effective Immunity to Dental Caries: Cellular Basis
- R01 DE 04224 Macrina, Francis L. Virginia Commonwealth University  
Studies on the Genetics of Oral Microflora
- R01 DE 04235 Thomas, Edwin L. St. Jude Children's Research Hospital  
Peroxidase in Saliva and Prevention of Oral Disease
- R01 DE 04265 Fitzgerald, Dorothea B. University of Miami  
Cariogenesis of Plaque Bacteria in Test Models
- R01 DE 04278 Cowman, Richard A. University of Miami  
Human Saliva-Streptococcal Metabolic Interactions
- R01 DE 04296 Pollock, Jerry J. S. U. New York Stony Brook  
Lysozyme-Cell Surface Interactions and Oral Defense
- R01 DE 004303 Nikias, Mata K. Columbia University  
Compliance Factors in Caries Prevention
- R01 DE 04321 McCabe, Mead M. University of Miami  
Cell Adherence of Dental Plaque Forming Streptococci
- R01 DE 04330 Curzon, Martin E. Eastman Dental Center  
Strontium and Dental Caries
- R01 DE 04385 Brown, Walter E. American Dental Assn. Health Fdn.  
Mechanism of Dental Caries

- R01 DE 04431 Little, Marguerite F. Eastman Dental Center  
Trace Element Influence on Caries In Vitro
- R01 DE 04464 Ellen, Richard P. University of Toronto  
Longitudinal Study of Dental Root Surface Caries
- R01 DE 04465 Banting, David W. University of Western Ontario  
Longitudinal Study of Dental Root Surface Caries
- R01 DE 04486 Wefel, James S. University of Iowa  
Kinetics and Mechanisms of Action of Fluorides
- R01 DE 04504 Burt, Brian A. U. of Michigan at Ann Arbor  
Plaque Bacteria as Predictors of Human Dental Caries
- R01 DE 04518 Levine, Michael J. S. U. of New York at Buffalo  
Streptococcus Sanguis Receptors--Salivary Glycoproteins
- R01 DE 04529 Hillman, Jeffrey D. Forsyth Dental Center  
Replacement Therapy of Dental Caries
- R01 DE 04542 Brown, Lee R. U. of Texas Health Sci Ctr, Houston  
Study of the Antimicrobial Effects of Fluoride
- R01 DE 04600 Fox, Jeffrey L. U. of Michigan at Ann Arbor  
Hydroxyapatite Remineralization: The Role of Fluoride
- R01 DE 04614 Liljemark, William F. U. of Minnesota of Mnpls-St Paul  
Adherence of Dental Plaque Bacteria to Hydroxyapatite
- R01 DE 04615 Whanger, Philip D. Oregon State University  
Fluoride-Cadmium Interaction in Dental Caries
- R01 DE 04616 Shearer, Thomas R. U. of Oregon Health Sci. Ctr.  
Fluoride-Cadmium Interaction in Dental Caries
- R01 DE 04666 Staat, Robert H. University of Louisville  
Dental Plaque Glucanohydrolases and Caries Formation
- R01 DE 04705 Jordan, Truman H. Cornell College  
Reactions of Titanium Fluoride with Hydroxyapatite
- R01 DE 04733 Taubman, Martin A. Forsyth Dental Center  
Secretory Immune Response and S. Mutans Enzyme Antigens
- R01 DE 04749 Rawls, Henry R. La. S. U. Med. Ctr., New Orleans  
F-Releasing and Acid Barrier-Polymer Caries Therapies
- R01 DE 04760 Calhoun, Noah R. Howard University  
Zinc Deficiency and Dental Caries



- R01 DE 04795 Minah, Glenn E. University of Maryland at Baltimore  
Characteristics of Cariogenic Dental Plaque
- R01 DE 04819 Silverstone, Leon M. University of Iowa  
Remineralization of Enamel Caries in Vitro
- R01 DE 04835 Clarkson, Brian H. University of Iowa  
Anti-Caries Mechanisms of Fluoride Complexes: In Vitro
- R01 DE 04903 Byers, Benjamin R. U. of Mississippi Medical Center  
Trace Metal Uptake in Cariogenic Streptococcus Mutans
- R01 DE 04904 Phillips, Ralph W. Ind. U.-Purdue U. at Indianapolis  
Fluoride Distr. in Teeth Using Silicate Cement Model
- R01 DE 04926 McIntire, Floyd C. University of Colorado Medical Ctr.  
Bacterial Coaggregation Mechanisms in Dental Plaque
- R01 DE 04938 Kolakowski, Donald L. U. of Connecticut Health Center  
A Study of Host Factors in Caries Susceptibility
- R01 DE 04957 Pieringer, Ronald A. Temple University  
The Role of Bacterial Metabolites in Oral Diseases
- R01 DE 05017 Linzer, Rosemary S. U. of New York at Buffalo  
Characterization of Surface Antigens of S Mutans
- R01 DE 05018 Chiu, Teh-Hsing University of Pittsburgh  
Cell Envelope Synthesis and Control of Oral Microbes
- R01 DE 05027 Yotis, William W. Loyola University Medical Center  
Binding of Fluoride by Cariogenic Bacteria
- R01 DE 05059 Schlesinger, David H. University of Illinois Medical Ctr.  
Protein Inhibitors of Calcium Phosphate Precipitation
- R01 DE 05067 Gron, Paul Forsyth Dental Center  
Interaction of Topical Fluoride with Human Enamel
- R01 DE 05102 Doyle, Ronald J. University of Louisville  
Potential Anticaries Agents
- R01 DE 05115 Silversin, Jacob B. Harvard University  
Diffusion of Preventive Health Measures to Schools
- R01 DE 05141 Kuramitsu, Howard K. Northwestern University  
Cariogenic Mechanisms of Gingival Plaque Bacteria
- R01 DE 05180 Daneo-Moore, Lolita Temple University  
Composition of S. Mutans in Different Growth Environment

- R01 DE 05253 Brudevold, Finn Forsyth Dental Center  
Measurements of Enamel Permeability as Related to Caries
- R01 DE 05289 Bawden, James W. U. of N. C., Chapel Hill  
F Binding by Early Enamel Matrix Proteins
- R01 DE 05327 Anbar, Michael S. U. of New York at Buffalo  
Polymeric Polyphosphonates on Dental Caries and Plaque
- R01 DE 05354 Brown, Walter E. American Dental Assn Health Fdn  
Prevention of Dental Caries
- R01 DE 05466 Mitoma, Chozo SRI International  
Biochemical Studies of a Sweetener, SRI Oxime V
- R01 DE 05476 Goodman, Murray U. of California San Diego  
Novel Peptide Derived Sweeteners
- R01 DE 05510 Curzon, Martin E. Eastman Dental Center  
Physico-Chemistry of Strontium in Caries Lesions
- R01 DE 05511 Hefferren, John J. American Dental Assn Health Fdn  
Evanston-Oak Park Fluoridation Study After 25 Years
- R01 DE 05518 Pullman, Ira New York University  
Mineral-Organic Interactions in the Oral Milieu
- R01 DE 05531 Ellison, Solon A. Columbia University  
Salivary Immune Factors
- R01 DE 05596 Rawls, Henry R. La. S. U. Med. Ctr., New Orleans  
Topically-Applied Polymers for Caries Prevention
- R01 DE 05606 Fives-Taylor, Paula M. U. of Vermont & St. Agric. College  
Pili of *S Sanguis* and Their Role in Adhesion
- R01 DE 05722 Arnold, Roland R. University of Louisville  
Salivary Lactoferrin and Regulation of the Oral Flora

-----Conference Grants-----

- R13 DE 05192 Jenny, Joanna University of Iowa  
Conference on Ethics in Human Dental Caries Research
- R13 DE 05389 Tanzer, Jason M. U. of Connecticut Health Center  
Symposium and Workshop on Animal Models in Cariology

----Young Investigator Awards----

- R23 DE 04771 Peri, Barbara M. University of Chicago  
Development of Immunity to Cariogenic Strep
- R23 DE 04795 Minah, Glenn E. University of Maryland at Baltimore  
Development of Fissural Dental Plaque
- R23 DE 05039 Bozzola, John J. Medical College of Pennsylvania  
A Ribosomal Vaccine Against Dental Caries
- R23 DE 05071 Choih, Sun-Jin U. of Minnesota of Mnpls-St Paul  
Oral Bacteria, Proteases and Human Salivary Proteins
- R23 DE 05240 Haber, Jerome Tufts University  
Immunological Studies--Caries and Peridontal Disease
- R23 DE 05357 Berkowitz, Robert J. University of Pennsylvania  
Maternal Transmission of Streptococcus Mutants
- R23 DE 05436 Ryan, Vivian W. Stevens Institute of Technology  
Salivary Proteins in Bacterial Aggregation/Adherence
- R23 DE 05501 Herzberg, Mark C. U. of Minnesota of Mnpls-St Paul  
Platelet-Streptococcal Interactions in Endocarditis
- R23 DE 05628 Cerklewski, Florian L. Oregon State University  
Influence of Trace Metals on Dental Health

----Training Grants----

- T32 DE 07014 Katz, Ralph V. U. of Minnesota of Mnpls-St Paul  
Postdoctoral Training in Dental Caries Research
- T32 DE 07026 Retief, Hugo U. of Alabama in Birmingham  
Caries Research



FY 1980  
Active Grants, Fellowships and Awards  
(alphabetical listing)

----Fellowships----

Appelbaum, Benjamin	University of Pennsylvania	F32	DE 05164
Molecular Basis of Plaque Formation			
Drinkard, Carol	U. of N.C., Chapel Hill	F32	DE 05257
Fluoride Effect on Proteins of Early Enamel Matrix			
Ferretti, Gerald A.	U. of Connecticut Health Center	F32	DE 05185
Effect of Fluoride on Bacterial Attachment to Enamel			
Habib, Charles M.	Tufts University	F32	DE 05204
Mechanisms of Dental Caries Removal by GK 101 & GK 101E			
Hicks, M. John	University of Iowa	F32	DE 05256
Effect of Calcifying Fluids on Acid-Etched Caries			
Kral, Timothy A.	Temple University	F32	DE 05227
Regulation of Autolysis in Streptococcus Mutans			
Lee, Sandra L.	Rush University	F32	DE 05136
Properties and Function of Bone Phosphoprotein			
McDowell, Thomas D.	Temple University	F32	DE 05189
Studies of the Autolytic System of Streptococcus Mutans			
McGivney, Anne L.	U.S. National Institute of Health	F32	DE 05237
Hybridoma Analysis of Actinomycete Coaggregation			
Parks, Lawrence C.	Temple University	F32	DE 05161
Cell Wall in the Adherence of Cariogenic Bacteria			
Thibodeau, Edward A.	University of Rochester	F32	DE 05220
Fluoride and Oral Antimicrobial Systems			
Wong, William	Temple University	F32	DE 05160
Lipoteichoic Acid Excretion by Cariogenic Streptococci			
Zero, Domenick T.	Eastman Dental Center	F32	DE 05193
Interactions Between Fluoride and Acquired Pellicles			

-----Research Career Development Awards-----

Ebersole, Jeffrey L.	Forsyth Dental Center	K04	DE 00075
Relationship of Secretary Immunity to Dental Caries			
Macrina, Francis L.	Virginia Commonwealth University	K04	DE 00081
Plasmids of Oral Bacteria--Biomedical Implications			
Michalek, Suzanne M.	U. of Alabama in Birmingham	K04	DE 00092
Cellular Basis of Immunity to Dental Caries			
Rawls, Henry R.	La. S.U. Med. Ctr., New Orleans	K04	DE 00050
Anticaries Therapies--Polymer & Physical Chemistry			
Skobe, Ziedonis	Forsyth Dental Center	K04	DE 00018
Ultrastructure of Amelogenesis & Odontopathic Bacteria			
Smith, Daniel J.	Forsyth Dental Center	K04	DE 00024
Effects of Immunization on Experimental Dental Caries			

-----Research Grants-----

Anbar, Michael	S.U. of New York at Buffalo	R01	DE 05327
Polymeric Polyphosphonates on Dental Caries and Plaque			
Arnold, Roland R.	University of Louisville	R01	DE 05722
Salivary Lactoferrin and Regulation of the Oral Flora			
Banting, David W.	University of Western Ontario	R01	DE 04465
Longitudinal Study of Dental Root Surface Caries			
Bawden, James W.	U. of N.C., Chapel Hill	R01	DE 05289
F Binding by Early Enamel Matrix Proteins			
Bleiweis, Arnold S.	University of Florida	R01	DE 02901
Cell-Wall Antigens of Cariogenic Streptococci			
Brown, Lee R.	U. of Texas Health Sci Ctr, Houston	R01	DE 04542
Study of the Antimicrobial Effects of Fluoride			
Brown, Walter E.	American Dental Assn Health Fdn	R01	DE 04385
Mechanism of Dental Caries			
Brown, Walter E.	American Dental Assn Health Fdn	R01	DE 05354
Prevention of Dental Caries			
Brudevold, Finn	Forsyth Dental Center	R01	DE 05253
Measurements of Enamel Permeability as Related to Caries			

Burt, Brian A.	U. of Michigan at Ann Arbor	R01	DE 04504
Plaque Bacteria as Predictors of Human Dental Caries			
Byers, Benjamin R.	U. of Mississippi Medical Center	R01	DE 04903
Trace Metal Uptake in Cariogenic Streptococcus Mutans			
Calhoun, Noah R.	Howard University	R01	DE 04760
Zinc Deficiency and Dental Caries			
Chang, Yung-Feng	University of Maryland at Baltimore	R01	DE 04116
Amino Acids and Cell Wall Biosynthesis			
Chiu, Teh-Hsing	University of Pittsburgh	R01	DE 05018
Cell Envelope Synthesis and Control of Oral Microbes			
Clarkson, Brian H.	University of Iowa	R01	DE 04835
Anti-Caries Mechanisms of Fluoride Complexes: In Vitro			
Cowman, Richard A.	University of Miami	R01	DE 04278
Human Saliva--Streptococcal Metabolic Interactions			
Curzon, Martin E.	Eastman Dental Center	R01	DE 04330
Strontium and Dental Caries			
Curzon, Martin E.	Eastman Dental Center	R01	DE 05510
Physico-Chemistry of Strontium in Caries Lesions			
Daneo-Moore, Lolita	Temple University	R01	DE 05180
Composition of S. Mutans in Different Growth Environment			
Doyle, Ronald J.	University of Louisville	R01	DE 05102
Potential Anticaries Agents			
Ellen, Richard P.	University of Toronto	R01	DE 04464
Longitudinal Study of Dental Root Surface Caries			
Ellison, Solon A.	Columbia University	R01	DE 05531
Salivary Immune Factors			
Evans, Richard T.	S.U. of New York at Buffalo	R01	DE 04061
Salivary Antibodies to S. Mutans: Induction and Effects			
Fitzgerald, Dorothea B.	University of Miami	R01	DE 04265
Cariogenesis of Plaque Bacteria in Test Models			
Fives-Taylor, Paula M.	U. of Vermont & St. Agric. College	R01	DE 05606
Pili of S Sanguis and Their Role in Adhesion			
Fleiss, Joseph L.	Columbia University	R01	DE 04068
Statistical Methods in Dental Research			



Fox, Jeffrey L.	U. of Michigan at Ann Arbor	R01	DE 04600
Hydroxyapatite Remineralization: The Role of Fluoride			
Freedman, Michael L.	U. of Connecticut Health Center	R01	DE 03758
Virulence and Competition Against S Mutans			
Fuerstenau, Douglas W.	University of California, Berkeley	R01	DE 03708
Interfacial Properties of Apatite and Tooth Materials			
Goodman, Murray	U. of California San Diego	R01	DE 05476
Novel Peptide Derived Sweeteners			
Gron, Paul	Forsyth Dental Center	R01	DE 05067
Interaction of Topical Fluoride with Human Enamel			
Handelman, Stanley L.	Eastman Dental Center	R01	DE 03713
Effect of Fissure Sealant on Progress of Dental Caries			
Hay, Donald I.	Forsyth Dental Center	R01	DE 03915
Tooth-Saliva Interface Phenomena and Dental Caries			
Hefferren, John J.	American Dental Assn Health Fdn	R01	DE 05511
Evanston-Oak Park Fluoridation Study After 25 Years			
Higuchi, William I.	U. of Michigan at Ann Arbor	R01	DE 01830
Quantitation of Enamel Demineralization Mechanisms			
Hillman, Jeffrey D.	Forsyth Dental Center	R01	DE 04529
Replacement Therapy of Dental Caries			
Jordan, Truman H.	Cornell College	R01	DE 04192
$\text{SnF}_2$ Reaction with Apatite and Other $\text{Ca}/\text{PO}_4$ Systems			
Jordan, Truman H.	Cornell College	R01	DE 04705
Reactions of Titanium Fluoride with Hydroxyapatite			
Kabara, Jon J.	Michigan State University	R01	DE 04077
Specific Dietary Lipids as Anticariogenic Agents			
Kashket, Shelby	Forsyth Dental Center	R01	DE 03430
Interactions of Saliva Proteins and Bacteria			
Kashket, Shelby	Forsyth Dental Center	R01	DE 03917
Fluoride and the Metabolism of Plaque Bacteria			
Kleinberg, Israel	S. U. New York Stony Brook	R01	DE 03993
Effect of Saliva on the Metabolism of Dental Plaque			
Knox, Kenneth W.	Institute of Dental Research, Sydney, A.	R01	DE 04174
Variations in the Surface Structure of Oral Bacteria			

Kolakowski, Donald L.	U. of Connecticut Health Center	R01	DE 04938
A Study of Host Factors in Caries Susceptibility			
Kuramitsu, Howard K.	Northwestern University	R01	DE 03258
Streptococcus Mutans: Dental Caries Mechanisms			
Kuramitsu, Howard K.	Northwestern University	R01	DE 05141
Cariogenic Mechanisms of Gingival Plaque Bacteria			
Le Geros, Racquel Z.	New York University	R01	DE 04123
Enamel Apatites Relating to Caries Susceptibility			
Levine, Michael J.	S. U. of New York at Buffalo	R01	DE 04518
Streptococcus Sanguis Receptors--Salivary Glycoproteins			
Liljemark, William F.	U. of Minnesota of Mnpls-St Paul	R01	DE 04614
Adherence of Dental Plaque Bacteria to Hydroxyapatite			
Linzer, Rosemary	S. U. of New York at Buffalo	R01	DE 05017
Characterization of Surface Antigens of S Mutans			
Little, Marguerite F.	Eastman Dental Center	R01	DE 04431
Trace Element Influence on Caries In Vitro			
Loesche, Walter J.	U. of Michigan at Ann Arbor	R01	DE 03011
Effect of Chemotherapy on Plaque and Microbial Ecology			
Macrina, Francis L.	Virginia Commonwealth University	R01	DE 04224
Studies on the Genetics of Oral Microflora			
Mandel, Irwin D.	Columbia University	R01	DE 01554
Host Factors in Caries Resistance			
Mayer, Robert M.	Ohio State University	R01	DE 03731
Studies on the Dextran Sucrase of Streptococcus Sanguis			
McCabe, Mead M.	University of Miami	R01	DE 04321
Cell Adherence of Dental Plaque Forming Streptococci			
McGhee, Jerry R.	U. of Alabama in Birmingham	R01	DE 04217
Effective Immunity to Dental Caries: Cellular Basis			
McIntire, Floyd C.	University of Colorado Medical Ctr.	R01	DE 04926
Bacterial Coaggregation Mechanisms in Dental Plaque			
Minah, Glenn E.	University of Maryland at Baltimore	R01	DE 04795
Characteristics of Cariogenic Dental Plaque			
Mitoma, Chozo	SRI International	R01	DE 05466
Biochemical Studies of a Sweetener, SRI Oxime V			

Moreno, Edgard C.	Forsyth Dental Center	R01	DE 03187
Transport in Enamel and Solubility of Apatites			
Nancollas, George H.	S. U. of New York at Buffalo	R01	DE 03223
The Kinetics of Mineralization of Teeth			
Nikias, Mata K.	Columbia University	R01	DE 04303
Compliance Factors in Caries Prevention			
Perry, Dennis	Northwestern University	R01	DE 03615
Streptococcal Surface Antigens and Dental Caries			
Phillips, Ralph W.	Ind. U.-Purdue U. at Indianapolis	R01	DE 04904
Fluoride Distr. in Teeth Using Silicate Cement Model			
Pieringer, Ronald A.	Temple University	R01	DE 04957
The Role of Bacterial Metabolites in Oral Diseases			
Pollock, Jerry J.	S. U. New York Stony Brook	R01	DE 04296
Lysozyme-Cell Surface Interactions and Oral Defense			
Pullman, Ira	New York University	R01	DE 05518
Mineral-Organic Interactions in the Oral Milieu			
Rawls, Henry R.	La. S. U. Med. Ctr., New Orleans	R01	DE 04749
F-Releasing and Acid Barrier-Polymer Caries Therapies			
Rawls, Henry R.	La. S. U. Med. Ctr., New Orleans	R01	DE 05596
Topically-Applied Polymers for Caries Prevention			
Roby, John F.	Iowa S. U. of Science & Tech.	R01	DE 03578
Biosynthetic Study of Dental Plaque Polysaccharides			
Rosan, Burton	University of Pennsylvania	R01	DE 03180
Microbiologic Studies of the Human Oral Streptococci			
Schachtele, Charles F.	U. of Minnesota of Mnpls-St Paul	R01	DE 03654
Studies on the Molecular Basis of Dental Caries			
Schlesinger, David H.	University of Illinois Medical Center	R01	DE 05059
Protein Inhibitors of Calcium Phosphate Precipitation			
Shearer, Thomas R.	U. of Oregon Health Sci. Ctr.	R01	DE 03536
Selenium Metabolic Effects and Dental Caries			
Shearer, Thomas R.	U. of Oregon Health Sci. Ctr.	R01	DE 03856
Fluoride-Selenium Interaction in Dental Caries			
Shearer, Thomas R.	U. of Oregon Health Sci. Ctr.	R01	DE 04616
Fluoride-Cadmium Interaction in Dental Caries			



Shockman, Gerald D.	Temple University	R01	DE 03487
Inhibition of Human Cariogenic Streptococci			
Shovlin, Francis E.	College of Med. & Dent. of N. J.	R01	DE 03867
Uptake of Hydroxyapatite Phosphorus by Oral Bacteria			
Silversin, Jacob B.	Harvard University	R01	DE 05115
Diffusion of Preventive Health Measures to Schools			
Silverstone, Leon M.	University of Iowa	R01	DE 04819
Remineralization of Enamel Caries in Vitro			
Smith, Eric E.	University of Miami	R01	DE 03118
Inhibition of Dextranucrases of Cariogenic Organisms			
Staat, Robert H.	University of Louisville	R01	DE 04666
Dental Plaque Glucanohydrolases and Caries Formation			
Tanzer, Jason M.	University of Connecticut Health Center	R01	DE 03758
Virulence Characterization in Streptococcus Mutans			
Taubman, Martin A.	Forsyth Dental Center	R01	DE 04733
Secretory Immune Response and S. Mutans Enzyme Antigens			
Thomas, Edwin L.	St. Jude Children's Research Hospital	R01	DE 04235
Peroxidase in Saliva and Prevention of Oral Disease			
Wefel, James S.	University of Iowa	R01	DE 04486
Kinetics and Mechanisms of Action of Fluorides			
Whanger, Philip D.	Oregon State University	R01	DE 04615
Fluoride-Cadmium Interaction in Dental Caries			
Wicken, Anthony J.	University of New South Wales, Kens.,A.	R01	DE 04175
Variations in the Surface Structures of Oral Bacteria			
Yotis, William W.	Loyola University Medical Center	R01	DE 05027
Binding of Fluoride by Cariogenic Bacteria			

----Conference Grants----

Jenny, Joanna	University of Iowa	R13	DE 05192
Conference on Ethics in Human Dental Caries Research			
Tanzer, Jason M.	U. of Connecticut Health Center	R13	DE 05389
Symposium and Workshop on Animal Models in Cariology			

----Young Investigator Awards----

Berkowitz, Robert J.	University of Pennsylvania	R23	DE 05357
Maternal Transmission of Streptococcus Mutants			
Bozzola, John J.	Medical College of Pennsylvania	R23	DE 05039
A Ribosomal Vaccine Against Dental Caries			
Cerklewski, Florian L.	Oregon State University	R23	DE 05628
Influence of Trace Metals on Dental Health			
Choih, Sun-Jin	U. of Minnesota of Mnpls-St Paul	R23	DE 05071
Oral Bacteria, Proteases and Human Salivary Proteins			
Haber, Jerome	Tufts University	R23	DE 05240
Immunological Studies--Caries and Peridontal Disease			
Herzberg, Mark C.	U. of Minnesota of Mnpls-St Paul	R23	DE 05501
Platelet-Streptococcal Interactions in Endocarditis			
Minah, Glenn E.	University of Maryland at Baltimore	R23	DE 04795
Development of Fissural Dental Plaque			
Peri, Barbara M.	University of Chicago	R23	DE 04771
Development of Immunity to Cariogenic Strep			
Ryan, Vivian W.	Stevens Institute of Technology	R23	DE 05436
Salivary Proteins in Bacterial Aggregation/Adherence			

----Training Grants----

Katz, Ralph V.	U. of Minnesota of Mnpls-St Paul	T32	DE 07014
Postdoctoral Training in Dental Caries Research			
Retief, Hugo	U. of Alabama in Birmingham	T32	DE 07026
Caries Research			

PART III

NATIONAL INSTITUTE OF DENTAL RESEARCH

ANNUAL REPORT

EXTRAMURAL PROGRAMS

October 1, 1979 - September 30, 1980

Edited By

Special Assistant for Program Coordination

Extramural Programs

*This document was prepared for administrative use at NIH. The comments and declarations of its contributors are their own and do not necessarily represent an official statement of the Institute.*

Compiled By

Dental Research Data Officer

National Institute of Dental Research

National Institutes of Health

Bethesda, Maryland





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## REPORT OF THE EXTRAMURAL PROGRAMS

NATIONAL INSTITUTE OF DENTAL RESEARCH

October 1, 1979 - September 30, 1980

by

Dr. Clair L. Gardner

Associate Director for Extramural Programs

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. The health scientist administrators who develop and manage these extramural programs encourage a high level of interest in oral biology throughout the scientific community. In the planning and implementation of specific research programs, they carefully consider recommendations from advisory committees, special consultants and other NIH staff. As a result of this collaboration, the NIDR Extramural Programs (NIDR-EP) have been able to maintain strong basic research programs. These programs have set the stage for current efforts to develop and expand coordinated clinical research programs in the fields of periodontal disease, oral soft tissue diseases, dental implantology, behavioral science and dental pain. The progress achieved in these areas is reflected in this report.

### PERSONNEL AND ADMINISTRATION

During FY 1980 there were personnel changes involving two full-time positions. Dr. David Wolff, a former NIH Grants Associate, accepted an appointment as a Health Scientist Administrator in the Soft Tissue Stomatology and Nutrition Program and Ms. Tanya McCoy accepted the position of Grants Management Specialist in the Grants Management Section of NIDR-EP. Before coming to the NIH, Dr. Wolff had been Professor of Microbiology at Ohio State University. The Grants Management Specialist position was formerly held by Ms. Robin Rosen, who resigned early in FY1980.

Continuing efforts were made to maintain a favorable work environment at NIDR-EP for all employees, including those with physical handicaps as well as other problems. For example, during the past year one employee voluntarily provided special training to a handicapped employee in the operation of a computerized travel reporting system. This effort was encouraged and applauded by her coworkers. Employees work well together routinely, and when short deadlines must be met and smooth teamwork is necessary, they show a special spirit of cooperation.

During FY 1980 NIDR-EP professional staff expended considerable effort on tasks related to the implementation of the Civil Service Reform Act. In these activities, they prepared comprehensive definitions of the major responsibilities of the managerial positions in question (GS13-15), described the importance of these responsibilities and set performance objectives and standards of achievement. In addition, they held periodic interviews to assess actual performance achievement in the light of the criteria which had been developed. Subsequently, a thorough review was made of available information to determine whether recommendations for merit pay increases were warranted.

During FY 1980 four NIDR-EP employees received NIH awards. Dr. Richard Christiansen, Chief, Craniofacial Anomalies Program, received the PHS Commendation Medal and Mr. Robert Ginsburg, Grants Management Officer, received an NIH Merit Award. Quality Increase awards were given to Ms. Mary Arnot and Ms. Frances Gaetano.

The Scientific Review Branch, established four years ago to separate review from program activities, continued to function in an effective manner. In FY 1980, staff of this Branch conducted 14 project site visits and convened 3 meetings of the NIDR Special Grants Review Committee, which reviewed 13 Institutional National Research Service Award (NRSA) applications requesting \$7.8 million and one Dental Research Institute/Center application requesting \$7.6 million. The Scientific Review Branch also conducted 4 No-Study-Section reviews. In addition, staff prepared summary statements of these 18 initial reviews for secondary review by the National Advisory Dental Research Council.

The Office of Centers and Special Programs provided fiscal and administrative support for the five Dental Research Institutes and Centers and provided staff to work with representatives of the Division of Research Resources (DRR) on the Minority Biomedical Support (MBS) Program. The Chief of this office also administered Short Term Training grants, served as the NIDR liaison to the National Institute on Aging (NIA) in the administration of the Geriatric Dentistry Academic Awards, and served as the NIDR representative to DRG to resolve problems of assignment and review.

#### STAFF ACTIVITIES

The Special Assistant for Program Coordination served as editor of the annual reports and of written material prepared for various purposes such as the Congressional Budget Justification, the NIDR Research Plan, the NIH Research Highlights and responses to Congressional inquiries. He also conducted staff meetings, represented the Institute as a member of the Diabetes Mellitus Coordinating Committee, and made several site visits for monitoring and programming. In addition, he made formal presentations at a joint AADS/AADR symposium (training), at the New York Academy of Dentistry Fifth Annual Seminar (periodontal disease), at the Annual Meeting of the Buffalo, New York OKU Society (NIDR clinical research), and at the Tenth Annual James A. English Symposium (summary speaker).



The Special Assistant for Research Manpower served as Chairman of the Staff Executive Committee, which provides secondary review of Fellowship applications, and of the Fellowship Advisory Committee, which advises the Director, NIDR, on NRSA Fellowship policies and practices. He also served as executive secretary of the Dental Research Institute and Special Programs Advisory Committee when it reviewed institutional fellowship grant applications; conducted site visits for program projects, center applications and research grants; and conducted No-Study-Section reviews of conference grant applications. In addition, he prepared written material on NRSA payback guidelines and on current and future manpower support (in reply to NSF request), and developed projections for clinical research training.

Extramural program staff made 53 visits to institutions and participated in 40 different scientific meetings to keep abreast of scientific developments, monitor research progress, and maintain close liaison with the scientific community. In addition to their informal contributions to these activities, staff made 16 formal presentations at different meetings. During FY 1980 staff participated in the publication of the proceedings of 4 State of the Art Workshops and one consensus development conference. They also attended 8 courses for professional development. Information on program priorities for federal funding was again disseminated by staff at the annual meeting of the American Association of Dental Schools, and the meeting of the American Association for Dental Research (AADR) in Los Angeles. At the AADR meeting, staff maintained a consultation room to inform scientists of research opportunities, resolve problems, and encourage young investigators to develop research plans.

NIDR-sponsored meetings supported by grant, contract or other funds, included one international meeting, 4 large scientific conferences, and one NIH consensus development conference. These funds were made available primarily for travel expenses incurred by participants. For the IADR meeting in Osaka, Japan, a block grant of \$75,000 was made to the organization to provide partial travel support for 75 NIDR grantees presenting papers. The consensus development conference (on the removal of third molars) was well attended and the published results have been widely disseminated and favorably received by the professional community. Two of the meetings were funded jointly with other institutes through arrangements made by the Fogarty International Center (FIC). The titles of the meetings are listed below.

1. International Association for Dental Research  
Annual Meeting, Osaka, Japan (Block travel grant) June 1980
2. International Conference on Human Herpes Viruses,  
Atlanta (Transfer of funds to FIC) Mar. 1980
3. National Conference on Oral Health Behaviors  
(Grant) April 1980
4. International Symposium on Current Trends in Prenatal  
Craniofacial Development, Bethesda April 1980



5. International Symposium on Calcium Binding and Calcium Function in Health and Disease, Madison, Wisconsin June 1980
6. NIH Consensus Development Conference on Removal of Third Molars, Bethesda Nov. 1980

#### PROGRAM EVALUATION ACTIVITIES

Program evaluation activities consumed a considerable amount of staff effort and funds during FY1980. All NIDR extramural programs except the Periodontal Diseases Program, which was evaluated in 1975, undertook the challenging task of developing succinct statements of program goals and objectives and of evaluative criteria for measuring their accomplishment. These statements were completed toward the end of FY1980 and made available for incorporation into the NIDR Long Range Plan being currently developed. Subsequently, they will be used to provide the framework for the formal program evaluations to be carried out during the next few years. In addition, staff also initiated a comprehensive analysis of the Institute's small grants program (R-23s) which is now 10 years old. Brief descriptions of these evaluation activities are presented in the paragraphs to follow.

Staff of the Soft Tissue Stomatology and Nutrition Program conducted 8 panel meetings during FY1980 to develop goals and objectives for the four major areas of the Program: oral soft tissue diseases, nutrition, salivary glands and their secretions, and mineralization. The resulting documents also contained evaluation criteria for each objective and a list of pertinent research approaches to be followed.

Staff of the Restorative Materials Program and their consultants also refined program goals, objectives and evaluation criteria. With special assistance from Dr. Richard Norman, who served as a full-time consultant, they developed working drafts and convened three separate panels of experts which prepared the final documents for each of the following subject areas: intracoronar restorative materials; prosthetic and maxillofacial materials; endodontics, implants, and technology.

Staff of the Pain Control and Behavioral Studies Program, with the collaboration of Dr. Donald Kruper, who served as a visiting scientist administrator, conducted 2 panel meetings during FY1980 to draft formal statements of the program goals, objectives and evaluation criteria. The panels consisted of a Pain Control Panel and a Behavioral Studies Panel composed of nationally recognized authorities in these fields. Subsequently, staff completed a revision of these statements.

Having already developed and refined program goals and objectives during the previous year, staff of the Craniofacial Anomalies Program became engaged in the preparation of a work scope for a contract to provide support services for the formal evaluation of this program. Subsequently, they participated in the issuance of the Request For Proposals, the review of the proposals and the final selection of the contractor. Since these tasks have now been done, the actual evaluation is scheduled for initiation soon and is expected to be completed during FY1981.

Statements of the program goals, objectives and evaluation criteria for the Dental Research Institutes/Centers (DRIC) Program were developed by an assembly of consultants familiar with the development and progress of this program. This group included NIDR staff, DRIC directors and former directors, and two former chairmen of the NIDR Special Grants Review Committee.

In FY 1971 the NIDR initiated a small grants program for relatively inexperienced but well-trained scientists in their initial attempts to obtain funding as independent investigators. Since that time the NIDR has made approximately 50 of these awards each year. To all appearances, this program has not only been highly successful within NIDR, but it has also stimulated the development of similar awards by other institutes. These awards have been standardized throughout the NIH and are now known as New Investigator Research Awards.

The NIDR program has been in operation long enough to warrant a thorough evaluation of its impact on the development and maintenance of dental research manpower and on the evolution of oral health research. Such an evaluation was recently initiated and is scheduled for completion in FY 1981.

#### CENTERS

The NIDR now supports eight centers. Five of these are non-categorical, university-based centers initiated during the 1960s; and three are specialized centers recently initiated to accelerate clinical research on periodontal diseases.

The non-categorical Dental Research Institutes and Centers (DRICs) at the Universities of Alabama, Michigan, North Carolina, Pennsylvania, and Washington supported 105 collaborative research projects during FY 1980. Although their activities were reduced due to increased costs, these centers continued to maintain an outstanding record of scientific achievement. During the past year, their senior investigators published 296 scientific papers (excluding abstracts) and had an additional 181 papers accepted for publication. Research training was provided for 49 research associates through direct participation in the research activities. Center investigators also served as preceptors for 69 fellows, many of whom conducted their research in the DRICs.

During FY 1980, renewal grants at the Universities of North Carolina and Washington were funded for an additional five years. The annual interim project site visits conducted at these and other centers were judged to be favorable from both an administrative and research point of view.

As indicated earlier in this report, planning activities to prepare for a formal evaluation of the DRICs were initiated during this year. Program goals, objectives and evaluation criteria were developed at a meeting held in April, 1980. Participants included NIDR staff, DRIC directors and former directors, and two former chairmen of NIDR's Special



Grants Review Committee (SGRC). The SGRC also held discussions related to the evaluation. These discussions resulted in a recommendation that the DRIC guidelines be updated and that new applicant institutions be invited to compete with renewal applicants for each 5 year period of support.

Research highlights of the DRIC Program are described elsewhere in this report and also in the categorical reports which follow this report. Thus, only administrative activities have been included here.

The three specialized Periodontal Clinical Research Centers recently established at Forsyth in Boston, SUNY at Buffalo, and Virginia Commonwealth in Richmond continued to make progress in identifying and characterizing the oral microflora and in elucidating the host response to specific pathogens. In their well-coordinated laboratory and clinical programs, they have followed the clinical course and microbiologic consistency of periodontal lesions and have begun to classify periodontal diseases by performing cluster analysis. They have also developed new methods to detect disease activity. Moreover, they have now assembled sufficient data to develop immunologic profiles of certain periodontal patients, and are also engaged in the development of improved therapeutic and preventive measures.

Investigators at these promising centers maintain frequent communication with each other and with other scientists throughout the world. At least once each year, investigators from all three centers meet as a group to discuss findings and exchange ideas.

#### RESEARCH FUNDING

During FY 1980, the NIDR Extramural Programs awarded research funds of \$34 million, which included \$24.7 million for research grants and career awards, \$1.3 million for contract research by the five categorical programs, and \$8 million for the Dental Research Institutes and Centers Program.

#### Grants

Table 1 presents data on the FY 1980 distribution of research grant funds by program and by type of grant. It does not include grants by the National Caries Program. Altogether, the Extramural Programs made 335 grant awards: 294 for research projects, 3 for scientific conferences, 5 for the university-based dental research institutes and centers, 3 for periodontal clinical research centers, 28 for research career development awards, one for a research career award, and one for a minority program. Of the 294 project awards, 237 were made for regular research grants (R01), 9 for program projects (P01), and 48 for the new investigator awards (R23).

Compared to last year, the total research grant funds awarded by NIDR-EP in FY 1980 increased numerically by \$650,000 or 1.9% but there was a decrease in the constant dollar value of these funds. In FY 1980, 29% of



the research grant funds was awarded for new grants and competing renewals, and 71% was awarded for noncompeting continuations and supplemental grants (the same proportions as the previous year). The new research awards included 39 regular grants, 14 new investigator awards, and 2 conference grants. The competing renewals included 24 regular grants, 2 program projects, and 2 university-based center grants.

Compared to the previous year, the level of funding by each program was essentially unchanged except for the Restorative Materials Program which increased its grant funding by 11.5%. However, even this increase probably does not signify a real increase in program activity.

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 \* N.I.D.R. EXTRAMURAL RESEARCH GRANTS FUNDED FY 80 \*  
 \* DATA AS OF SEPTEMBER 30, 1980 - BY TYPE & CODE \*  
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TYPE/ CODE	PERIODONTAL		CRANIOFACIAL		RESTORATIVE		STOMATOLOGY		PAIN CONTROL		INSTITUTES		TOTALS	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
1 K04	3	\$109,591	1	\$36,936	1	\$38,961	1	\$39,204	0	\$0	0	\$0	6	\$224,692
R01	9	\$621,589	10	\$745,384	7	\$856,387	12	\$874,160	1	\$110,962	0	\$0	39	\$3,208,482
R13	0	\$0	0	\$0	1	\$15,000	1	\$3,500	0	\$0	0	\$0	2	\$18,500
R23	2	\$85,695	2	\$84,236	2	\$81,147	5	\$164,199	3	\$133,646	0	\$0	14	\$548,923
TYPE TOTALS	14	\$816,875	13	\$866,556	11	\$991,495	19	\$1,081,063	4	\$244,608	0	\$0	61	\$4,000,597
2 P01	0	\$0	2	\$455,346	0	\$0	0	\$0	0	\$0	0	\$0	2	\$455,346
P50	0	\$0	0	\$0	0	\$0	0	\$0	0	\$0	2	\$3,020,413	2	\$3,020,413
R01	4	\$351,453	10	\$870,960	4	\$237,121	4	\$329,426	2	\$150,018	0	\$0	24	\$1,938,978
TYPE TOTALS	4	\$351,453	12	\$1,326,306	4	\$237,121	4	\$329,426	2	\$150,018	2	\$3,020,413	28	\$5,414,737
3 R01	0	\$0	0	\$0	0	\$41,172	0	\$0	0	\$0	0	\$0	***	\$41,172
TYPE TOTALS	0	\$0	0	\$0	0	\$41,172	0	\$0	0	\$0	0	\$0	***	\$41,172
5 K04	6	\$232,829	6	\$228,409	5	\$191,457	5	\$169,478	0	\$0	0	\$0	22	\$822,173
K06	0	\$0	0	\$0	0	\$0	1	\$32,508	0	\$0	0	\$0	1	\$32,508
P01	1	\$522,176	4	\$1,271,731	0	\$0	1	\$198,312	1	\$202,422	0	\$0	7	\$2,194,641
P50	3	\$1,710,819	0	\$0	0	\$0	0	\$0	0	\$0	3	\$4,955,587	6	\$6,666,406
R01	34	\$2,635,869	47	\$3,629,091	22	\$1,479,826	47	\$3,321,497	24	\$1,457,245	0	\$0	174	\$12,523,528
R13	0	\$0	0	\$0	0	\$0	0	\$0	1	\$1,804	0	\$0	1	\$1,804
R23	6	\$202,902	9	\$247,527	4	\$109,312	11	\$273,514	4	\$123,528	0	\$0	34	\$956,783
S06	1	\$39,816	0	\$0	0	\$0	0	\$0	0	\$0	0	\$0	1	\$39,816
TYPE TOTALS	51	\$5,344,411	66	\$5,376,758	31	\$1,780,595	65	\$3,995,309	30	\$1,784,999	3	\$4,955,587	246	\$23,237,659
GRAND TOTALS	69	\$6,512,739	91	\$7,569,620	46	\$3,050,383	88	\$5,405,798	36	\$2,179,625	5	\$7,976,000	335	\$32,694,165

THIS REPORT DOES NOT INCLUDE THE NATIONAL CARES PROGRAM.  
 TYPES: 1 NEW AWARDS  
 2 COMPETING CONTINUATION AWARDS  
 3 SUPPLEMENTAL AWARDS  
 5 NON-COMPETING CONTINUATION AWARDS

CODES:  
 P01 PROGRAM PROJECTS  
 P50 INSTITUTE AWARDS  
 K04 CAREER DEVELOPMENT AWARDS  
 K06 CAREER AWARDS

R01 REGULAR RESEARCH GRANTS  
 R13 CONFERENCE GRANTS  
 R23 SPECIAL DENTAL AWARDS  
 S06 MINORITY PROGRAMS

## Contracts

Collaborative research funded by contract and by interagency agreement during FY 1980 totalled \$1.3 million, which was approximately the same as the FY 1979 level. These funds were distributed among three categorical extramural programs as shown in Table 2.

Table 2

### FY 1980 DISTRIBUTION OF CONTRACT SUPPORT BY PROGRAM

Program	No. of Contracts and Interagency Agreements		Amount (\$000,s)	Percent of Total
	<u>Active</u>	<u>Funded FY80</u>		
Craniofacial	5	3	\$156	12.2
Restorative	2	2	677	52.9
Stomatology	8	6	447	34.9
	—	—	—	—
Totals	15	11	\$1,280	100.0

Active craniofacial anomalies projects included studies on the long term effects of orthodontic treatment, the effects of surgery of the face and jaws on oral function, and the metabolism of pregnant women who have previously delivered offspring with cleft palate. Stomatology projects included immunologic studies of recurrent herpes simplex virus infections and recurrent aphthous ulcers in humans and studies on the early detection of human oral carcinomas. Restorative materials research dealt with bonding agents and with materials for dental fillings and prosthetic appliances.

## TRAINING

Table 3 summarizes the distribution of research training funds awarded by NIDR during FY 1980. A total of nearly \$4 million was provided for 102 separate awards including 63 individual fellowships, 27 institutional fellowship grants, 2 senior fellowships and 10 short-term training grants. Not only was this level of activity greater in dollar magnitude than the previous year (by \$600,000), but it also served a larger number of individuals and provided an increased variety of training approaches. FY 1980 saw the initiation of two new types of NRSA awards, the Senior Fellowship and the Short-Term Training Grant, which resemble earlier training programs. The ten newly awarded short-term training grants will provide up to 3 months of introductory research training for approximately 60 dental students, and the senior fellowship awards will enable two mid-career scientists to enlarge their current research capabilities or make major career changes. It should be mentioned here that three



Geriatric Dentistry Academic Awards were made to dental schools by the National Institute on Aging (NIA). Although the funds were provided by NIA, the NIDR provided consultation during the initiation and development of these awards. The geriatric dentistry awards are expected to develop the curriculum on aging in dental schools and support the development of research programs.

Through cooperative agreements with other NIH organizational components, the NIDR has also participated in efforts to provide a broad range of training opportunities for minority students. During the past 3 years, 20 students have received NIDR training funds under the Minorities Biomedical Support (MBS) Program through an arrangement with the NIH Division of Research Resources. This agreement also enabled the NIDR to contribute to the support of four high school students under the Summer Research Apprenticeships for Minority Students. A second agreement with the National Institute of General Medical Sciences provides for the support of postdoctoral dental fellows through the Minorities Access to Research Careers (MARC) Program.

The new awards and cooperative arrangements described above lend much needed flexibility and breadth to our current research training efforts. As a result, the outlook for developing research manpower programs appropriate for the future is promising.

Table 3: Distribution of NIDR Research Training Funds in FY 1980 (\$ in Thousands)

Type of Award	Caries		Perio.		Cranio.		Restor.		Stomat.		Pain		Total	
	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.
Fellowships	11	\$210	20	\$333	13	\$205	4	\$ 77	8	\$147	7	\$124	63	\$1,096
Institutional Fel. Grants	2	209	7	748	6	686	6	413	4	328	2	207	27	2,590
Senior Fellowships	.	.	.	.	.	.	.	.	1	35	1	32	2	67
Short-Term Tr. Grants	.	.	.	.	.	.	.	.	.	.	.	.	10	137
<b>TOTALS</b>	<b>13</b>	<b>\$419</b>	<b>27</b>	<b>\$1,081</b>	<b>19</b>	<b>\$891</b>	<b>10</b>	<b>\$490</b>	<b>13</b>	<b>\$510</b>	<b>10</b>	<b>\$363</b>		<b>\$3,890</b>

## RESEARCH HIGHLIGHTS

- Periodontal Disease Research. Highlights included findings in clinical periodontology, in microbiology and immunology, and in basic studies on connective tissue.

Clinical studies. Cluster analysis, a method which groups patients by disease characteristics was used in a preliminary study of 22 patients to classify periodontal disease. Three clusters were found: a major one with breakdown in some sites, but improvement in others; a second cluster with advanced bone loss but no active destruction (arrested disease); and a third group with rapid destruction only.

The majority of 153 sites studied for a one year period showed no bone loss but 10% showed definite loss and a few sites improved. Gingival attachment measurements gave similar results. These findings provided the first evidence that disease activity may be cyclical.

Sensitive assays were devised for prostaglandin and for antibody to A. actinomycetemcomitans in gingival fluid.

Microbiology. Over a one year period, the most numerous groups of organisms in periodontal sites were consistently the most numerous; organisms present in low numbers showed the same consistency. The flora was often characteristic, but different from one pocket to another with the same level of disease.

The suspected pathogen B. gingivalis, formerly B. asaccharolyticus, is implicated in adult periodontitis because it predominates during disease, stimulates antibodies, secretes toxins and causes disease in animals. Similar evidence implicates A. actinomycetemcomitans in localized juvenile periodontitis (LJP).

Oral strains of the black pigmented Bacteroides, which are prevalent in periodontal diseases, were distinguishable from non-oral strains by their serology, DNA homology, agglutination of red cells and attachment to oral cells. Such data enabled investigators to propose that the term Bacteroides gingivalis refer only to oral species and B. asaccharolyticus refer only to the similar, but serologically distinguishable non-oral species. B. gingivalis was formerly referred to as B. asaccharolyticus or B. melaninogenicus subsp. asaccharolyticus.

On the basis of fermentation studies, there are at least 10 biotypes of A. actinomycetemcomitans, but a given patient with LJP nearly always harbors only one biotype.

A. actinomycetemcomitans was susceptible to tetracycline, minocycline, and chloramphenicol, but only partially susceptible to ampicillin, erythromycin and penicillin. Since this organism is said to cause endocarditis occasionally, the use of penicillin for such infections should be questioned.

Preclinical in vitro studies on antibacterial agents against dental plaque have developed promising chlorhexidine analogues and salicylanilide compounds.

Immunology. More than 50% of patients with periodontal disease and a family history of diabetes mellitus had depressed neutrophil chemotaxis, whereas only one of 20 control patients with periodontal disease but no family history of diabetes showed depressed chemotaxis.

Four research teams have reported that LJP patients have high serum AB to A. actinomycetemcomitans, whereas other patients have low titers. Thus, it may be possible to diagnose this condition by immunologic data.

Immunologic profiles of patients with gingivitis, adult periodontitis, and LJP were developed from data assembled during the past decade. In gingivitis, serum antibody and lympho-proliferative responses to Actinomyces are elevated, but responses to other species are not. In contrast, patients with adult periodontitis have elevated serum AB and blastogenic responses mainly to B. gingivalis. Patients with LJP, on the other hand, have elevated antibody to A. actinomycetemcomitans and impaired neutrophil function, neither of which is evident in the other two diseases.

Bone and Collagen Metabolism. The origins of the bone-forming osteoblast cells and the bone-destroying osteoclast cells were studied by transplanting embryonic quail bones onto the chorioallantoic membranes of chick embryos. Osteoblasts originated only from the grafted quail bone, whereas the osteoclasts came from mononuclear chick cells.

Bone. Several studies indicated that bone metabolism can be regulated by influencing osteoclasts. In one study both parathyroid extract and prostaglandin E2 stimulated osteoclasts to proliferate and bone resorption was increased. In another study, however, dichloromethylene diphosphonate increased the number and size of osteoclasts, but bone resorption was decreased.

Studies of collagen metabolism showed that gingival tissues contain a pool of soluble collagen which turns over rapidly and a pool of fibrillar collagen that turns over slowly. In previous studies, these pools were not recognized. In diabetic animals the soluble pool turned over even more rapidly but the turnover of fibrillar collagen was greatly reduced. The net effect of diabetes was to decrease collagen metabolism.

- Craniofacial Research. Developmental biology studies dealt with the migration and differentiation of neural crest cells, which give rise to the skeletal and connective tissue structures of the craniofacial region. The effect on neural crest cells of extracellular matrix components including glycosaminoglycans, collagen and glycoprotein were emphasized.

Studies on the regulation of vascular development showed that fibronectin, a cell surface glycoprotein is a potent chemotactic agent for



endothelial cells. Other studies have dealt with substances which prevent endothelial proliferation. This research is important for understanding embryogenesis as well as tumor growth.

Studies on the etiology and pathogenesis of cleft lip and palate included research on mechanisms of palatal shelf movement and closure. The results suggest that scientists will be able to predict the types of agents which interfere with normal closure and thus may be able to prevent clefting. Studies on chemical changes in the extracellular matrix during palate closure suggested that alterations of macromolecules may provide the force necessary for normal closure.

In studies on the role of phenytoin (Dilantin) in cleft lip, mice given teratogenic doses of the drug showed central nervous system toxicity similar to that observed in humans being treated for epilepsy. However, when pregnant mice were treated with oxygen immediately after receiving a teratogenic dose, they did not show nervous system toxicity and the rate of phenytoin-induced cleft lip and palate was reduced three-fold.

Treatment. Although many cleft palate repairs are completed in children less than 2 years of age, some surgeons delay the final operation until the child is 5 years old. At this age growth is further advanced and there is less growth retardation from scarring. The placement of a prosthetic device to close the palate during the waiting period facilitates feeding and speech development.

Studies on the relationship of neuromuscular activity to bone remodeling were conducted to improve the treatment of craniofacial malformations. These studies showed that new skeletal relationships established by surgery remain stable only when muscles are repositioned so that they do not produce unbalanced forces.

Mechanical forces applied to cultured bone-forming cells activated enzymes associated with cell growth. Such studies of the biochemical response to mechanical forces will lead to physiologic approaches to orthodontic tooth movement. In vivo studies showed that orthodontic forces applied to teeth induced the production of proteinaceous substances which caused resorption in adjacent bone.

In studies on the effects of orthodontic treatment on periodontal disease and temporomandibular joint function, investigators compared patients treated orthodontically 25 years earlier to a control group. No significant differences in any aspect of dental health were observed.

- Restorative Materials. The clinical performance of nine high-copper amalgams was rated and the results correlated with their physical properties. All except one was superior to the low-copper control. When these alloys were ranked according to marginal fracture the top-rated five had equally good scores.

Clinical studies of resin restorations indicated that the new, modified cavity preparations with no mechanical retention were preferable to the conventional preparations which require the removal of tooth structure.

Research on the role of creep, corrosion and microstructure in the marginal fracture of amalgam restorations indicated that low creep values predict clinical performance, but high creep values may not.

A new device which analyzes and measures wear in vitro showed that the low wear rate of amalgam remained constant throughout the range of contact stresses seen in human mastication, whereas the wear rate of a composite changed as stress increased. When the stress reached a certain level, the composite showed catastrophic surface failure.

Endodontics. In newly developed evaluation methods, teflon tubes containing test materials were implanted into rat subcutaneous tissue and teflon cups containing test materials were implanted into guinea pig mandibles. The results showed that the new methods can determine whether materials are biocompatible with the specific types of tissues they will contact in clinical endodontics.

Implants. Thirty-seven endosseous implants made of a titanium core coated with a porous high density polyethylene (PHDPE) were evaluated in 14 dogs for up to 20 months. Clinical and histologic results indicated that these implants were firmly ankylosed for a time, but the ankylosing bony ingrowth became resorbed later and was replaced by soft tissue.

The effect of variations in elastic modulus and in surface composition on the performance of blade-shaped implants was studied in baboons. Pyrolytic carbon, alumina and carbon-coated alumina specimens were followed for 2 years. Eighteen of 22 implants were judged to be successful by clinical and radiographic criteria. Neither surface composition nor modulus were significant variables in this study.

- Nutrition. Hamsters fed a high level of dietary protein excreted large amounts of calcium causing the weight and calcium content of the long bones to be reduced; similar findings are seen in human osteoporosis.

Rats deprived of Vitamin A when nursing developed deep dentinal carious lesions in structural defects believed to have been caused by the earlier deficiency.

In animal experiments, low dietary zinc caused alveolar bone loss, retarded tooth and bone development, increased numbers of mast cells in the buccal mucosa, and prolonged retention of formed elements by buccal epithelial cells.

Soft Tissue. The chance of developing a secondary bacterial infection during a viral attack was enhanced by the ability of pathogenic staphylococci to adhere to virus infected cells.

The antiviral drug acyclovir prevented ganglionic latency when applied topically to a herpesvirus infection in mice up to three hours after infection.

The application of iontophoresis (weak electrical current) to the antiviral drug Idoxuridine (IDU) aided penetration of the drug into the skin of patients with herpes labialis infections so that the lesions caused less discomfort and healed more rapidly than controls.

The flow of soluble materials across dentin into the pulp of the dog tooth was increased by vasoconstrictors used in anesthetic solutions. Thus during operative procedures on deep carious lesions, bacterial toxins could reach damaging levels in the pulp tissue.

Salivary Secretions. A protein normally produced only by developing salivary glands can be induced or increased in glands of older animals by the drug isoproterenol; thus, the cells producing this protein may be identified as "proacinar" cells.

Investigators studying the neuroglandular junction recently developed a noninvasive method in which electrical activity in the tissue is monitored optically using potentiometric dyes rather than microelectrodes.

The biochemical phenotype of salivary gland cells has been found to be stable in long-term cultures; rat parotid cells continued to synthesize alpha-amylase for 46 days.

Mineralization. Parathyroid hormone may act on bone cells by altering membrane permeability and allowing calcium to enter the cell as a mediator of hormone action.

A freeze fracture study of the cartilage growth plate of long bones showed that the extracellular matrix vesicles observed by electron microscopy are not artifacts. When incubated in calcifying solution, these vesicles formed clusters of apatite crystals similar to those observed in vivo.

Bone-forming cells on the tension side of the periodontal ligament of teeth undergoing orthodontic movement contained intracellular vesicles loaded with collagen microfibrils. Many of these cells were undergoing mitosis.

Fluoride. Metabolic alkalosis in rats increased their resistance to fluoride toxicity. In rats on drinking water with different fluoride levels the fluoride content of plasma and of developing enamel was independent of the fluoride intake but dependent upon the acid-base status of the animal.

Gingival crevice fluid in dogs was found to contain the same fluoride concentrations as plasma, a finding which may explain why fluoride levels are high in enamel toward the gingiva and in plaque. Thus, gingival crevice fluoride may prevent dental caries.



- Treatment of Post-Operative Dental Pain. Placebo-induced pain relief (mediated by endorphins) was more effective in patients whose pain was severe than in patients whose pain was mild. These observations challenge the popular notion that placebos primarily affect pain that is mild or imagined.

Patients suffering from post-operative dental pain responded to morphine in an all-or-none fashion. Therefore, the dose for each individual should be titrated to obtain maximal relief with minimum morphine.

Surgical Techniques for Oral-Facial Pain Relief. Investigations to develop surgical procedures to relieve intractable oral-facial pain, in patients with head and neck cancer, led to the unexpected finding that severing the trigeminal nerve tract in monkeys did not produce dental analgesia. Thus, the trigeminal nucleus caudalis is not the exclusive brain stem relay station for the transmission of oral, facial and dental pain impulses.

Sensory Innervation of Rat Molars. Morphologic and functional studies showed that the dentin of developing and mature rat molars contain sensory nerve endings which probably serve as the primary sensory transmitters.

Dental Fear and Anxiety. Two techniques, tape-recorded relaxation instructions and electromyographic (EMG) biofeedback training, reduced anxiety during routine dental treatment, but musical programming had no effect. In EMG biofeedback subjects learn to control muscle tension by observing a visual display of their muscle activity. Eight minutes of EMG biofeedback training was more effective in reducing anxiety and pain than the relaxation instructions.

Children's Perception of Dento-facial Appearance. Investigators studied 500 children 10-13 years of age to determine how they judged dental appearance. The children found a broad range of conditions acceptable and showed objectivity in judging their own teeth. Only 20% found their own teeth unattractive. Over-jet (the degree to which the upper teeth extend beyond the lower teeth) caused dissatisfaction among girls, whereas crowding was the major cause of dissatisfaction in boys. Girls who were satisfied with their teeth practiced better oral hygiene than girls who were not.

Prepared by A. A. Rizzo

## ANNUAL REPORT FY 1980

### PERIODONTAL DISEASES PROGRAM

#### INTRODUCTION

Periodontal diseases constitute a major health problem throughout the world. Millions of Americans have already lost their teeth because of these diseases and many more will become edentulous unless effective prevention and treatment measures can be developed. The steadily increasing longevity of the population makes the control and prevention of periodontal diseases even more urgent and challenging for public health workers and researchers.

The goal of the Periodontal Diseases Program is to develop preventive measures which can be initiated at an early age and continued throughout life. 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 encompasses a wide variety of subject matter, which includes microbiologic studies on the identification and nature of the suspected pathogens, immunologic studies on the complex host response system activated by the disease, as well as studies to develop new therapeutic approaches. The host response studies emphasize the cellular and biochemical mechanisms involved in inflammation and tissue destruction. The effort to develop practical preventive measures suitable for the general public involves coordinated clinical and laboratory studies.

#### ADMINISTRATION

During FY 1980 the program awarded a total of \$6,512,739 for 57 regular research grants, 3 specialized clinical research centers, 1 program project, and 8 new investigator research awards. Nineteen regular research grants, 2 new investigator research grants and 1 program project were active but did not receive FY 1980 funds. A total of \$747,716 was awarded for 7 institutional training grants supporting 38 trainees and fellows, and an additional \$332,601 was awarded for 20 individual fellowships. A total of \$342,420 was expended for 9 career development awards.

Awards for the 3 specialized centers established to develop coordinated programs of basic and clinical research on periodontal diseases totaled \$1,710,819 in FY 1980. These centers made commendable progress in identifying and characterizing the oral microflora and in elucidating the specific host response to certain pathogenic organisms. In addition to these efforts, they are also attempting to develop improved therapeutic and preventive measures.

Table I shows the distribution of research and training funds during FY 1980.

Table I: DISTRIBUTION OF FUNDS DURING FY 1980

A. RESEARCH GRANTS

	<u>ACTIVE</u>	<u>FUNDED IN 1980</u>	<u>FUNDS</u>
Microbiology	17	14	\$1,592,202
Inflammation and Immune Response	29	21	1,279,893
Bone Metabolism	15	10	649,854
Tissue Structure and Metabolism	15	10	798,162
Prevention, Diagnosis, and Treatment	3	2	139,389
Clinical Research Centers	3	3	1,710,819
Career Development Awards	<u>9</u>	<u>9</u>	<u>342,420</u>
Total	91	69	\$6,512,739

B. TRAINING

Institutional Grants	8	7	\$ 747,716
Individual Fellowships	<u>21</u>	<u>20</u>	<u>332,601</u>
Total	29	27	\$1,080,317

Grand Total \$7,593,056

STAFF ACTIVITIES

Staff visited 11 institutions to program, monitor, and evaluate research and attended 11 meetings to keep abreast of scientific developments and to maintain close liaison with the scientific community.

A. Site Visits: Initial Review, Monitoring, and Programming

Forsyth Dental Center, Boston	October 1979
University of Washington, Seattle	October 1979
Virginia Commonwealth University, Richmond	November 1979



University of Southern California, Los Angeles	January 1980
Louisiana State University, New Orleans	February 1980
University of Utah, Salt Lake City	March 1980
University of New Mexico, Las Cruces	March 1980
University of West Virginia, Morgantown	March 1980
University of Washington, Seattle	May 1980
Virginia Commonwealth University, Richmond	June 1980
Forsyth Dental Center, Boston	June 1980

B. Meetings

American Association of Periodontology, Annual Meeting, Seattle	October 1979
Third Molar Consensus Development Conference, Bethesda	November 1979
Sixth Annual Periodontal Symposium, Los Angeles	January 1980
Amniocentesis Consensus Development, Bethesda	February 1980
Oral Biology & Medicine Study Section, New Orleans	February 1980
International Association for Dental Research, Los Angeles	March 1980
District of Columbia Dental Society Annual Meeting, Washington, D. C.	April 1980
Greater Washington Periodontal Society Seminar, Washington, D. C.	May 1980
Specialized Clinical Research Centers for Periodontal Diseases, Annual Meeting, Buffalo	May 1980
Special Study Section Meeting, San Francisco	July 1980
University of Iowa Symposium, Cedar Rapids	September 1980

C. NIDR Programs Advisory Committee Annual Meeting May 1980

D. Subcommittee on Periodontal Diseases December 1979  
May 1980

E. Seminar Sponsored

"Antigens of Actinomyces Viscofus"  
(Dr. Michael Reed, University of Mississippi) May 1980

RESEARCH HIGHLIGHTS

The research highlights outlined in this report were derived from studies in clinical periodontology, in oral microbiology and immunology and in basic connective tissue metabolism. The clinical research section describes studies on cluster analysis to classify periodontal diseases, monitoring studies on the clinical course of periodontal disease, and new methods to detect disease activity. The microbiology section includes projects to monitor the consistency of the flora in different periodontal sites over time, extensive studies on the important Bacteroides genus, and studies implicating two specific organisms as pathogens. The immunology

section includes studies relating neutrophil function to periodontal disease, significant studies on the human host response to two specific pathogenic organisms and the development of immunologic profiles of patients with gingivitis, adult periodontitis and localized juvenile periodontitis. Finally, the connective tissue section outlines basic investigations on the origin of osteoblasts and osteoclasts, on the control of osteoclast proliferation and function, and on the turnover rate of different pools of collagen.

### Clinical Studies

Current clinical classification of periodontal diseases is based on subjective rather than objective indices. Although these methods are widespread and generally applicable, they are fraught with limitations. Despite stringent controls, subjective measurements almost always yield variable results which frequently lead to confusion in interpretation. To develop objective criteria, investigators at Forsyth Dental Center are using cluster analysis, which groups patients according to common disease characteristics. In a preliminary study of 22 patients, the Forsyth investigators used various microbiological and immunological measurements which enabled them to separate the patients into 3 distinct clusters. The major cluster consisted of 12 young patients with sites actively breaking down as well as sites which were apparently improving spontaneously, probably the most prevalent form of destructive periodontal disease. The second cluster consisted of 6 patients who have arrested periodontal disease, i.e., advanced bone loss but no periodontal destruction during the monitoring period. The third group consisted of older patients with rapidly destructive periodontal disease throughout the dentition.

The investigators hesitate to accept this classification because their initial group was small and the technique is relatively untested. Nevertheless, the results are promising. Future studies with a larger group of subjects and additional discriminating features may enable them to define new clinical syndromes with confidence.

Whether periodontal disease is a chronic disease progressing constantly but slowly or whether it is characterized by intermittent exacerbation and remission is still not known. Only accurate, sensitive and reproducible methods of measuring the disease activity can supply the answer. Such methods would be extremely helpful to clinicians in deciding when to institute active therapy and in evaluating its efficacy. Since such methods have not yet become available, investigators at Forsyth Dental Center have used refined versions of two traditional approaches to identify sites of active destruction. One is a radiographic technique in which standardized radiographs were used to spot changes in the height of the alveolar bone. The second, which was based on the clinical measurement of attachment level, used the Ramfjord technique. With the standardized radiographic method, the mean bone changes in 153 individual sites in 16 untreated patients with advanced destructive periodontal

disease were monitored for one year. The majority of the sites showed no change; 10% showed clear evidence of active destructive diseases, and a few sites appeared to have improved. Some patients showed all these types of bone activity in different sites: no change, loss of bone, increase of alveolar bone.

Attachment levels were measured with periodontal probes at monthly intervals and the data subjected to regression analysis. The results were computerized to permit immediate comparison of the current measurements with those of previous visits and to determine whether a site was undergoing significant loss of attachment. Data showed that the majority of sites diagnosed clinically as sites of advanced disease did not undergo destructive changes during the monitoring period. A few sites showed loss of attachment, but others showed significant gain in attachment. These results suggest that disease activity in individual sites is cyclical. The similarity between these observations and those found by radiographic measurement supports the conclusion that only a small percentage of periodontal sites undergoes active breakdown during any given period. An important observation was that a significant loss of attachment in some sites was followed by attachment gain. According to the authors, this is the first solid evidence of a long-suspected cycle of destruction and repair in progressive, destructive periodontal disease. Since regression analysis appears to be promising, it is being routinely incorporated into all new clinical experimental designs at Forsyth Dental Center.

Two laboratory methods developed by the Forsyth investigators to measure disease activity also show promise. One of these can detect as little as 4 picograms of prostaglandin ( $PGE_2$ ) in 0.1 ml of gingival fluid. Samples of gingival fluid taken from deep periodontal pockets of gingivitis patients contained 4 to 5 times more prostaglandin than samples from shallow pockets. The second method enables investigators to measure significant antibody activity to strain Y<sub>4</sub>, Actinobacillus actinomycetemcomitans, in a dilution of 1:4000 of gingival fluid. The scientists believe that the new techniques will be useful in the rapid diagnosis of periodontal patients.

### Microbiology

Advances in microbiology during FY 1980 included findings on the consistency of the periodontal flora over time, on the relation between specific organisms and specific disease syndromes, and on the basic taxonomy of suspected pathogens. Progress was also made in devising new selective growth media for the suspected pathogen A. actinomycetemcomitans and in the study of antibacterial agents against periodontal organisms. These studies are summarized in the following paragraphs.

To determine how consistent the microbiologic composition is in individual sites of periodontal disease, investigators at Forsyth Dental Center used immediate darkfield examination and selective media cultural techniques to monitor untreated advanced periodontal lesions for one year. Repeated sampling showed that a given pocket usually contained the same groups of predominant organisms and the same groups of poorly



represented organisms as was observed in earlier samples. Data from these studies also indicated that the microbiota is characteristically different in diseased pockets from different individuals even though the level of severity may be almost identical. These data suggest a low order of transmissibility from site to site within the same mouth.

Last year's annual report implicated the oral organisms B. asaccharolyticus (now known as B. gingivalis) in adult periodontitis, and Actinobacillus actinomycetemcomitans (Y-4) in juvenile periodontitis. The evidence for pathogenicity was based on 5 criteria: 1) the number of organisms is high in diseased sites but low or nonexistent in healthy sites; 2) the organism evokes an immune response in the host; 3) pure cultures of the organism cause periodontal disease in animals; 4) the organism produces harmful substances which can trigger tissue destruction; 5) elimination of the organism from diseased sites arrests the disease process. During the past year evidence against the two suspected pathogens has been supported by new findings. Table 2 summarizes the accumulated data.

Table 2. SUMMARY OF EVIDENCE AGAINST TWO SUSPECTED PATHOGENS\*

	<u>Bacteroides Gingivalis</u> (formerly <u>B. asaccharolyticus</u> )	<u>Actinobacillus</u> <u>Actinomycetem-</u> <u>comitans</u> (Y-4)
DISEASE ASSOCIATION	Predominates in adult periodontitis Scarce in healthy sites Absent in LJP	Predominates in localized juvenile periodontitis
HOST RESPONSE	Serum Antibody (AB) Salivary AB	Serum AB Salivary AB Specific anti-toxic AB
ANIMAL PATHOGENICITY	Perio. disease in rats Ligature lesions in monkeys and beagle dogs Skin infections in guinea pigs	Perio. disease in rats
PATHOGENIC MECHANISMS (Toxic Substances Produced)	Collagenase Fibrinolysin Hydrogen sulfide Methyl mercaptan	Ammonia Fatty acids Inhibits phagocytosis
EFFECT OF ELIMINATION	By surgery - improvement By antibiotics - improvement	Arrested disease

\* Data primarily from Periodontal Clinical Research Centers at Forsyth and SUNY Buffalo, and the University of Pennsylvania Center for Oral Health Research.

During the past year, investigators at SUNY, Buffalo, have extended their investigations into the ecology, adherence, antigenic, and genetic heterogeneity of the black pigmented Bacteroides, since several species of this genus are prevalent in different types of periodontal disease. For example, to understand the colonization patterns of the B. melaninogenicus/asaccharolyticus group, the investigators determined the adherence of these organisms to erythrocytes and to buccal epithelial cells.

One striking finding was that oral B. asaccharolyticus strongly agglutinated erythrocytes from 16 different species whereas non-oral B. asaccharolyticus failed to agglutinate any of these erythrocytes. This finding gave rise to a rapid, accurate method of distinguishing oral and non-oral strains. The agglutination of rabbit erythrocytes by both oral and non-oral B. melaninogenicus subsp. intermedius was strong but that of other species was variable and often weak. Unlike oral B. asaccharolyticus strains which adhered to human buccal epithelial cells in saline, non-oral B. asaccharolyticus and non-oral B. melaninogenicus subsp. intermedius did not. Pretreatment of the cells with serum or saliva prevented the oral B. asaccharolyticus cells from adhering. Thus, there are different attachment sites for human buccal epithelial cells on the surface of oral and non-oral B. asaccharolyticus strains. These and other similar studies have led the investigators at SUNY Buffalo and at the University of Connecticut to propose the following terminology:

1. Bacteroides gingivalis to refer to the human oral strains commonly referred to in the literature as B. asaccharolyticus or B. melaninogenicus subsp. asaccharolyticus.
2. B. asaccharolyticus to refer to a distinct species obtained from non-oral sites.
3. B. gingivalis of oral origin can be distinguished from the non-oral B. asaccharolyticus by differences in antigenicity, DNA homology, adherence to human buccal epithelial cells, and specific patterns of erythrocyte agglutination.
4. Bacteroides melaninogenicus subsp. melaninogenicus, Bacteroides melaninogenicus subsp. intermedius and Bacteroides melaninogenicus subsp. levii are three antigenically and biochemically distinct organisms. Antigenically similar strains of Bacteroides melaninogenicus subsp. intermedius have been found in both oral and non-oral locations.
5. Bacteroides macacae to refer to catalase-positive, black pigmented Bacteroides found in periodontal sites in Macacae arctoides monkeys.

Investigators at SUNY, Buffalo, have further characterized the gram-negative A. actinomycetemcomitans that is associated with severe oral and non-oral infections. Ten biotypes of A. actinomycetemcomitans could be established on the basis of their ability to ferment different amounts



of dextrin, maltose, mannitol, and xylose. Only one biotype was found in all the oral sites of each individual studied, except for one LJP patient who harbored 2 biotypes.

The susceptibility of A. actinomycetemcomitans to a wide variety of antibiotics was also tested. Tetracycline, minocycline, and chloramphenicol inhibited more than 96% of the strains tested, but other antibacterial agents, including ampicillin, erythromycin, and penicillin G, were much less active. For example, only 50% of A. actinomycetemcomitans strains were inhibited by 4 mg/ml of penicillin, the therapeutic level encountered in normal treatment. Since this organism is a common isolate from actinomycosis lesions and is occasionally reported as the etiologic agent in fatal cases of bacterial endocarditis, the Buffalo investigators question the use of penicillin in patients with A. actinomycetemcomitans infection. Since all A. actinomycetemcomitans strains may not be destroyed by the standard penicillin prophylaxis, these patients could develop subacute bacterial endocarditis. These investigators suggest that antibiotics of the tetracycline group be used to treat A. actinomycetemcomitans infections.

In preclinical in vitro studies, investigators at SUNY, Buffalo, have developed agents which can either disperse or inhibit the formation of bacterial plaque. They have synthesized nine analogs of chlorhexidine which can inhibit bacterial growth and dental plaque formation. The analogs were made by replacing the p-chlorophenyl groups of chlorhexidine with straight or branched alkyl chains. Each of these compounds had equal or greater potency than chlorhexidine in inhibiting plaque activity against both A. viscosus and A. naeslundii. The Buffalo group has also been working on salicylanilides, another group of antimicrobial agents. One of these compounds, 3, 5, 4-tribromosalicylanilide (TBS), a particularly effective antimicrobial agent, is now undergoing required toxicity studies before being tested in human patients.

Investigators at Forsyth Dental Center have developed a selective medium for isolating A. actinomycetemcomitans. Trypticase soy agar (BBL), supplemented with 5% sheep blood, 8 mg/ml malachite green, and 128 mg/ml bacitracin and incubated in an atmosphere of air + 10% CO<sub>2</sub>, is selective for actinomycetemcomitans and Haemophilus aphrophilus. These two microorganisms can be separated by the catalase reaction, which is positive for A. actinomycetemcomitans and negative for H. aphrophilus.

Spirochetes, or treponemes, are ubiquitous in periodontal lesions, where they often make up from 30 to 40% of the subgingival microflora, but studies of these organisms have been hampered by difficulties in isolation and identification. The oral spirochetes have been divided into three morphologic groups--small, intermediate, and large. The small group can be cultured routinely; but so far only one member of the intermediate group, Treponema vincentii (Borrelia), has been cultivated. Since acute necrotizing ulcerative gingivitis (ANUG) is believed to be caused by the large and intermediate groups, studies of these organisms are clearly indicated. Investigators at Virginia Polytechnic Institute and Virginia



Commonwealth University continue to try to isolate and characterize these organisms. Thus far, they have cultured treponemes from 72% of the supragingival and 79% of the subgingival samples from patients with periodontitis. The large treponemes were more frequent in samples from patients with severe adult periodontitis than in those from patients with moderate periodontitis or juvenile periodontitis. The investigators have also cultured seven species of spirochetes which do not belong to previously described species.

### Immunology

Several studies have demonstrated that patients with localized juvenile periodontitis also have impairments in neutrophil chemotaxis and phagocytosis, but no other medical abnormalities. Therefore, investigators have suspected that the neutrophil impairment accounts for the susceptibility of these patients to periodontal breakdown. Since reduced neutrophil chemotaxis has also been documented in patients with diabetes mellitus, who are believed to be more susceptible to periodontal destruction, investigators at SUNY at Buffalo have begun several studies to correlate neutrophil function, periodontal disease and diabetes. In one project, they studied neutrophil chemotaxis in two groups of adults with comparable levels of periodontal disease. Neither group had diabetes, but those in one group had a strong family history of diabetes. The findings showed that 13 of 24 patients with a family history of diabetes had depressed neutrophil chemotaxis, whereas only one of 20 age and sex matched control patients without such a family history showed depressed chemotaxis.

Recent immunologic findings may soon make possible a definitive diagnosis of localized juvenile periodontitis from laboratory tests. Investigators at SUNY, Buffalo, Forsyth Dental Center, and the University of Pennsylvania have all reported that patients with localized juvenile periodontitis have high serum levels of IgG antibody to A. actinomycetemcomitans whereas edentulous and normal subjects and patients with periodontitis and ANUG have low titers. The investigators at SUNY, Buffalo, have chemically purified this serum IgG and have shown that it precipitates specifically with antigens of A. actinomycetemcomitans, inhibits the leucocidin from A. actinomycetemcomitans, and reacts specifically with the ELISA test (enzyme-linked immunosorbent assay) with antigens of A. actinomycetemcomitans. This antibody was specific not only for the genus Actinobacillus but also for the serotypes of actinomycetemcomitans taken from the subgingival plaque of patients with LJP. Three such serotypes have been described. Most of the patients with LJP have antibodies directed against the major serotype specific antigen. In one family with LJP, the investigators found antibodies to the same serotype antigen among individual family members, a finding which suggests familial transmission of the infection.

Immunologic profiles of different types of periodontal patients are given in Table 3, which summarizes their immune responses and phagocytic cell function. Studies during the past decade have shown definitive differences between the immune responses of patients with periodontal disease and those of normal healthy control patients. These studies have established that periodontal infections are associated with significant systemic immune reactions. Furthermore, it is becoming increasingly clear that clinically distinct forms of periodontal disease can be characterized by immunologic data. In gingivitis, there are low levels of serum antibodies to many organisms but elevated antibody to Actinomyces species, a predominant subgingival organism in this condition. In most cases, the lymphoproliferative response to A. viscosus is also elevated whereas the response to B. asaccharolyticus, an organism not common in gingivitis, is negative. In contrast, patients with adult periodontitis have elevated serum antibodies as well as lymphoproliferative responses to B. gingivalis (asaccharolyticus), an organism often isolated in large numbers from these patients. In gingivitis and adult periodontitis, related conditions which differ in severity but affect most of the population, neutrophil chemotactic function is nearly always normal. However, subjects with juvenile periodontitis have depressed neutrophil chemotactic responses which presumably account for their unusual susceptibility to periodontal disease. Patients with LJP also have markedly elevated antibody levels to A. actinomycetemcomitans, which is prominent in their lesions.

Table 3. IMMUNOLOGIC PROFILES OF PERIODONTAL PATIENTS\*

RESPONSE OR FUNCTION	PATIENT GROUP		LOCALIZED JUVENILE PERIODONTITIS
	GINGIVITIS	ADULT PERIODONTITIS	
Serum Antibody (AB)	Elevated AB to <u>Actinomyces viscosus</u>	Elevated IgG AB to <u>A. viscosus</u>	Elevated IgG precipitins to <u>A. actinomycetemcomitans</u>
	Low levels of AB to many oral organisms	Markedly elevated IgG AB to <u>B. gingivalis</u>	Elevated antitoxin AB to <u>A. actinomycetemcomitans</u>
Cellular Immunity	Pos. for <u>A. viscosus</u> , 90%	Pos. for <u>A. viscosus</u> , 80%	Strong pos. for: <u>A. viscosus</u>
	Pos. for dental plaque	Pos. for <u>B. gingivalis</u> , 60%	<u>B. gingivalis</u>
	Neg. for <u>B. gingivalis</u> , 85%	Strong pos. for dental plaque	<u>Capnocytophaga</u>
Neutrophil Chemotaxis	Normal	Normal	Depressed in 70-80%

\* Data from article entitled "Systemic Immune Response to Oral Anaerobic Organisms" by Genco et al.



Investigators at Forsyth Dental Center and the University of Pennsylvania have modified the indirect ELISA to determine antibody against A. actinomycetemcomitans so that they can now detect IgG in dilutions of 1:4000 of 0.1 ml of serum. Similar antibody levels can also be measured when the leukotoxin, carbohydrate, or lipopolysaccharide of the organism are used as antigens.

In human clinical immunology, it is often impossible to obtain a sufficient number of samples in a short period of time. To solve this problem, investigators at Virginia Commonwealth University developed a method of freezing peripheral blood lymphocytes from human subjects and storing them in liquid nitrogens. Cells thus cryopreserved showed almost 100% viability and functional activity when they were studied later for blastogenesis or polyclonal antibody activity. The new technique enables investigators to compare samples taken at different times from one patient and to compare samples taken from different patients at different times.

### Bone and Collagen Metabolism

The formation and resorption of bone throughout the body are continuing interrelated processes which remain in equilibrium in a healthy adult. However, this steady state can be disrupted by periodontal disease which causes a net loss of the alveolar bone supporting the teeth, a loss that frequently progresses until the teeth cannot be saved. Past research indicates that the net loss of bone is due not only to increased resorption but also to a slower rate of formation. Thus, the program supports basic studies on many aspects of bone metabolism.

The origin of the osteoblasts and osteoclasts responsible for the formation and resorption of bone remains controversial. Last year we reported that investigators at the University of Massachusetts found evidence that mononuclear cells may give rise to functioning osteoclasts. Additional evidence for the hematopoietic origin of osteoclasts was recently presented by investigators at Washington University and the Jewish Hospital at St. Louis, who used the quail-chick chimera model to determine the origin of several types of bone cells. In this system, bone rudiments of embryonic quails were transplanted onto the chorio-allantoic membrane of chick embryos where tissues from both species continued to grow and differentiate. The advantage of the model is that the cells of the quail and chick can always be distinguished by differences in their nuclear morphology. The experiments showed that osteoblasts and osteocytes of these grafts were exclusively derived from osteoprogenitor cells resident in the quail bone graft. Osteoclasts, on the other hand, originated from the mononuclear cells of the chick and were carried by the blood into the transplanted bone rudiments of the quail. In another set of experiments, these investigators found that mononuclear phagocytes, like osteoclasts, showed clear zones at the sites where they resorbed bone particles.

Recently investigators at the University of Utah have shown that some factors can regulate the resorptive activity of bone by controlling the function of osteoclasts. In one experiment, they observed that the number and size of osteoclasts were increased after dichloromethylene diphosphonate had been administered to growing rats. Since it has been established that diphosphonate is a potent inhibitor of bone resorption, this result puzzled the investigators. However, subsequent studies explained this apparent contradiction by demonstrating that the osteoclasts from the treated animals had smaller ruffled borders than those from controls, which indicated less bone-resorbing activity per cell, and that the area of resorption enclosed by adjacent osteoclasts was smaller in the treated animals than in the controls. Thus, even though osteoclasts were more numerous in the diphosphonate-treated animals, their total resorptive activity was less than in the controls.

In studies of how parathyroid extract and prostaglandin  $E_2$  cause bone resorption, investigators at Harvard Dental School used mouse calvaria in tissue culture to show that these substances stimulate an increase in the population of osteoclasts. By autoradiography of thymidinelabeled osteoclasts, they showed that this increase is due to mitosis.

Recent tissue culture experiments on bone have led investigators at Harvard Dental School to postulate that the amount of hydroxproline excreted in the urine is not an accurate index of either bone resorption or bone formation unless supported by other data. When embryonic chick bone was grown in tissue culture, a large amount of newly synthesized collagen was apparently degraded without ever being incorporated into bone. Radioactive labeling experiments indicated that approximately one-half of the newly synthesized collagen was retained in the bone; the other half was degraded and released into the medium. Only a very small amount of the collagen released into the medium was derived from the breakdown of structural collagen present in the bone tissue before explantation. About 85% of the hydroxproline in the tissue culture medium was dialyzable as small peptides or prehydroxproline.

It has been widely accepted that collagen in periodontal tissues undergoes rapid turnover because these tissues are in a continuous state of healing and repair. However, investigators at the University of Southern California have shown that gingiva contains different forms of collagen with different metabolic rates; these include a pool of soluble collagen which turns over rapidly and a pool of fibrillar collagen that turns over slowly. Previously, investigators had failed to recognize these different pools of collagen and did not deal with them separately in turnover studies.

Investigators at the University of Southern California have also studied the metabolism of different collagen pools in the gingiva of rats with streptozotocin-induced diabetes. The newly synthesized soluble pool of gingival collagen turned over even more rapidly than that of nondiabetic controls. However, the metabolism of the older pool of fibrillar collagen



in the diabetic rats, as well as in the controls, was greatly reduced. The net effect of diabetes on collagen metabolism was to decrease the turnover rate.

#### MEETINGS SPONSORED

The new NIDR Programs Advisory Committee, composed of the Subcommittees on Caries and Periodontal Diseases, met once and the Subcommittee on Periodontal Diseases met twice during the FY 1980. The NIDR Programs Advisory Committee reviewed the history, current status, and future of training in relation to both caries and periodontal diseases. The Committee stressed the need for more clinical researchers in both caries and periodontal research.

At its first meeting, the Subcommittee on Periodontal Diseases discussed slow-release mechanisms and their relevance to periodontal diseases. Dr. Robert Langer reviewed the history of the development of delivery systems and described examples of these systems, including the mechanism of drug release in each one. Dr. Dale Mirth summarized his investigations on the controlled release of fluorides, and Dr. Max Goodson described the slow release of tetracycline from hollow fibers placed in periodontal pockets.

At the second meeting, Dr. George Cahill reviewed the current status of diabetes, Dr. Robert Gottsegen described the clinical aspects of periodontal diseases in diabetic patients, and Drs. Walter Loesche and Robert Genco summarized microbiological and immunological investigations as they relate to periodontal disease in diabetic patients. Drs. Lorne Golub, Victor Terranova, and Harish Reddi presented data on different aspects of connective tissue metabolism in diabetes.

#### Publications

During FY 1980, the proceedings of the International Symposium on "Phenytoin-Induced Teratology and Gingival Pathology", held at the University of North Carolina in May, 1979, were published by Raven Press. Editors of the volume are Drs. Thomas M. Hassell, Malcolm C. Johnston and Kenneth H. Dudley. A summary of these proceedings appeared in the Journal of American Dental Association, 99:652-654, 1979.

#### FUTURE PLANS

The Institute will continue its strong ongoing programs of basic research into the biologic processes underlying oral health and disease. These will include studies related to the identification and characterization of both pathogenic and nonpathogenic microflora in the oral cavity, studies related to immune mechanisms which may result in periodontal destruction, and studies related to soft and hard tissue destruction.

Since the lack of objective measurement techniques has hindered our ability to evaluate the progression of periodontal disease, we will



continue to support the newly initiated technologies of cluster analysis and regression analysis.

The successful measurement of extremely low antibody titers to A. actinomycetemcomitans in crevicular fluid may be useful in diagnosing certain periodontal conditions. It is, therefore, imperative that such studies be continued and expanded so that easily applied, rapid methods of diagnosing the diseases and assessing current disease activity will be possible.

#### SUMMARY

During FY 1980 the Program made research awards totaling \$6.5 million for 3 specialized clinical research centers, 1 program project, 57 regular research grants, and 8 young investigator research awards. For training, the Program awarded \$747,716 for grants supporting 38 trainees and \$332,601 for 20 fellowships. Periodontal disease research highlights during FY 1980 included significant findings in clinical periodontology, in oral microbiology and immunology, and in basic studies on connective tissue.

Clinical Studies. In studies to develop an objective classification of periodontal disease, investigators used cluster analysis, a method which groups patients according to common disease characteristics. In a study of 22 patients, the investigators found 3 clusters. In the major cluster, some periodontal sites were breaking down, but others were improving spontaneously. The second cluster showed advanced bone loss but none occurred during the monitoring period (arrested disease). In the third group there was rapid destruction with no repair. In studies to monitor the course of disease, the majority of the 153 sites studied showed no bone loss in a one year period; 10% showed definite loss and a few sites appear to improve. Attachment level measurements followed for the same period gave similar results. These findings provided the first definite evidence that disease activity in individual sites may often be cyclical. Scientists have also devised two sensitive assays to detect disease in specific periodontal sites: a method which detects as little as 4 picograms of prostaglandin in 0.1 ml of gingival fluid, and an assay which measures antibody to the suspected pathogen A. actinomycetemcomitans in a dilution of 1:4000 of gingival fluid.

Periodontal Microbiology. The microbial flora in different periodontal sites was consistent over a one year period. The same groups of organisms tended to be predominant or be present in low numbers repeatedly throughout the year. The flora was often characteristic and different from one pocket to another with the same level of disease severity, even in the same individual.

The accumulated evidence implicating two suspected pathogens was summarized in a table. B. gingivalis, formerly B. asaccharolyticus, was implicated

in adult periodontitis because it predominates during active disease, stimulates specific antibodies in the serum and saliva of affected patients, secretes toxic substances, and causes periodontal disease in animals. Similar evidence implicates A. actinomycetemcomitans in localized juvenile periodontitis (LJP).

To understand the colonization patterns of the black pigmented Bacteroides group, which are prevalent in several types of periodontal disease, scientists compared the characteristics of oral and extraoral strains. Oral strains were distinguishable from non-oral strains because of their serology, DNA homology, ability to agglutinate red cells and to attach to oral cells. Such data enabled investigators to classify organisms of interest and propose the following terminology:

Bacteroides gingivalis to refer to oral strains only. This strain was formerly referred to as B. asaccharolyticus or B. melanogenicus subsp. asaccharolyticus. B. asaccharolyticus to refer to a similar, but distinctly different species obtained from non-oral sites.

Fermentation studies of A. actinomycetemcomitans have established 10 biotypes of this organism; in nearly all instances a given patient with LJP harbors only one biotype. This organism was highly susceptible to the antibiotics tetracycline, minocycline, and chloramphenicol, but only partially susceptible to ampicillin, erythromycin and penicillin. Since this organism is occasionally reported as the cause of bacterial endocarditis, the investigators question the use of penicillin for such infections. Preclinical in vitro studies to develop antibacterial agents against dental plaque have synthesized chlorhexidine analogues with greater potency than chlorhexidine itself, as well as a promising salicylanilide compound now undergoing toxicity tests.

Immunology. In a research project to correlate impaired neutrophil chemotaxis with periodontal disease and diabetes mellitus, investigators found that more than 50% of patients with periodontal disease and a family history of diabetes had depressed neutrophil chemotaxis, whereas only one of 20 age and sex-matched control patients without such a family history showed depressed chemotaxis.

Investigators from four research institutions have reported that LJP patients have high levels of serum AB to A. actinomycetemcomitans, whereas other categories of patients have low titers. Thus, it may be possible to make a definitive diagnosis of this condition by means of immunologic data alone.

Studies during the past decade have established that periodontal infections are associated with host immune responses which differ in patients with different types of periodontal disease. Thus, it has recently become possible to develop immunologic profiles of patients with gingivitis, adult periodontitis, and LJP. Data supporting this

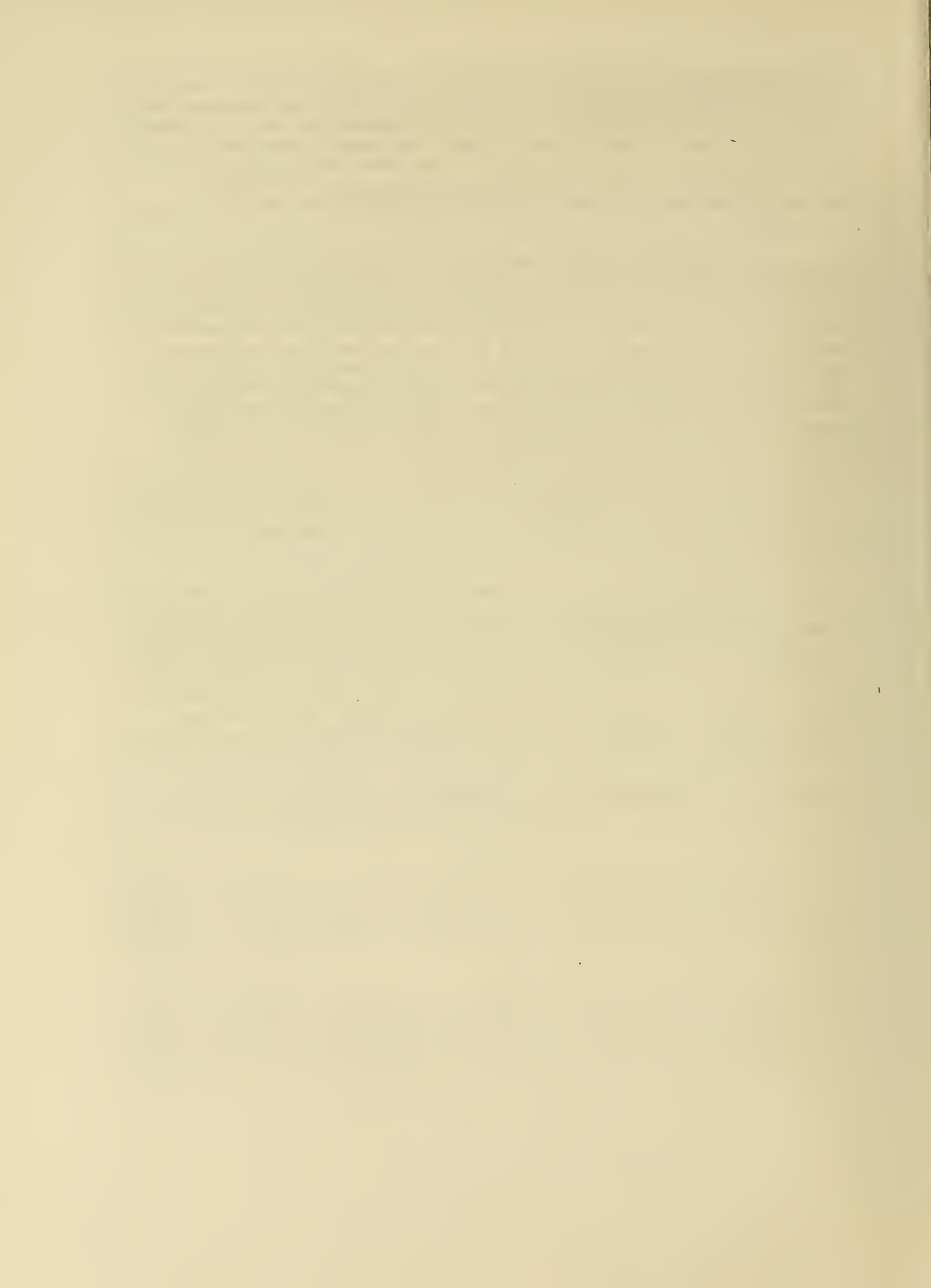
view was summarized in a table. In gingivitis, serum antibody and lympho-proliferative responses to Actinomyces species are elevated, but responses to other organisms are not. In contrast, patients with adult periodontitis have elevated serum AB and blastogenic responses mainly to B. gingivalis. Patients with LJP on the other hand have markedly elevated antibody levels to A. actinomycetemcomitans, and impaired neutrophil function, neither of which is evident in the other two types of patients.

Bone and Collagen Metabolism. To study the origins of the bone-forming osteoblast cells and the bone-destroying osteoclast cells, investigators transplanted bone rudiments of embryonic quails onto the chorioallantoic membranes of chick embryos. In this system cells from both species grow and differentiate together, but can be distinguished from each other by nuclear morphology. The experiments showed that osteoblasts originated only from the grafted quail bone, whereas the osteoclasts were derived from mononuclear chick cells. Several studies indicated that bone resorptive activity can be regulated by controlling the osteoclasts. One study showed that both parathyroid extract and prostaglandin E<sub>2</sub> increased bone resorption by stimulating osteoclasts to proliferate. In contrast, dichloromethylene diphosphonate also caused an increase in the number and size of the osteoclasts, but the net effect of its influence was to decrease bone resorption. Apparently, this compound depresses osteoclast function in some way.

Investigators studying collagen metabolism have found that gingival tissues contain a pool of soluble collagen which turns over rapidly and a pool of fibrillar collagen that turns over slowly. In previous turnover studies, the presence of different pools has not been taken into account. In diabetic animals the soluble pool of collagen turned over even more rapidly than normal animals, but the turnover of the older pool of fibrillar collagen in the diabetic rats was greatly reduced. The net effect of diabetes on collagen metabolism was to decrease the turnover rate.

Prepared by P. F. Parakkal and A. A. Rizzo





## ANNUAL REPORT 1980

### CRANIOFACIAL ANOMALIES PROGRAM BRANCH

#### INTRODUCTION

The Craniofacial Anomalies Program Branch supports research and research training related to the prevention, diagnosis, etiology, and treatment of craniofacial malformations. The areas of research include cleft lip/palate, other congenital anomalies involving oral or craniofacial structures, disfigurements resulting from surgery or accidents, malocclusion of teeth and jaws, and the effects of anatomic malformation on oral function.

To achieve the program objectives of reducing the morbidity and mortality associated with craniofacial anomalies, more knowledge is needed at both the laboratory and clinical levels. Consequently, the Program provides research funds for studies ranging from basic investigations in developmental biology to epidemiological surveys. Clinical studies in growth and development and treatment are also supported. During FY 1980, a contractor was selected to provide support for a formal evaluation of the Craniofacial Anomalies Program. This evaluation is expected to be completed during the coming year.

#### ADMINISTRATION

In FY 1980 \$8.8 million was awarded for 84 research grants, which included 6 program projects, 67 regular research grants, and 11 new investigator awards. Three research contracts were awarded for \$156,596. The Program also awarded \$891,236 to support 59 research trainees, including 46 fellows on 6 institutional training grants (\$685,619), and 13 individual postdoctoral fellows (\$205,617). In addition, 7 research career development awards were made at a cost of \$265,385, and 10 professional student short-term training programs were funded for \$136,721.

Table 1 shows the distribution of grant support according to funding mechanism. Approximately 20% of the grant funds were used for program projects, and 60% for regular grants. Table 2 shows the distribution of research grants by program category. Although the overall level of funding in FY 1980 was similar to FY 1979, the distribution of funds for specific categories within the Program did show some changes. One of these was the initiation of several grants in acquired craniofacial defects, an area which the Institute wishes to develop further. Table 3 shows the distribution of grants by scientific approach. Only minor differences are apparent between the current figures and those for last year.

TABLE 1. FY 1980 RESEARCH AND TRAINING SUPPORT BY FUNDING MECHANISM  
(in thousands of dollars)

	<u>No. of Grants</u>		<u>Cost</u>	<u>Percent</u>
	<u>Active</u>	<u>Funded</u>		
Program Projects (P01)	6	6	\$1,727	19.7
Regular Research Grants (R01)	78	67	5,245	59.9
Special Research Grants (R23)	12	11	332	3.8
Career Development Awards (K04)	7	7	265	3.0
Institutional Training Grants (T32)	7	6	686	7.8
Individual Fellowships (F32)	13	13	206	2.4
Student Short-Term Grants (T35)	10	10	137	1.6
Research Contracts	5	3	156	1.8
	<u>138</u>	<u>123</u>	<u>\$8,754</u>	<u>100.0</u>

TABLE 2. FY 1980 ACTIVE RESEARCH GRANTS BY PROGRAM CATEGORY

	<u>Number</u>	<u>Cost</u>	<u>Percent</u>
I. Craniofacial Anomalies-General	26	\$1,641	22.6
II. Cleft Lip/Palate	19	1,791	24.6
III. Other Congenital Anomalies	8	804	11.1
IV. Malocclusion	40	2,862	39.4
V. Acquired Defects	3	169	2.3
	<u>96*</u>	<u>\$7,267</u>	<u>100.0</u>

\*Includes P01, R01, and R23

TABLE 3. FY 1980 ACTIVE RESEARCH GRANTS BY SCIENTIFIC APPROACH

	<u>Number</u>	<u>Cost</u>	<u>Percent</u>
I. Etiology	50	\$3,369	46.4
II. Diagnosis and Treatment	29	2,506	34.5
III. Pathology	17	1,392	19.1
	<u>96*</u>	<u>\$7,267</u>	<u>100.0</u>

\*Includes P01, R01, and R23; 12 grants received no funds in FY 1980.



## STAFF ACTIVITIES

During FY 1980, staff activities included visiting institutions, monitoring grants and contracts, communicating with researchers, participating in scientific meetings, and publications. Through these professional activities, staff was able to maintain close communication with scientists working in the field of craniofacial anomalies. These activities included:

### A. Site Visits: Initial Review, Monitoring, Programming & Communication

Eastman Dental Center, Rochester, New York	Nov 1979
University of Michigan, Ann Arbor	Nov 1979
University of California, Los Angeles	Feb 1980
Forsyth Dental Center, Boston	Feb 1980
New England Primate Center, Framingham	Feb 1980
Boston University, Boston	Feb 1980
Eastman Dental Center, Rochester, New York	Feb 1980
University of Pittsburgh, Pittsburgh	Feb 1980
University of Iowa, Iowa City	Mar 1980
University of Southern California, Los Angeles	Mar 1980
Harbor General Hospital, Torrance, California	Mar 1980
University of Michigan, Ann Arbor	Apr 1980
University of Tennessee, Memphis	May 1980
Indian Health Service, Shiprock, New Mexico	May 1980
Columbia University, New York City	May 1980
Georgetown University, Washington, D. C.	Jun 1980
University of Pennsylvania, Philadelphia	Aug 1980

### B. Meetings

Biennial Meeting of the Edward H. Angle Society of Orthodontists, South Carolina. (Participant)	Oct 1979
Annual Meeting of the American Dental Association, Dallas.	Oct 1979
American Association for the Advancement of Science, San Francisco. (Participant)	Jan 1980
Symposium on the Role of Dentistry in Interdisciplinary Treatment of Genetic Diseases, sponsored by the Medical University of South Carolina. (Participant)	Mar 1980
Annual Meeting of the American Association of Dental Research, Presented Symposium on Temporomandibular Joint, Los Angeles.	Mar 1980
Fifth Annual University Seminar, sponsored by the New York Academy of Dentistry, New York City. (Participant)	Mar 1980

- 16th Annual Dental Students Research Conference, sponsored by the American Dental Association and Procter and Gamble, Co., University of Iowa, Iowa City. (Participant). Apr 1980
- 80th Annual Meeting of the American Association of Orthodontists, New Orleans. (Participant). Apr 1980
- International Conference on Oral Biology, Tokyo. Jun 1980
- International Symposium on Oral Physiology, Tokyo Jun 1980
- International Association for Dental Research, Chaired the Symposium, "Craniofacial Biology - Congenital Defects," and Moderated the Symposium, "Normal and Abnormal Craniofacial Development," Osaka, Japan. Jun 1980
- C. Sponsored Meetings and Seminars:
- Sponsored the "NIH Consensus Development Conference for Removal of Third Molars," Bethesda. Nov 1979
- International Symposium on Current Research Trends in Prenatal Craniofacial Development, Bethesda. Apr 1980
- Sponsored the Seminar, "The Role of Prostaglandin (E-2) in Bone Remodeling: Immunohistochemical and Quantitative Evidence," presented by Dr. Zeev Davidovitch, Bethesda. May 1980
- D. Staff Development
- Project Officers Civil Rights Contract Compliance, Bethesda. Nov 1979 & Dec 1979
- Equal Employment Opportunity - Current Assessment, Bethesda. June 1980
- Performance Assessment Workshop, Bethesda. Sep 1980
- E. Publications
- "Psychosocial Aspects of Craniofacial Disfigurement," A State of the Art Assessment; American Journal of Orthodontics, pp. 410-420. Oct 1979
- "Removal of Third Molars Consensus Development Conference," Journal of Oral Surgery. Jan/Feb 1980
- "Removal of Third Molars Consensus Development Conference," Journal of the American Dental Association, Vol. 100, pp. 579-580. Apr 1980

## RESEARCH HIGHLIGHTS

### A. Developmental Biology

Neural crest cells are important in the development of normal craniofacial anatomy because of their important contribution to the primordial tissues which differentiate into the bone, muscles, connective tissues, neurons, and endocrine elements of the craniofacial region. Thus, the complex biological phenomena involved in the migration and differentiation of these embryonic cells are under continual study.

The main route of migration of cephalic neural crest cells from the neural tube is through two narrow, cell-free spaces, one just under the surface ectoderm, the other just outside the neural tube. During migration, they traverse, but do not intermix with, mesodermally derived mesenchymal cells lying just outside the cell-free spaces. These spaces contain abundant extracellular matrix material which consists mainly of three components: sulfated and nonsulfated glycosaminoglycans, collagen, and glycoprotein. Texas Tech University scientists found that these components are modified by the mesodermally derived mesenchymal cells which pass through the cell spaces during different stages of embryonic development. In young embryos, in which the neural folds are beginning to fuse, the cell-free space lateral to the neural tube is enlarged and contains an extracellular matrix rich in hyaluronate. Under the electron microscope, this matrix looks like fibrillar strands extending beneath the ectoderm. During the next few hours, mesodermally derived mesenchymal cells fill in this space and reduce it to two narrow channels. The strands of matrix seen earlier are replaced by beadlike patches of amorphous material within the cell-free spaces, and accumulations of similar material are associated with the thick basement membrane of the surface ectoderm. It is this type of matrix material which is encountered by the initial wave of migrating cephalic neural crest cells. As these cells proceed laterally from the neural tube, they appear to remove the basement membrane of the surface ectoderm. This observation has led investigators to believe that the crest cells not only utilize the basement membrane as a migratory substrate but may also remove from it information which has been programmed there by the surface ectoderm or by the mesodermally derived mesenchymal cells. In further research the scientists hope to clarify the exact nature of the interactions between migrating crest cells and basement membrane.

To simplify their study of this complex, naturally occurring system, the Texas Tech investigators are developing a three-dimensional culture model system. Neural tubes isolated from chick embryos are placed onto a three-dimensional lattice of repolymerized, hydrated collagen. A monolayer or sheet of neural crest cells then grows out onto the collagen gel from the neural tube explant. This sheet of cells is similar to the sheet of crest cells which migrate away from the neural tube in vivo. Unlike neural crest cells in the usual two-dimensional culture systems, the crest cells explanted onto the



collagen gels resemble the in situ crest cells in several respects: they develop migratory-like appendages at the leading edge, retain broad areas of cell-cell contact, and remain undifferentiated. The investigators believe that this system will enable them to test the inductive capacities of various surrounding tissues normally encountered by these cells.

Investigators at Massachusetts General Hospital have also been studying the effects of extracellular matrix macromolecules on the regulation of cell migration and differentiation. The results of their work on the role of hyaluronate have led them to hypothesize that when hyaluronate is confined within tissues it gives rise to swelling pressures that cause the structures to separate and thus to produce the cell-free spaces mentioned earlier in this report. They also postulate that hyaluronate interacts with the surface of migratory cells to prevent cell interactions which might lead to differentiation. More recently these scientists have studied V-2 carcinomas, which are invasive in rabbits but not in nude mice. Hyaluronate concentrations were 3 to 4 times higher in the rabbit tumor than in the mouse tumor. This finding suggests that hyaluronate is important in tumor cell migration as well as in normal development. If so, the administration of hyaluronidase might be helpful in preventing metastasis.

Scientists at the University of Southern California are continuing to look for ways to determine the regulatory steps in neovascularization. Previously they had reported that vascular endothelial cells in culture secrete various proteases, including plasminogen activator. Now they have found that endothelial cells also secrete elastase, the only enzyme which can break down elastin, a component of several connective tissues. Since elastase can hydrolyze several proteins, it may contribute to the invasiveness of new vascular tissues. Recent studies have also shown that fibronectin, a cell surface glycoprotein synthesized by most cells, is a potent chemotactic agent for endothelial cells. In microgram quantities, it can stimulate endothelial cells to migrate across a filter. The degree of stimulation is dose dependent.

In other studies, these investigators obtained evidence that cultured endothelial cells also produce a substance which inhibits DNA synthesis and prevents the cells from further proliferation. This substance may thus be important in regulating growth and determining the final quantity and distribution of blood vessels in tissues and organs. Without such mechanisms of control during embryogenesis, blood vessels might not grow into developing tissues at normal rates, and malformation could result. Biological control of vascularization is also important in the study of tumor growth and treatment.

Investigators at the University of Chicago have continued their efforts to characterize the molecular nature of odontogenic epithelial-mesenchymal interactions in vitro. First, they maintained incisor primordia from 16-day rat embryos in organ culture for up to 7 days and then treated them overnight with a low dose of the thymidine analog, 5-bromodeoxyuridine (BrdU), a drug known to prevent differentiation into teeth. Since previous work had indicated that this

mode of blocking differentiation is mediated through modifications in the extracellular matrix, the scientists tried to characterize the rate of biosynthesis and the molecular makeup of the matrix components. Using radiolabeled precursors, they found that the overall rates of collagen and proteoglycan biosynthesis are more depressed in BrdU-treated organs than in untreated counterparts. Furthermore, both treated and untreated incisor rudiments elaborated the same types of proteoglycans, but the collagen moieties differed. After 7 days in vitro, Type I was the predominant collagen species in the untreated cultures, whereas a mixture of Types I and III was found in the BrdU-treated cultures, a profile somewhat similar to that observed in freshly dissected, less-differentiated cultures. Thus, the thymidine analog caused both qualitative and quantitative alterations in the molecular composition of the odontogenic extracellular matrix. The investigators believe that BrdU-mediated odontogenic arrest is related to the modifications at the epithelial-mesenchymal interface. Studies are in progress to pursue this hypothesis.

## B. Cleft Lip and Palate

Etiology and Pathogenesis. The secondary palate is formed by the fusion of the two palatal shelves, which grow down from the maxillary processes and become vertically positioned on either side of the tongue. The secondary palate is formed when the palatal shelves assume a horizontal position above the tongue and fuse with each other and the overlying nasal septum. If the basic mechanisms by which this shelf movement and palatal closure take place were understood, investigators could foresee what types of agents or conditions would interfere with the normal processes and cause cleft palate. To determine what changes occur in the various components of the palatal shelves during the process of normal palatal closure, scientists are conducting further studies.

The palatal shelves are wedge-shaped structures composed of a core of mesenchymal cells and extracellular molecules covered with epithelium. Among the extracellular matrix molecules are collagen and glycosaminoglycans. Investigators at the University of Michigan used computer-assisted techniques designed for describing patterns to determine the mesenchymal and epithelial cell distribution before, during, and after palatal shelf closure. They found a dramatic decrease in mesenchymal cells in the interior of most of the shelf during closure, but highly localized increases in epithelial cell density at the surface. These changes led them to postulate that localized epithelial changes dictate the direction of form change whereas the force necessary to move the palatal shelves results from changes in the extracellular matrix which displaces the mesenchymal cells and matrix in the direction indicated by the epithelial changes. To test this hypothesis, the investigators varied osmotic conditions on normal palatal shelves and on shelves in which the extracellular molecules had been chemically altered. Results of these experiments support the hypothesis that alterations in the properties of the extracellular matrix may provide the force for palatal closure.



In a related approach to this problem, researchers at Boston Biomedical Research Institute measured the level of glycosaminoglycans in the developing palatal tissue of normal mouse embryos and in embryos with cleft palate whose mothers had been treated with cortisone. The levels of glycosaminoglycan were measured at the time of palatal shelf elevation and after fusion in a strain of mouse (CD-1) extremely sensitive to cortisone-induced cleft palate. Injection of pregnant females with cortisone on days 11 to 14 of pregnancy produced a cleft palate rate of 96 to 100%. For comparison, the investigators also studied a mouse strain (C57 BL/6J) which is much less sensitive to cortisone-induced cleft palate.

In the untreated CD-1 embryos the investigators found that hyaluronic acid is a major component at the time of palatal shelf elevation and fusion. Since this glycosaminoglycan binds large amounts of water, finding it in high concentrations would support the hypothesis that the extracellular matrix provides hydrostatic force for palatal shelf movement. As palatal development proceeds after fusion, the level of hyaluronic acid decreases and another type of glycosaminoglycan, the sulfated glycosaminoglycans, predominate. Since these compounds do not bind water as strongly as hyaluronic acid but do play a role in cartilage development, it seems reasonable that they should appear after palatal fusion has taken place. Thus, the normal biochemical pattern seems consistent with the major biological events taking place.

In the embryos of the CD-1 strain whose mothers were treated with cortisone, marked changes were found in the glycosaminoglycan content of developing palatal tissue. Hyaluronic acid was decreased from 48% to 27% of that of the untreated controls during the period when shelf elevation and fusion take place, and the levels of the sulfated glycosaminoglycan were actually higher than in normal controls during this period. Subsequently the sulfated glycosaminoglycans fell to below normal. The initial effect of cortisone treatment appears to be to derange the pattern of glycosaminoglycan synthesis in palatal tissue. As a result, the tissues no longer contain a large amount of hyaluronic acid, with its extremely high water-binding property but, instead, contain high levels of sulfated glycosaminoglycans. This change in glycosaminoglycan composition would be expected to cause a radical reduction in tissue turgor and might thereby cause the loss of an elevating force essential for shelf fusion.

In the cortisone-resistant mice, the glycosaminoglycan pattern resembled that of the untreated embryos. Cortisone treatment caused only a slight decrease in hyaluronic acid (88% of normal) during the period of shelf elevation; normal values were seen at other times. These results support the hypothesis that hydrophilic glycosaminoglycans, especially hyaluronic acid, provide force for palatal shelf elevation and indicate that significant alterations in these components may cause clefting.

Using a different approach, scientists at the Institute for Developmental Research of the University of Cincinnati are testing the hypothesis that palate shelf movement is effected partly by contraction



of cytoplasmic processes and partly by migration of cells. Previous studies in embryo culture had shown that the neurotransmitter serotonin synthesized in the palate appeared to regulate rotation of the anterior end of the palate by stimulating cell contraction.

More recently, these workers have shown that palate mesenchymal cells migrate specifically towards a chemoattractant factor in medium from neuroblastoma cultures. Pretreatment of the palate cells with  $10^{-5}M$  serotonin caused a 100% increase in the number of cells moving towards the chemoattractant(s). Thus, this experiment and the previous work on palate explants support the hypothesis that serotonin mediates palate elevation by stimulating cell contraction and increasing cell migration.

Protein carboxymethylation, which is involved in mammalian chemotaxis, is believed to be a common intermediate step in the transduction of biological signals from cell surface receptors. Since serotonin stimulates cell movement, the Cincinnati scientists reasoned that this neurotransmitter might affect protein carboxymethylation in developing palatal tissues. When whole palates or cultured palate cells were exposed to serotonin, protein carboxymethylation increased 60 to 70%. These results are consistent with the possibility that serotonin acts as an extracellular biological signal which produces macromolecular modifications which, in turn, lead to palate cell movement and shelf rotation.

In other experiments on cell migration, the effect of the antiepileptic but teratogenic agent, phenytoin, was analyzed. Toxic concentrations of phenytoin added to cultures of palate mesenchyme cells markedly inhibited the chemotaxis produced by the neuroblastoma medium. The investigators concluded that the production of cleft palate by phenytoin may be related to its inhibition of cell migration.

Studies on the role of phenytoin in the production of cleft lip and palate in mice are continuing at the University of North Carolina. Previous studies had demonstrated that phenytoin is teratogenic in A/J mice. Pregnant animals of this strain, which had received intraperitoneal injections of 75 mg/kg of phenytoin in the morning of gestational day 10, routinely had litters with nearly 100% cleft lip and palate. The animals showed ataxia, disorientation, and other signs of acute phenytoin toxicity affecting cerebellar-vestibular functions as well as such CNS effects as hyperactivity and sedation. These signs of acute toxicity are also seen in human patients treated intravenously for status epilepticus of the grand mal type. Severe cases of toxicity may involve CNS depression and cardiovascular collapse. Although a lower teratogenic dose of 50 mg/kg did not produce the extreme cerebellar-vestibular components of toxicity, the investigators believe that some degree of cardiorespiratory depression occurs.

Thus the scientists suspected that the teratogenicity of phenytoin may be partly related to cardiorespiratory depression in the pregnant mother. To test this possibility, they placed one group of pregnant animals in a hyperoxic environment (50%  $O_2$  - 50%  $N_2$ ) after phenytoin

injections to determine whether oxygen treatment reduced maternal toxicity and the associated birth defects. Animals given 60 mg/kg of sodium phenytoin but not treated with oxygen showed visible CNS effects including irritability, hyperactivity, ataxia, and sedation. Moreover, 84% of their viable fetuses (104 out of 124) had cleft lip with or without cleft palate, and six percent of the implantation sites showed fetal resorption. In contrast, pregnant animals placed in hyperoxia after phenytoin injection showed no sign of CNS toxicity and the rate of phenytoin-induced cleft lip and palate was reduced more than three-fold. Only 25% of the 124 viable fetuses showed clefting and resorptions were evident in only 3% of the implantation sites.

Thus their evidence supports the possibility that phenytoin causes cardiorespiratory depression which can be overcome by elevating respiratory oxygen. In future studies, the investigators will maximize the beneficial effects of hyperoxia by optimizing variables such as the  $O_2-N_2$  ratio, humidity, temperature, and time of oxygen exposure. The investigators are also exploring whether any mechanisms are responsible for the teratogenicity of phenytoin, such as the interference of the drug or its metabolites with enzymes important in oxidative metabolism.

Patient Management. Surgical repair of cleftpalate sometimes leaves persistent oro-nasal fistulas which are believed to adversely affect speech development. To determine how these fistulas contribute to speech problems, investigators at the University of Arizona have conducted special studies in children with repaired clefts and oro-nasal fistulas. In these studies temporary obturation of the fistula was used to differentiate between nasal air leaks associated with the fistulas and those associated with failure of the repaired palate to close the posterior passage into the nose. These findings indicate that the fistulas do contribute to the audible emission of air from the nose during speech, to impaired articulation, and to reduced intelligibility of speech. The findings may also help to resolve a controversy regarding the sequence of surgery for cleft palate. Some surgeons have advocated repairing the soft palate early in life but delaying repair of the hard palate until the child is approximately five years of age to avoid damage that would restrict growth of the upper jaw. However, the current findings indicate that leaving the hard palate open could significantly impair speech development because even the small opening of an oro-nasal fistula has a detrimental effect. If this surgical approach were to be used, some kind of prosthetic device to close the hard palate cleft temporarily should be considered.

Workers at the Carolina Cleft Lip and Palate Center in Charleston are conducting studies on infants with cleft lip and palate treated by a combined surgical and prosthetic approach. When the infants are about one month old, a preliminary surgical lip closure is performed and a pin-retained artificial palate is inserted. At six months, lip repair is completed and the soft palate is repaired, but the pin-retainer artificial palate is left undisturbed until the child is approximately six years of age. At that time, permanent surgical repair of the hard palate is completed.



A study of 31 subjects treated by this surgical-prosthetic method revealed that the insertion of the palatal pin-retained prosthesis made feeding easier. In many cases, nonconventional feeding methods involving syringes and eye-droppers were no longer necessary. Normal bottle feeding with a premature nipple became possible. Eighty-four percent of parents reported feeding to be easier after insertion of the prosthesis. They also observed a drastic reduction in the loss of fluid and food through the nose. Moreover, the children with prostheses began using strained baby foods and junior foods at the same age as normal noncleft children and also showed a slight improvement in mid-facial growth and arch alignment.

### C. Other Craniofacial Anomalies

At the Center for Craniofacial Anomalies, University of California, San Francisco, patients with facial asymmetries, premature closure of cranial sutures, and other congenital anomalies are being studied to develop improved methods of treatment. The treatment under current study is based on the principle that neuromuscular activity is a major factor in bone remodeling and in the development of new bony structures. Thus, treatment planning depends on an analysis which incorporates both neuromuscular and morphological elements.

One condition being studied is hemifacial microsomia, a congenital defect consisting of asymmetrical deficiency of facial structures, often with severe underdevelopment of the ear, cheek-bone, and mandible. Electromyographic studies of such patients indicated that the masseter muscle is sometimes missing on the underdeveloped side, in which case the mandibular gonial angle does not develop and the temporal muscles may vary in size, thickness, and position. After surgical and orthopedic treatment to replace a missing mandibular ramus and coronoid process, the temporal muscle sometimes increased in size. Compensatory neuromuscular mechanisms are often observed in patients with facial asymmetries. For example, a facial muscle such as the platysma can be recruited abnormally in certain jaw and facial movements and the anterior temporalis can be recruited during protrusion. This use of the temporal muscle is not seen in normal control subjects. In some patients, certain jaw or facial movements are lacking because of failure to synergistically recruit mandibular muscles which are not recruited correctly. Since restoration of adequate function is a major concern in these cases, understanding of the neuromuscular compensations which are possible will enable clinicians to treat severe deficiency malformations more successfully.

Detachment of a muscle is a common empirical procedure in the treatment of craniofacial deformations, but subsequent function and stability are matters of some concern. To study experimentally produced conditions which unbalance muscular forces acting at the margins of adjacent bones, other researchers at the San Francisco Center used rhesus monkeys. The imbalance was induced by detaching a muscle of one side of the suture. Bone formation on one side of the suture increased and neuromuscular response changed significantly; the background level of activity was produced until the muscle reattached.



Five weeks after a craniofacial muscle was detached in the experimental animal, reattachment of the muscle progressed to such an extent that functional stimuli were again transmitted to the bone margins. These results suggest that new form established by surgical methods will remain stable after this five-week period only when care is taken that the reattached muscles do not deliver unbalanced forces to the facial bones.

#### D. Malocclusion

Bone Growth and Remodeling. Orthodontists have traditionally used mechanical forces applied continuously both to realign the dentition and to alter the dimensions and growth of the facial bones. In recent years, discontinuous or intermittent forces have attracted interest because they are believed to be physiologically more appropriate and more efficient in remodeling the skeletal tissues.

Investigators at the University of Connecticut have devised an experimental system to study the biochemical changes which occur when mechanical forces interact with skeletal tissue. This experimental model has two components, a mechanical force and cells cloned from an osteoblastoma. Since these bone-forming cells were cloned, every cell is identical and can be expected to react to mechanical stimulation in precisely the same way. In one experiment, the cells were grown in glass tubes and approximately once every two seconds were compressed by light hydrostatic forces 1/6 of atmospheric pressure. This interval was chosen to be consistent with a slow chewing cycle.

The cells showed a significant response to the intermittent mechanical compression. The enzyme ornithine decarboxylase (ODC), one of the earliest indicators of cell growth, increased in activity up to 60%. Thus, the mechanical forces appeared to stimulate growth.

To further explore this phenomenon, the investigators examined the cell membrane, which previous work had indicated to be a critical factor in the transduction of environmental signals into cellular responses. They found that the intermittent force applied to the cell caused a redistribution of positively charged ions. Using a radioisotope (<sup>86</sup>Rubidium) and an analog of the cardiac drug digitalis (Ouabain), the investigators showed that the mechanical force indirectly activated a key enzyme in the cell membrane. The activation of this enzyme in the cell membrane and the associated redistribution of ions is believed to be part of the mechanism by which the cell perceives a mechanical force.

Researchers at the University of Florida are studying the biochemical events that occur in the tissue around orthodontically treated teeth and that result in bone resorption. Earlier experiments had demonstrated that extracts prepared from such sites in rats can stimulate bone resorption in vitro; this suggests that orthodontic tooth movement stimulates chemical mediation of the bone resorption. Stimulation of bone resorptive activity peaked seven days after appliance activation

and was a direct function of the amount of force applied: the greater the force, the higher the level of resorptive activity in the extracts. This bone-resorptive activity was also shown to be heat stable, non-dialyzable, and osteoclast-mediated. Recent work has demonstrated that root resorption and bone resorption are similar in their response to applied force. This observation suggests that both root resorption and bone resorption are stimulated by the same biologically active substances.

The investigators are now studying the chemical and mechanical nature of the bone resorptive activity. Extracts from sites of orthodontic tooth movement contain an active large molecular weight fraction (65,000 D) which is pronase digestible and therefore proteinaceous. Fractionation by molecular weight by means of column chromatographic techniques has yielded preliminary data which indicate significant amounts of bone resorptive activity in multiple fractions. This suggests multiple mediators of such activity.

Resorption of the roots of primary teeth is an important but poorly understood biologic process. Scientists at the University of Pennsylvania are using immunohistochemical methods to localize cyclic nucleotides around resorbing primary teeth in kittens. They have found that this degradative process is initiated by mononuclear cells which normally populate these sites. Many of these cells contain high amounts of cyclic nucleotides. Later in the process, root resorption is performed by large multinucleated cells which contain even greater amounts of nucleotides. These cells also seem to be "bathed" in extracellular nucleotides, apparently as a result of the progressive dissolution of the extracellular matrix around the resorbing root.

The histochemical method for localizing nucleotides was also applied to jaw sections from cats treated by rapid palatal expansion. Tissue responses in young and old animals were compared to determine why the response of adult subjects to orthodontic forces is sluggish. In the young cats, osteoblasts, cells which form new bone, responded to the tensile forces by marked increases in their cyclic nucleotide contents, whereas osteoblasts in the old animals were unresponsive and did not show such increases. This observation focuses attention on molecular events which may be responsible for the different clinical response to force application to teeth seen in young and adult patients.

Clinical investigators at Tufts University are continuing to study the application of pulsating forces to achieve tooth movement in human orthodontic patients. Traditionally, continuous forces have been employed. In recent tests, the scientists confirmed data collected during previous years. Tooth movement over the latter half of the test periods averaged .0077 mm per hour to produce 2.7 mm of movement of the test teeth in approximately 350 hours.

The pulsating forces consisted of a 32-ounce force applied for 0.2 seconds followed immediately by a sustained force of 6 to 10 ounces applied for 1.4 seconds. Tests were conducted during sleeping hours, which averaged approximately 7.5 hours. Every two weeks, the amount



of bodily movement, the amount of tipping of each tooth, and the degree of mobility were measured. These measurements were compared with analogous measurements of control teeth to which 20 ounces of continuous force had been applied by a rubber band-actuated plunger. The results showed that the pulsed teeth averaged 3.7 mm for the entire test period, the control teeth only 1.0 mm. Patients reported that the force pulsations were barely perceptible and did not cause pain or interference with sleep.

Investigators are measuring the long-term effects of using the Obwegeser technique to surgically correct mandibular prognathism. This method repositions the mandible posteriorly by moving it either vertically or horizontally or by rotating it. The centroid of the chin was used as the point of measurement to analyze the effects of these corrective measures. Changes in both upper and lower lip were studied by measuring the cross-sectional changes in each lip as well as the change in shape.

Distal repositioning of the mandible and rotation of the chin caused changes in the upper lip, including an increase in the area of the lip as well as in its horizontal length. The extent of these alterations was directly related to the amount of distal repositioning and/or rotation.

The changes in the lower lip were also caused by the combined effect of distal repositioning of the chin and rotation of the chin during surgery. If the rotation were clockwise, (towards a closed bite position), the tissue of the free-standing lower lip redistributed itself so that the lip was depressed, curled outward, and exposed less cross-sectional area of tissue. These changes were brought about by relatively small amounts of upward positioning of the mandible during surgery. The final shape and position of the lips in closure depended upon the exact way they were brought into new contact with each other and upon the stiffness characteristics of the separate upper and lower lips.

Observations on the effects of surgery made after five to seven years were similar to those observed after one year. The findings of this study will enable clinicians to accurately predict changes in the facial profile as a result of surgery.

In a retrospective study at the University of Illinois, periodontal health, temporomandibular joint function, and occlusion were evaluated in 96 subjects treated orthodontically 10 to 35 years earlier. These results were compared with those of a similar group of 103 adults with untreated malocclusions.

The two groups did not differ significantly in the incidence of overall periodontal disease. However, the orthodontically treated group had a greater percentage of teeth with mild-to-moderate periodontal disease and included more subjects with at least 40% of their teeth affected by periodontal disease than the untreated group. Both groups showed



minimal evidence of severe periodontal disease. This finding is believed to reflect the fact that the subjects were from a high socioeconomic level and thus had a high level of dental awareness and good oral hygiene habits. The prevalence of periodontal disease was higher in orthodontic subjects treated with extraction therapy than in subjects treated with a nonextraction approach. There was no difference in the prevalence of signs/symptoms of temporomandibular joint dysfunction in the two groups, nor in the prevalence of nonfunctional occlusal contacts. A mandibular shift from the retruded (centric) contact to the intercuspal or habitual position was prevalent in both groups, but the average amount of shift was greater in the control group. This study provided no evidence that orthodontic therapy is beneficial in preventing periodontal disease.

#### E. Acquired Craniofacial Defects.

In order to estimate the magnitude of the problem of acquired craniofacial defects in the United States, Program staff initiated a study of data from the National Center for Health Statistics. The study indicated that approximately 10 million craniofacial injuries requiring medical attention occur in the United States each year, and that half of these injuries caused a restriction of personal activity for an average duration of 7 days. Hospitalization was required for 569,000 persons (5.4%), for which a total of 354,000 surgical procedures were necessary. The total estimated costs for treatment of the craniofacial injuries of the hospitalized group approximated \$1200 million, which includes \$400 million in lost productivity. These data emphasize the magnitude of the problem and indicate the need for increased research on the treatment and prevention of such injuries.

#### FUTURE PLANS

In FY 1981 craniofacial anomalies research currently supported by the NIDR will be formally evaluated and recommendations will be made for future research. In addition, staff, together with consultants, will develop a long-range craniofacial program plan which will be incorporated into the overall long-range plan for the entire NIDR. Meanwhile, projects with immediate promise will be continued or expanded, as described below.

In FY 1981, developmental biology studies on cell proliferation, cell migration and differentiation will be continued. Since neural crest cells contribute the primordium for the development of craniofacial structures, studies of the mechanisms by which these cells migrate within the extracellular matrix will be expanded. Since each tissue in the body has a characteristic extracellular matrix, which is believed to be essential for the normal orderly development of cells and tissues, future studies will attempt to determine how matrix substances interact with cells and influence their movements and general behavior. These studies will also examine cellular responses to hormones and growth factors and will explore the cell surface receptor number, binding affinity, and the mobility of the receptor-matrix complexes. Such studies are expected to yield important insights

into the control of craniofacial development and malformation.

In FY 1981 studies on the role of dilantin in the causation of cleft lip and palate will be expanded with emphasis on the mechanisms of action of this drug. There will also be an expansion of oral cleft studies in which tissue culture systems are used. One unique system employing isolated embryonic mouse heads permits investigators to study the events which occur during palate elevation, including the deposition of proteoglycans at the epithelial mesenchymal interface and the pattern of mesenchymal cell distribution.

Since treatment of craniofacial malformations rests in part on the remodeling of skeletal tissues, studies of the basic mechanisms by which applied forces cause remodeling will be continued. These studies will explore the hypothesis that bone resorption associated with tooth movement results from the local production chemical mediators, such as prostaglandin and collagenase, secreted by macrophages and other cells. The findings obtained may enable clinicians to control remodelling more effectively.

In 1981 human and animal studies will be conducted to improve the diagnosis, treatment and prognosis of dentofacial disfigurement and to evaluate the increasingly popular orthognathic surgery approach to this problem. A special effort will be made to develop an improved record system for disfigurement cases so that research on diagnostic and treatment questions can be better addressed. Workshops to be funded in FY 1981 include an assessment of progress in orthodontic engineering research and an international conference on genetics.

#### SUMMARY

Awards made by the Craniofacial Anomalies Program Branch in FY 1980 totaled \$8.8 million, which included \$5.2 million for individual research grants and \$1.7 million for program projects. Seven career development awards were made for \$265,345. Forty six fellows were supported through 6 institutional training grants at a cost of \$685,619, and 13 individual postdoctoral fellowships were awarded, totaling \$205,617. Research contracts totaled \$153,596. Ten professional student short-term Training Programs were funded for \$136,721.

Research in developmental biology was highlighted by studies involving the mechanisms responsible for migration and differentiation of neural crest cells. Since these cells are among the most important contributors to the developing craniofacial region they are of special interest. The effect on neural crest cells of extracellular matrix components including glycosaminoglycans, collagen and glycoprotein were emphasized.

Studies involving the regulation of vascular development have shown that fibronectin, a cell surface glycoprotein synthesized by most cells, is a potent chemotactic agent for endothelial cells. Other studies have dealt with substances preventing endothelial proliferation. This research is of importance in understanding control of embryogenesis as well as tumor growth and control.



Studies relating to etiology and pathogenesis of cleft lip and palate continue to be a major focus of the program area. Research on mechanisms responsible for palatal shelf movement and closure has produced results which suggest that scientists will someday be able to predict the types of agents which interfere with normal palatal development and thus they may be able to prevent cleft palate. Attention has focused on chemical changes occurring in the extracellular matrix during palate closure. It is thought that alterations of macromolecules in the extracellular matrix may provide the force necessary for palatal shelf movement during closure.

Studies on the role of Dilantin in the production of cleft lip were continued. Mice given teratogenic doses of the drug showed signs of acute central nervous system toxicity similar to those observed in humans being treated for epilepsy. When pregnant mice were placed in an environment high in oxygen immediately after receiving a teratogenic dose of phenytoin they did not exhibit signs of central nervous system toxicity and the rate of phenytoin induced cleft lip and palate was reduced more than threefold. These findings offer another approach to the study of the mechanisms responsible for the teratogenicity of phenytoin.

Although a large portion of cleft palate repairs are completed before 2 years of age, some surgeons prefer to delay the final operations until 5 years. At this age growth is further advanced and there is less retardation due to surgically-induced scar tissue. Studies relating to this surgical approach have shown that the placement of a prosthetic device to close the palate during the period prior to surgical closure is important for proper feeding and normal speech development.

Studies on the relationship of neuromuscular activity to bone formation and remodeling are being conducted in order to provide data which will allow clinicians to more successfully treat craniofacial malformations. These studies have shown that new skeletal relationships established by surgery will remain stable only when care is taken to insure that repositioned muscles do not produce unbalanced forces.

Studies of the biochemical changes which occur when cultured bone forming cells are subjected to mechanical forces have shown that these forces activate enzymes associated with cell growth. Knowledge of the mechanisms by which cells perceive and respond to mechanical forces is important in order to develop more efficient physiologically acceptable approaches to orthodontic tooth movement.

Related studies dealing with bone response to orthodontic forces have shown that material causing resorption is produced in bone adjacent to teeth to which forces are applied. Chemical analysis suggest that this material is proteinaceous and may in fact be composed of several different proteins.

Studies on the long-term effects of orthodontic treatment compared periodontal disease, temporomandibular joint and occlusal function in orthodontically treated patients to a comparable group of untreated individuals. The treated group which had, on the average, completed



their treatment more than 25 years earlier showed no significant difference from the untreated group in any aspect of dental health. Additional studies are being conducted to confirm and extend these results.

Prepared by R. L. Christiansen and J. D. Niswander

## RESTORATIVE MATERIALS PROGRAM BRANCH

### INTRODUCTION

The Restorative Materials Program serves as a primary focus at the NIDR for supporting research and development in dental biomaterials and instrumentation. Since the sequelae of oral diseases are damaged tissues, there is a continuing need for materials to repair these tissues and restore function and appearance.

Through grants, contracts and interagency agreements, the Program funds research in the development of new and improved materials. These research areas fall into eight categories: 1) restorative filling materials for repairing teeth; 2) bonding agents, adhesive coatings and cements to prevent decay on the chewing surfaces of teeth and at the margins of fillings; 3) intraoral prostheses for replacing missing teeth and other oral tissues; maxillofacial prostheses to replace defects resulting from congenital abnormalities, surgery, or accidents; 4) artificial tooth implants to replace missing 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 to improve dental care; 8) improved restorative materials for prevention. Special emphasis is given to clinical studies of implants and maxillofacial materials.

### ADMINISTRATIVE

Table I shows the distribution of funds for research and research training in FY 1980. During FY 1980 the Restorative Material Program Branch awarded a total of \$3.7 million to support 46 grants and 2 interagency agreements for research on restorative filling materials, bonding agents, oral and facial prostheses, artificial tooth implants, endodontics, transplants/replants and general studies. These awards included 6 research career development awards. The program also awarded a total of \$413 thousand to support 6 training grants and \$77 thousand for 4 individual postdoctoral fellowships.

FY 1980 Distribution of Funds

Restorative Materials Program Branch

<u>Program Area</u>	<u>No. of Projects</u>		<u>Obligated Funds</u> (Thousands)	<u>% of Funds</u>
	<u>GRANTS*</u>			
	<u>Active</u>	<u>Funded</u>		
Filling Materials	18 1/2	13 1/2	\$ 664	22
Bonding Agents	2	2	95	3
Prostheses (Oral)	13	11	799	26
Prostheses (Facial)	4	1	95	3
Implants	11	9	836	27
Transplants & Replants	3	3	112	4
General Studies	4	3	180	6
Prevention	1/2	1/2	53	2
Endodontics	4	3	216	7
Total	60	46	\$ 3,050	100

CONTRACTS AND INTERAGENCY AGREEMENTS

Filling Materials	1 1/4		394	58
Bonding Agents	1/4		114	17
Prostheses (Oral)	1/2		169	25
Prostheses (Facial)	0		0	0
Total	2		\$ 677	100

TRAINING GRANTS

Institutional Grants	6		413	84
Fellowships	4		77	16
Total	10		\$ 490	100

GRAND TOTALS 72 \$ 4,217

\* Includes 6 Research Career Development Awards for \$230 thousand.



## STAFF ACTIVITIES

During FY 1980 Program staff made visits to research institutions to program and monitor grants and contracts. Through these professional activities and participation in scientific meetings they were able to stay abreast of scientific developments and to maintain close liaison with scientists working in the area of restorative materials. These professional activities are listed below.

### A. Site Visits: Initial Review, Monitoring, Programming and Communication

Tulane University	Jan 1980
Northwestern University	Apr 1980
University of California (Berkeley)	Aug 1980
University of California (San Francisco)	Aug 1980
University of the Pacific (San Francisco)	Aug 1980
USPHS Hospital (San Francisco)	Aug 1980
National Bureau of Standards (Gaithersburg, Md.)	Aug 1980

### B. Scientific Meetings

ADA Annual Meeting, Dallas (monitored exhibit)	Oct 1979
ADA Council on Dental Materials, Instruments and Equipment, Chicago	Nov 1979
Greater New York Dental Meeting, N.Y. (monitored exhibit)	Nov 1979
Fifth Annual Yankee Dental Congress, Boston (monitored exhibit)	Jan 1980
East Coast Dental Society Meeting, Miami (monitored exhibit)	Feb 1980
American Association of Dental Schools, Los Angeles	Mar 1980
American Association of Dental Research, Los Angeles	Mar 1980
American National Standards Committee, Los Angeles	Mar 1980
ANSC MD 156 Subcommittee for Dental Implants, Los Angeles	Mar 1980
D.C. Dental Society Annual Meeting, Washington, D.C. (monitored exhibit)	Apr 1980

### C. Administrative

Performance Appraisal Training - Phase I, Bethesda	Oct 1979
Interagency Materials Group, NSF, Washington, D.C.	Oct 1979
STEP Module, "Introduction to Extramural Programs of NIH", Bethesda (Dr. Valega served on the faculty)	Dec 1979
"New Concepts in Prosthetic Surgery and Implant Dentistry", Continuing Education Course, LSU, New Orleans	Jan 1980
NIH Civil Rights Compliance Seminar, Bethesda	Feb 1980
Workshop for Supervisors on Dealing with Job Performance Problems, Bethesda	Feb 1980

D. Additional Staff Activities

Dr. Valega was invited to become a member of the NIH Staff Training in Extramural Programs (STEP) committee.

Dr. Reese was invited to become a member of the Fellowship Committee of the American Association for Dental Research.

The staff prepared an exhibit entitled "Clinical Evaluation of Dental Restorative Materials" which was displayed at appropriate national meetings, as noted under "B". The exhibit will also be displayed at other meetings.

A conference in June 1978, entitled, "Clinical Evaluation of Dental Materials", was supported jointly by the Program, by the Council on Dental Materials, Instruments and Equipment of the American Dental Association and by the Bureau of Medical Devices of the Food and Drug Administration. The staff assisted in the preparation of the proceedings which were published in June 1980 as NIH Publication No. 80-1147, edited by Harold O. Wyckoff, Jr., D.D.S. and Dorothy G. Weigl, M.S.

To prepare for the formal evaluation planned for the Restorative Materials Program, staff and their consultants devoted considerable effort to the refinement of program goals, objectives and evaluation criteria. With special assistance from Dr. Richard Norman, a well-known dental materials scientist and educator, who served as a full-time consultant, they developed working drafts and convened three panels of experts to prepare the final documents on the different subject areas included in the Program. A panel was assigned to each of the following subject areas: intracoronar restorative materials; prosthetic and maxillofacial materials; endodontics, implants, and technology.

The first two panels met informally at the 1980 AADR meeting in Los Angeles, and each of these initial meetings was followed by a 2-day meeting in Bethesda in May 1980. The third panel met as a group in July 1980. The documents from all three panels have now been finalized, and will form the basis for the formal program evaluation. The individual meetings to refine program goals and objectives are listed below.

Panel 1:	Intra-coronar Restorative Materials, Los Angeles Bethesda	Mar 1980 May 1980
Panel 2:	Prosthetics & Maxillofacial Materials, Los Angeles Bethesda	Mar 1980 May 1980
Panel 3:	Endodontics, Implants, Instruments & Equipment, Bethesda	Jul 1980

## AWARDS

Dr. Kaare Langeland, the principal investigator of an NIDR research grant on the biocompatibility of endodontic materials, received the Pulp Biology Award June 6, 1980, at the Annual Meeting of the International Association of Dental Research in Osaka. Dr. Langeland is Professor and Head, Department of Endodontics, at the University of Connecticut. He is the first researcher ever to receive this honor.

## RESEARCH HIGHLIGHTS

### Clinical Performance of Copper Amalgams

At the University of North Carolina Dental Research Institute, scientists have evaluated the clinical performance of nine different amalgams with copper contents ranging from 9% to 30% and have correlated the results with their physical properties as determined in the laboratory. Approximately 50 restorations of each amalgam were inserted into Class 1, 2, and 5 cavity preparations; a conventional composition containing 4% Cu served as a control. Over a twelve-month period, a total of 523 restorations were placed by a team of three clinicians.

After one and two years, standardized black and white photographs of these restorations were compared and scores for marginal breakdown were given. The two-year results are shown in Table 2. All of the alloys except one showed less marginal breakdown than the conventional alloy. The scores of the 5 best amalgams did not differ statistically. A sixth amalgam had an intermediate score, and the lower scores of the other three did not differ significantly from that of the conventional control. The seven top-ranked alloys contained the highest copper levels, were free of the gamma-2 phase, and showed distinctly lower creep values than those of the remaining three. Nevertheless, within the group of seven gamma-2-free alloys there was no correlation between creep and marginal breakdown and little or no correlation between compressive strength and clinical performance, regardless of the time period.

With one exception, the ranking of these alloys after two years of clinical service was the same as after one year. Therefore, the investigators believe that in the future it may be possible to predict the long-range performance of amalgam restorations after only one year of evaluation, provided that high-resolution photography is used and experimental conditions are favorable. However, for the present, they see at least 2 years of follow-up clinical evaluations as a minimum. In this study, clinical observations will be extended to 5 years to determine whether the clinical corrosion rate will remain constant.



Table 2. Amalgam Alloys Ranked According to Marginal Fracture

Alloy	% Cu	Creep (%)	Marginal Fracture Index* (2 years)
Tytin	13	0.07	2.67
Indiloy	13	0.06	2.94
Dispersalloy	12	0.25	2.96
Phasealloy	18	0.37	3.07
Cupralloy	19	0.22	3.17
Sybraloy	30	0.02	4.00
Aristaloy CR	13	0.28	4.84
Optaloy II	9	1.77	5.06
Velvalloy	4	1.00	5.15
Micro II	9	1.40	5.50

\*Lower number means less marginal breakdown.

#### Amalgam Creep and Marginal Fracture.

The 1979 Annual Report included investigations on the relation of "creep" and corrosion to the marginal fracture which occurs clinically in silver amalgam fillings. Creep is measured in the laboratory as the extent of the deformation that occurs when amalgams are subjected to pressure. In continuing research, LSU investigators have studied the effect of creep, corrosion, and amalgam microstructure on the marginal fracture of clinical restorations. In this laboratory project, they measured creep and corrosion in four experimental amalgams: (1) a conventional amalgam called Amalgam A; (2) amalgam A with 10% Gamma 1 phase added; (3) amalgam A with 10% Gamma 2 added; and (4) amalgam A with 10% CuSn additive. The investigators attempted to hold the amount of the Ag<sub>3</sub>Sn phase in these amalgams constant and to vary the relative percentage of Gamma 1, Gamma 2, and CuSn.

The addition of 10% Gamma 1 and 10% Gamma 2 increased the creep rate 44% and 88% respectively, but the addition of 10% CuSn decreased the creep rate. The addition of Gamma 2 also increased the tin-corrosion index, whereas the addition of either Gamma 1 or CuSn reduced it.

These results indicate that in a given amalgam the creep rate may or may not be related to the corrosion index. When the creep and corrosion characteristics of A, A + Gamma 2, and A + CuSn were compared, a decrease in creep was associated with a decrease in the tin-corrosion index. Both of these reductions were due to a reduction in the Gamma 2 phase, which is the most creep- and corrosion-prone phase. Since copper- and gold-containing amalgams with little or no Gamma 2 phase have reportedly shown improved marginal integrity over conventional amalgams, the LSU investigators concluded that low creep is associated with improved clinical marginal integrity via the mechanism of low corrosion. On the other hand, when the content of Gamma 1 is increased, high creep rates may be associated with improved marginal integrity and low corrosion.

High creep is usually associated with marginal fracture, but the LSU results indicate that high creep values without fracture are also possible. Thus, high creep may not necessarily be a predisposing condition for fracture. On the other hand, low creep values are always associated with improved marginal integrity. These data suggest that low creep values are predictive of clinical performance whereas high creep values may not be.

### Composite Cavity Preparations

Investigators at the University of North Carolina have evaluated the clinical performance of resin restorative materials placed in modified cavity preparations with no mechanical retention and in conventional preparations with mechanical retention. In modified preparations, the enamel walls diverge so that the cavo surface angle is greater than 90 degrees. The investigators sought to determine whether chemical etching would provide sufficient retention in the modified preparations and whether it is necessary in the conventional preparations. The study included three resin systems: an unfilled acrylic resin, an autopolymerizing composite resin, and a photo-polymerizing composite resin. A total of 479 restorations were placed by a team of three clinicians.

After three years only one restoration failed through loss of retention. The composite resins placed in the modified cavity preparations showed greatly improved color matching characteristics. The results clearly indicated that conservative cavity preparations with no mechanical retention are preferable to conventional cavity preparations which require the removal of tooth structure for incisal and gingival retention. Moreover, the modified preparations could often be completed without local anesthesia. Acid etching generally had no effect on marginal leakage when composites were used in conventional cavity preparations. However, as expected, the use of acrylic resins in unetched preparations caused deep inter-facial penetration of fluids to increase significantly.

### Amalgam and Composite Wear

Investigators at the University of Connecticut are developing a device to analyze and measure the wear of amalgams and composites in vitro. So far, they have studied the extent of wear in restorative materials placed in sliding contact with pins carved from human enamel. Sliding contact was achieved by the use of a pinion-rotating-diaphragm disc sliding-wear test apparatus. The results showed that composite restoratives and dental amalgams differ remarkably in the wear rate when different levels of contact stress (normal load per unit area) are applied. Throughout the range of stresses associated with human mastication, the rate of wear of dental amalgam remained virtually constant, whereas that of a composite depended dramatically on the contact stress. The experiments run at 37.0°C, used a constant sliding velocity to explore the effect of various contact stresses on the rate of sliding wear of the two materials. The results showed a striking wear rate



( $0.39 \text{ um hr}^{-1}$ ) which was independent of changes in contact stress over the range seen in the mouth (1.0 to  $1.4 \text{ Kgf mm}^{-2}$ ). The composite, however, showed a wear rate which increased more than a thousand-fold from  $0.15 \text{ um hr}^{-1}$  at stresses below  $1.3 \text{ Kgf mm}^{-2}$  to more than  $200 \text{ um hr}^{-1}$  at a stress of  $1.4 \text{ Kgf mm}^{-2}$ . Thus, the rate of resistance to wear of the two materials depends on the stress level at which the comparison is made. At lower stress the composite seems to be more wear resistant than the amalgam; however, at only a slightly higher stress, the amalgam maintains its resistance to wear and the composite undergoes catastrophic surface failure. Since the stress levels in vitro are comparable to the highest ranges experienced in vivo, the investigators believe that the poor resistance to wear of posterior composite restorations may well be due to the same type of severe surface failure observed in the laboratory. Analogous changes in the mode of surface failure as a function of contact stress may have been observed by other investigators at the University of Michigan in single and double pass sliding tests.

The advantages of the University of Connecticut device is that in a single experiment it provides data not only for predictions but also for analysis of the processes responsible for physiological wear. In subsequent studies these investigators will explore how wear is affected by other factors including pH, temperature, test duration, surface finish, and aging of the specimen.

#### Biocompatibility of Endodontic Materials.

Appropriate experimental methods to determine the biocompatibility of materials used in dentistry have long been needed. To be significant, such methods should test specifically for the unfavorable reactions that might be expected to occur clinically. With its special needs, the field of endodontics has provided a challenge to toxicologists that is just now being addressed by investigators at the University of Connecticut. To evaluate endodontic filling materials in vitro and in vivo, these scientists have developed promising new methods which enable them to test endodontic sealers separately and in combination with solid cores of the filling materials. The two methods under study, which test for biocompatibility and for the ranking of the toxicity level, consist of implantation into rat subcutaneous tissue and into bone to more closely simulate the endodontic situation in human patients. The sealers used in this study are AH26, Kerr Sealer, and Kloroperka N.Ø; and the filling materials were gutta percha points and a new endodontic material called hydron.

In the subcutaneous method, a 7 mm length of narrow teflon tubing containing test substances is inserted into the subcutaneous tissue of rats by means of a needle of slightly larger inside diameter that serves as a sleeve-like carrier. After insertion, the needle is withdrawn while a plunger holds the teflon tube in place. The distal end of the teflon tube remains open to expose the material to the tissue, but the proximal end is closed to avoid spillage of unset material into surrounding tissues. This method poses much less danger of contaminating tissue from spillage or external sources than did the incisional implantation



technique used in earlier experiments. Preliminary evidence indicates that the current method provides a clean control along the sides of the teflon implant and a distinct experimental area where the material contacts the tissue. Since this method can be carried out with minimal complications, it appears to be suitable for large-scale routine testing of materials.

In the intraosseus method, teflon cups containing endodontic materials are implanted into the mandibular bones of guinea pigs. Spillage of material does not usually occur when the teflon cup is introduced with the special applicator. With this method, test materials become transported from the cup into canals in the surrounding bone, both to the side of the cup and in front of the cup beyond the drilling site. This significant finding reconfirms the fact that sealers placed in contact with vital tissue will diffuse into the tissues and cause a persistent inflammatory response where the sealant contacts tissue. Thus the lack of biocompatibility of all sealers so far tested has once again been demonstrated. In contrast, bone growth into the empty cups, or into cups previously filled with calcium hydroxide, provides evidence that this methodology distinguishes between favorable and unfavorable materials.

Hydron, a hydrophilic gel, poly (2 hydroxy ethyl methacrylate) with barium sulfate added for radiocontrast, was used to show that the implant methods are effective in demonstrating unfavorable reactions. Both subcutaneous and bone implant tests showed that Hydron caused inflammation in the tissue it contacted, was transported in vessels, and was taken up by macrophages and foreign body cells. These preliminary results disagree with the manufacturer's claim that the material is biocompatible.

#### Polyethylene-coated Titanium Implants.

In their continuing animal tests of dental implants, investigators at the University of South Carolina have evaluated endosseous implants made of a titanium core coated with a porous, high-density polyethylene (PHDPE). A specific aim of the research is to determine why porous coated dental implants fail.

Thirty-nine PHDPE-coated tooth roots, which served as free-standing posterior dental implants in 14 dogs for periods ranging from 4 to 20 months, were evaluated clinically; 37 were also studied histologically. The clinical evaluations consisted of monthly examinations to monitor mobility, pocket depth, width of keratinized gingiva around the implant, condition of the gingiva, plaque accumulation and radiographic appearance. For the histologic study, block sections of the mandible were removed and the tissues prepared for microradiography, undecalcified ground section histology, and conventional decalcified paraffin histology.

Clinically, mobility and/or radiographic signs of bone loss were the most reliable indicators of implant failure. Criteria for failure included mobility measurements greater than 1/2 mm and radiographic bone loss of more than about 1 mm. Twenty-one of the 39 PHDPE implants showed mobility

of less than 1/2 mm and were therefore judged to be clinically acceptable. The remaining 18 were considered failures because of mobility and radiographic bone loss. Two of these failures were related to the dislodgement of the titanium core from the porous coating. Implant status could be correlated with pocket depth measurement but not with the amount of plaque, the gingival appearance, or the degree of bleeding around the neck of the implant. These clinical signs were unremarkable until advanced stages of failure became evident.

Nine of the 21 clinically acceptable implants were found to be unacceptable histologically. Histologic examination indicated that the bony ingrowth into the porous roots that held the tooth firm by ankylosis had been resorbed and replaced by fibrous tissue and inflammatory cell infiltrate. Implants immobile at one examination were found to have significant mobility at the next examination one month later with little change in radiographic appearance. Bone loss was observed, however, as the mobility subsequently increased. This failure differs from periodontal disease processes which involve crestal bone initially and apical progression subsequently. Instead, these observations are consistent with an overload failure of the bony ingrowth attachment mechanism. High loads applied during mastication combined with a relatively small area of root surface are predisposing factors for this type of failure.

These studies are significant because they provide a better understanding of the biologic response to different types of artificial tooth roots. The results suggest that the chemical and physical nature of porous coated endosseous dental implants is such that they can become firmly ankylosed for a time and may function well during this period but that the ankylosing bony ingrowth into the pores may become resorbed later and be replaced by soft tissue.

#### Carbon and Alumina Implants.

Investigators at Tulane University are conducting research into tooth implants to determine how variations in elastic modulus and in composition affect the clinical performance of dental implants. Implants of identical blade shape were fabricated from pyrolytic carbon, from alumina, and from alumina coated with 4000A of carbon. The carbon and carbon-coated alumina specimens had the same shape and surface chemistry, but the elastic modulus of the carbon-coated alumina specimen was 25 times higher than that of the carbon specimens. The higher the modulus, the stiffer the material. The alumina and carbon-coated alumina specimens had the same shape and elastic modulus but differed in surface composition. Thus the two variables, elastic modulus and surface composition, could be tested separately.

Eight implants of each of the 3 types (24 transplants) were surgically inserted into 12 adult female baboons. The implants were inserted bilaterally into molar sockets four months after the second and third molars had been extracted. Each animal hosted two different implant types. Thus, the eight implants of each type were placed into eight



different animals. To normalize any potential variables associated with the surgical technique, four of the eight were placed on the right side and four on the left side. After insertion, the implants remained free standing from approximately three months. Subsequently, each implant served as an abutment tooth for a three-unit gold fixed bridge. The performance of the implants was evaluated clinically and radiographically every three months for 2 years.

After 2 years, 18 implants were judged to be successful by clinical and radiographic criteria and 4 were considered failures. At that time, the animals were sacrificed and the entire mandibles were removed and mounted in a special fixture for mechanical testing. Loads were applied to the implants and to natural teeth for measurements of implant mobility and strain in the mandibular bone adjacent to the implant. The mandibular specimens were then prepared for histologic evaluation by being embedded in plastic and sectioned with a diamond saw. A finite element analysis was conducted to determine the effect of elastic modulus on stress distribution in the bone. Two-dimensional and three-dimensional models were developed to represent the anatomical structure of the baboon mandible containing an implant. Different elastic moduli for the implants and various loading conditions were used to test the models. The results showed that the carbon implants produced significantly higher stresses in the crestal portion of the mandible than the alumina implants.

The 18 implants judged clinically and radiographically successful included all 3 types of specimens. Results of mechanical testing showed that the displacement per unit load (a measure of mobility) of all these successful implants was 5 to 10 times less than that of the adjacent natural teeth. Histologic evaluation showed that implants having no measurable clinical mobility were attached to the mandibles by direct bone-to-implant contact. The strain measurements will be correlated with the results of the finite element analysis. The four implant specimens judged to be failures included two carbon implants, one carbon-coated alumina implant, and one alumina implant.

These results indicate that implants of identical shape and surface composition will perform equally well even though they may have greatly different elastic moduli. They also indicate that implants of the same design and elastic modulus may perform well in spite of different surface compositions. Thus, in this study neither surface composition nor modulus seemed to be significant variables.

#### FUTURE PLANS

When last year's plans to initiate a broad program of contract-supported clinical trials of tooth implants were reconsidered, they were deemed inadvisable at this time. During this fiscal year, staff and their consultants met and decided that before new clinical trials are initiated, the results of the ongoing clinical study of tooth implants being conducted by the Veterans Administration at 5 centers in the Los Angeles area should be studied. Meanwhile, staff will continue to support im-



plant research by the traditional investigator-initiated research grant. To stimulate new implant projects, staff will also issue a Request for Applications in the NIH Guide to Grants and Contracts.

The project for the clinical evaluation of restorative materials at the U.S. Public Health Service Hospital in San Francisco will be renewed. This project has been supported by an Interagency Agreement for the past 5 years. In the next project period, the investigators will continue to evaluate the clinical performance of a wide spectrum of amalgam alloy formulations. One of the objectives of this study is to determine the effect of polishing on the longevity of amalgam restorations and the value of early statistical measures of clinical performance in predicting amalgam longevity. The long-term clinical evaluation of composite resins for the restoration of posterior teeth will also continue. In this study the occlusal wear of posterior composites will be measured. In addition, the time required for placing and finishing posterior composites will be measured and compared with that of amalgams. A project will be initiated to determine whether the current increased incidence of nickel sensitivity can be correlated with the addition of nickel in intraoral alloys. In addition, a project will be initiated to improve ceramic-metal combinations for use in crown and bridge dentistry.

Early in FY 81, the NIDR will initiate a new interagency research agreement with the National Bureau of Standards to standardize patient radiographs so that films taken at different times in the same individual can be compared. The diagnostic system envisioned will use a dental x-ray source detector and an image-processing computer which will enable investigators to observe and record accurately the progress of such common dental pathologies as periodontal disease and caries. This project is being developed through collaboration with an intramural scientist in the Diagnostic Methodology Section of the NIDR.

A new exhibit, "Restorative Dental Materials: Alternatives to Gold", has recently been completed. Designed to inform dental practitioners of current trends in research on restorative materials, it will be shown first at the 1980 American Dental Association annual meeting and subsequently at other professional meetings.

#### SUMMARY

During FY 1980 the Restorative Materials Program Branch awarded 4.2 million for 46 research grants, 2 interagency agreements, 6 research career development awards, 6 training grants and 4 individual postdoctoral fellowships.

The research supported during FY 1980 included a study at the Dental Research Institute at the University of North Carolina in which investigators rated the clinical performance of nine different high copper amalgams and correlated the results with their physical properties. All except one was superior to the conventional control alloy. When the investigators listed these alloys in a ranking order according to their marginal fracture index, the top-rated five had equally good scores.

Researchers at University of North Carolina also evaluated the clinical performance of resin restorations placed in non-retentive cavity preparations. The results indicated that the new, modified cavity preparations with no mechanical retention were preferable to the conventional cavity preparations which require the removal of tooth structure for incisal and gingival retention.

At LSU, investigators are continuing research on the role of creep, corrosion and amalgam microstructure in the marginal fracture of clinical restorations. Data obtained indicate that low creep values are predictive of clinical performance, whereas high creep values may not be.

Investigators at the University of Connecticut have developed a device to analyze and measure the wear of amalgams and composites *in vitro*. The results showed that the low rate of wear of dental amalgam remained constant throughout the entire range of contact stresses associated with human mastication, whereas the wear rate of a composite showed a dramatic dependence on the contact stress. When the stress reached a certain level, the composite showed catastrophic surface failure. This analysis explains their poor resistance to wear in posterior restorations.

Other scientists at the University of Connecticut have developed promising methods to evaluate endodontic filling materials *in vivo*. In these studies the investigators simulated the human endodontic situation by implanting teflon tubes containing endodontic materials into rat subcutaneous tissue and teflon cups containing test materials into guinea pig mandibular bones. The results obtained so far indicate that the new methods can readily determine whether materials are biocompatible with the specific types of tissues they will contact in clinical endodontic therapy.

In implant research at the University of South Carolina, researchers evaluated endosseous implants made of a titanium core coated with a porous high density polyethylene (PHDPE). Thirty-seven of these, implanted in 14 dogs for from 4 to 20 months, were evaluated clinically and histologically. The results suggest that porous coated endosseous dental implants can become firmly ankylosed for a time and function well during this period, but the ankylosing bony ingrowth into the pores may become resorbed later and replaced by soft tissue.

At Tulane University, researchers studied the effect of variations in elastic modulus and in surface composition on the clinical performance of dental implants placed in baboons for 2 years. Alumina and carbon-coated alumina specimens were tested. Eighteen of 22 implants were judged to be successful by clinical and radiographic criteria. Neither surface composition nor modulus seemed to be significant variables in this study.

In preparation for a formal evaluation, Program staff conducted three panel meetings to develop goals, objectives and evaluation criteria for the program. In May 1980, two panel meetings were held: Panel I - Intra-Coronal Restorative and Panel II - Prosthetic and Maxillofacial Materials. The third panel, on Endodontics, Implants and Technology, was held in July of 1980. The documents developed by the panels will contribute to the

initial stages of the program evaluation, and to both immediate and long range planning for the program.

To increase technology transfer to practitioners, staff developed an exhibit entitled "Clinical Evaluation of Dental Restorative Materials", and displayed it at meetings attended by large numbers of clinicians.

Prepared by T. M. Valega and J. A. Reese



## ANNUAL REPORT FY 1980

### SOFT TISSUE STOMATOLOGY AND NUTRITION PROGRAM BRANCH

#### INTRODUCTION

The Soft Tissue Stomatology and Nutrition Program Branch supports research in four major areas: oral soft tissue diseases, nutrition, salivary glands and their secretions, and mineralization. The program's 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 mechanism of mineralization, with special emphasis on the cells and regulatory systems which affect the structure, function and repair of bones and teeth.

#### ADMINISTRATION

During FY 1980, the program awarded 88 research grants at a cost of \$5,405,798, 5 contracts at a level of \$374,332, and one interagency agreement for \$72,803. Table 1 illustrates the distribution of grant and contract funds by subject category.

During this fiscal year, 4 training grants received \$327,799 for the support of 19 postdoctoral trainees. Funds were also provided for 8 individual postdoctoral fellowships (\$147,196), one senior fellowship (\$35,000), 6 career development awards (\$208,682) and 1 career award (\$32,508). Support (\$8,000) was also provided for the Minority High School Students Program.

During FY 1980, Program staff were active in the work of the NIH Nutrition Coordinating Committee and its Subcommittee on Nutrition Education. They presented verbal and written accounts of NIDR's nutrition activities, including specific written reports on current nutrition research projects. Program staff also represented the NIDR on the NIH Digestive Diseases Committee and on the NIH Cystic Fibrosis Coordinating Committee.

During this fiscal year, the two contracts jointly sponsored by NIDR and the National Institute of Allergy and Infectious Diseases to determine the clinical efficacy of the antiviral compound 9-hydroxy-ethoxymethyl guanine were continued. The interagency agreement with the Veterans Administration Hospital in East Orange, New Jersey to improve the detection of early cancerous lesions in the oral cavity was funded for its final year.

Eight panel meetings were conducted during FY 1980 to develop goals and objectives for the four (4) major areas of the Soft Tissue Stomatology and Nutrition Program Branch. The resulting document which contained evaluation criteria for each objective and a list of possible research

areas for each objective was forwarded to the Director's office for use in developing the Institute's long range plan.

TABLE I. DISTRIBUTION OF ACTIVE GRANTS & CONTRACTS FY 80

	Projects Funded FY 1980	FY 1980 Funds Expended
RESEARCH GRANTS		
Nutrition	11	\$ 761,757
Salivary Secretions	18	951,216
Soft Tissue	25	1,622,553
Mineralization	<u>34</u>	<u>2,070,272</u>
TOTALS	88	\$5,405,798
CONTRACTS		
Herpes Infection	2	\$ 101,075*
Oral Cancer	1	72,803
Apthous Stomatitis	2	250,439
Fluoride	<u>1</u>	<u>22,818</u>
TOTALS	6	\$ 447,135
TRAINING		
Senior Fellows	1	\$ 35,000
Individual Fellows	8	147,196
Institutional Training Grants	4	327,799
Minority High School Students Program	<u>1</u>	<u>8,000</u>
TOTALS	14	\$ 517,995
GRAND TOTALS	108	\$6,370,928

\* Program's share of a joint effort with NIAID

## STAFF ACTIVITIES

### Monitoring, Evaluation and Programming Visits

1. Baylor College of Medicine, Houston  
December 13, 1979 (Project site visit)
2. University of Alabama, Birmingham  
December 13, 1979 (Programming visit)
3. University of Minnesota, Minneapolis  
March 13, 1980 (Project site visit)
4. University of California, Los Angeles  
March 19, 1980 (Project site visit)
5. Mayo Clinic, Rochester  
April 28-29, 1980 (Project site visit)
6. University of Washington, Seattle  
May 5-6, 1980 (Project site visit)
7. Detroit School of Dentistry, Detroit  
May 15, 1980 (Programming visit)
8. University of South Florida, Tampa  
June 27, 1980 (Programming visit)
9. University of Colorado, Denver,  
September 15-16, 1980 (Project site visit)
10. University of North Carolina Dental Research Center,  
September 25-26, 1980, Chapel Hill (Project site visit)
11. Duke University Comprehensive Cancer Care Center,  
Chapel Hill, September 26, 1980 (Programming visit)

### Scientific Meetings

1. American Association for Advancement of Science,  
San Francisco  
January 3-8, 1980
2. Conference on Human Herpes Viruses, Atlanta  
March 17-21, 1980
3. AADR Meeting, Los Angeles  
March 20-23, 1980
4. FASEB Meeting, Anaheim, California  
April 13-16, 1980



5. American Society for Microbiology, Miami, May 11-14, 1980
6. Gordon Conference on Food and Nutrition, New London, July 27-August 1, 1980

## RESEARCH HIGHLIGHTS

### Nutrition

University of Connecticut workers have studied the effects of high and low dietary protein levels on tooth and bone mineralization in the golden hamster. The diets contained either 12, 18 or 36% casein as the source of protein and constant concentrations of other trace nutrients. They were made equicaloric with dextrin. Urinary calcium excretions at both the low (12%) and intermediate (18%) protein levels were similar to those of the control, but significantly higher calcium excretion was observed in the high casein (36%) intake group. Growth rates and body weights were similar in all groups after 9 months on the different diets. The high-protein diet caused no change in the weight of the total skeleton, but it significantly reduced the weight and calcium content of the diaphyses of the long bones. This weight loss was due to a lower content of mineral and organic matrix, a finding also observed in osteoporosis. The investigators also found that after 9 months periodontal disease had developed in all the hamsters studied regardless of the level of protein intake.

The same workers also collaborated with investigators at the University of California at Berkeley in a study of dietary protein-related calciuria in human subjects. They had observed earlier that urinary calcium excretion was increased within 30 minutes of intake, peaking at 2 hours after the meal. They concluded that this effect was due to increased calcium reabsorption by the kidney rather than to increased intestinal absorption. They had also observed that urinary zinc levels increased with the high protein diet. In other studies, the Connecticut group demonstrated that calciuria could not be induced by an increase in renal acid excretion nor by the ingestion of only sulfur-containing amino acids equivalent in amount to the amino acids in the high protein diets. They therefore concluded that the mechanism of the calciuria was neither increased acidification of the urine nor sulfur-containing amino acid activity. However, they did observe that the loss of bone calcium during high protein intake was correlated with increased levels of serum insulin. The investigators will now attempt to determine the significance of the elevated levels of serum insulin. If they can confirm that these levels are related to the amount of renal calcium reabsorption, they may be able to control calcium loss by modifying the diet.

The effects of Vitamin A deficiencies on tooth development and bone formation have been studied at the University of Alabama. When compared with rats nursed by normal dams, rats that had been nursed by dams deprived of Vitamin A showed a significant increase in deep dentinal carious lesions in the molars. To determine the biologic basis for this unusual pattern of caries susceptibility, the investigators examined the

structure and function of the salivary glands and the structure and biochemistry of the teeth from the offspring deprived of Vitamin A. The salivary glands of these pups were structurally and functionally normal, but the developing teeth showed a defect in formation associated with an increased amount of glycosaminoglycans in the dentin and predentin; these alterations were most severe after tooth eruption. The investigators concluded that these pathologic alterations in tooth structure interfered with the normal calcification process and thus led to the increased susceptibility to dental caries.

Several investigators have been studying the effect of low dietary zinc levels on oral health. In one case, zinc deficiency in mice fed 5 ppm zinc per day for 12 months was correlated with significant alveolar bone loss. This finding indicated that low zinc diets may aggravate periodontal disease. In another study, the ingestion of a zinc-deficient diet (10 ppm Zn) by young guinea pigs retarded both bone and tooth development. Such low zinc levels apparently interfere with glycosaminoglycan metabolism and this in turn affects membranous bone during early development. In a third zinc deficiency project, investigators fed weanling rats a diet containing 0.4 ppm zinc per day for 4 weeks and studied its effects on mast cell counts in the buccal mucosa at the occlusal level. The mucosa of these zinc-deficient rats contained 17.3 mast cells per unit area of tissue whereas the same amount of tissue in animals fed adequate zinc (40 ppm) contained 5.2 mast cells. This increase in mast cells was observed in the superficial subepithelial zone of the lamina propria. In a related study, the buccal epithelial cells of zinc-deficient rats were found to retain nuclei and cytoplasmic organelles longer than the same cells of rats on diets adequate in zinc. The investigators postulate that inadequate hydrolytic enzyme activity in the zinc-deficient tissue inhibits the removal of these subcellular organelles.

### Soft Tissues

Serious staphylococcal infections sometimes occur after episodes of influenza or local herpetic infections. Recent findings by an investigator at Duke University have shed light on what mechanisms may be involved in these bacterial superinfections. The experiments showed that protein A, found in pathogenic Staphylococcus aureus, enables the organism to bind more strongly to influenza A-infected cells in the presence of anti-influenza serum than nonimmune serum. The isolated staphylococcal protein A was also found to inhibit complement-mediated cytolysis of antibody-coated influenza-infected cells. Thus, protein A can serve as a mechanism for localizing pathogenic staphylococci onto virus-infected cells where the organisms can then initiate a local bacterial infection. Protein A can also aggravate the existing viral disease by inhibiting the antiviral immune defense system. These findings explain how certain bacteria, such as S. aureus, cause bacterial superinfections and how such infections may increase morbidity and even mortality from the viral infection.

Investigators at New York University Medical Center induced orofacial herpes virus infections in mice in order to study the establishment of latency as well as the effect of various antiviral compounds in vivo.



They found that when acyclovir, an antiherpes drug, was applied topically up to 3 hours after the initiation of the experimental herpesvirus infection, ganglionic latency in mice was prevented. In previous studies, this group had shown that neither the anti-herpetic drug adenine arabinoside (ARA-A), nor a herpes virus immune serum prevented the latent infections.

In another study of anti-herpesvirus therapy, a group at the Medical College of Georgia has used iontophoresis to enhance the penetration of drugs into virus-infected tissues. In this method, a weak electrical current of a charge similar to that of the therapeutic drug is applied to the drug and the tissues at the desired topical site. As a result, the drug enters the tissue more effectively than by topical application only and higher tissue concentrations are reached. In a recent clinical study, the investigators applied 0.1% idoxuridine (IDU) by iontophoresis to early herpetic lip lesions. Previously, IDU applied topically to such lesions had not been effective. With iontophoresis, however, the duration of the lesion and the discomfort caused by the herpes virus infection were sharply reduced. In the fourteen human subjects treated with IDU within 1-1/2 days of onset, the average duration of the lesions was reduced from almost 9 days to less than 3.5 days. This preliminary clinical trial will be expanded and repeated in the next project year.

Using dogs, an investigator at the School of Dentistry, Medical College of Georgia, has developed a technique to measure the rate of fluid movement across dentin into the pulp chamber, thus making it possible to study fluid flow and pulp pressure related to dental pain. The investigators have found that when pulpal blood flow is reduced by the action of vasoconstrictors, the concentration of any substance diffusing across dentin rises rapidly in the pulp chamber. This finding relates directly to the dental practice of using local anesthetics or other drugs which contain vasoconstrictors. If the blood flow is reduced or stopped during cavity preparation, bacterial toxins and other toxic materials present in deep carious lesions could flow through the dentin and accumulate in the pulp at high enough concentrations to cause irreversible damage.

### Salivary Secretions

Investigators at the University of Alabama are determining what controls structural development and biochemical differentiation in the major salivary glands. According to the results, postnatal development of the three major salivary glands in rats usually occurs at different times and at different rates. For example, the sublingual gland is already present at birth and contains adult mucous cells, whereas the parotid and submandibular glands do not undergo significant development and differentiation until after birth. Moreover, unlike the sublingual gland, both the parotid and the submandibular glands respond to the beta-adrenergic agonist (stimulant) isoproterenol with structural and biochemical changes. Morphologic studies and biochemical analyses of specimens of the submandibular gland showed that a particular protein normally produced only by very young developing glands could be induced or increased in glands of older animals by isoproterenol. The induced protein was associated with cells which resembled those observed in young developing glands. Thus, the investigators concluded that these



cells were "proacinar" cells, which had been stimulated directly by isoproterenol to a higher level of protein synthesis.

Scientists at the University of Pennsylvania have used the salivary gland of the snail to study the ionic conductances of the intra- and extralumenal membranes of acinar gland cells. Among other important observations, they found that the resting potential of the acinar gland cell depends upon the potassium concentration in both the intra- and extralumenal space. They also observed that the action potentials can be blocked by cadmium ions in either the intra- or extralumenal space. Since cadmium is a potent blocker of calcium ions, this finding suggests that calcium is normally responsible for carrying some of the electric current inward during the generation of the action potentials.

Since it has been difficult to study the relatively small acinar cells with conventional microelectrodes, investigators have recently developed an optical method of monitoring electrical activity in an excited tissue, in a noninvasive fashion, by means of potentiometric dyes such as merocyanine-rhodamine dye NK2367. This technique enables the scientists not only to monitor electrical activity but also electrical pathways in specific tissues, a task which would be almost impossible with conventional microelectrodes. These technical advances will make it possible in the future to link stimulation and secretion in a simple model system and will make it easier to study the specific effects of agonists (stimulants) and antagonists on the neuroglandular junction. The new potentiometric dye methods may also be useful in studying the salivary gland in higher animals.

Investigators at the University of Southern California are studying the kinetics of secretory protein production in long-term cultures of salivary gland cells. They recently found that in a 24-hour period, rat parotid cells synthesize and secrete 2 to 4 times their intracellular content of alpha-amylase. The cells maintained this rate of synthesis for at least ten days and continued to produce amylase after 46 days in culture. The scientists have also demonstrated that parotid cells in culture for at least seven days or more can be subcultured if they are first treated with collagenase and hyaluronidase. Since these subcultured cells retain their ability to synthesize amylase, the findings indicate that the biochemical phenotype of secretory cells is stable in cell culture. The findings of this study extend the usefulness of salivary gland cultures and provide a system for expanded studies of secretory cell development and function.

Investigators at the University of Georgia have found that the fluoride concentrations of gingival fluid from the crevices of canine teeth in dogs show a positive linear correlation with fluoride levels in plasma. When corrections are made for evaporation loss, the fluoride concentration in gingival crevice fluid is almost identical with that in plasma. Since the fluoride levels of tooth surfaces increase toward the gingival margin and fluoride levels of dental plaque near the gingival margin are many times higher than whole saliva, gingival crevice fluoride may contribute to the fluoride content of both enamel and plaque. Thus, gingival crevice fluoride may be directly important in preventing dental caries.

## Mineralization

An investigator at the University of Kansas Medical Center and the Kansas City Missouri Veterans Administration Hospital has conducted biochemical and physiological studies of isolated bone cells in culture. These studies indicate that high concentrations of calcium in the incubation media caused changes similar to those initiated by parathyroid hormones in vivo. The high calcium levels stimulated hyaluronate synthesis and inhibited substrate decarboxylation in osteoblast-like cells to the same extent and following the same course as parathyroid hormone. Moreover, parathyroid hormone did not produce its expected biochemical change in media devoid of calcium. These studies indicate that parathyroid hormone may act on bone cells by altering membrane permeability, thus enabling calcium to enter the cells to mediate hormone action. In related studies, the same investigator has found that isolated cells, kept for 4 days in culture, synthesize only the type I collagen molecule and precursors as did cells in the intact cultured calvaria. As the cells remained longer in culture, however, they formed small amounts of type III collagen. This alteration in phenotype was accompanied by a decreased ability of parathyroid hormone to stimulate cyclic AMP formation. These data suggest that the change in collagen type in older cells is due to an alteration caused by aging in culture.

In a comprehensive freeze fracture study of the cartilage growth plate of long bones, investigators at the University of Kansas Medical Center have obtained data which confirm previous electron microscopic observations that extracellular matrix vesicles are present in mineralizing tissues. Since the freeze fracture method avoids alterations from fixation and dehydration, this method has established that the matrix vesicles are not artifacts of conventional electron microscopic preparation. These findings also demonstrate that the vesicles are free from attachment to cells, and their structural characteristics indicate that they probably bud from the plasma membrane of cells associated with mineralization, in this case, from chondrocytes. In a companion study, these investigators also found that matrix vesicles isolated from tibial and femoral epiphyseal plates of rachitic rats and incubated in a calcifying solution, formed clusters of apatite crystals about the vesicles. These findings do not agree with earlier results with rabbit and calf matrix vesicles which seemed to accumulate only non-crystalline calcium phosphate. The new system needs refinement but it offers a promising method for studying vesicle-induced crystalline apatite formation similar to that observed in the in vivo bone system.

In studies conducted at Texas Tech University on periodontal ligament cells in an orthodontic model system, important cellular responses have been observed on the tension (bone formation) and compression (bone resorption) sides of the tooth. The most significant ultrastructural feature of cells on the tension side of the periodontal ligament was the presence of intracellular vesicles containing collagen microfibrils. These cells, which are found in modest numbers in unstimulated periodontal ligament, are believed to be involved in either collagen secretion or degradation. After orthodontic forces were applied, the number of these cells increased dramatically. Many cells undergoing mitosis were also



observed to contain collagen fibers, a possible indication that a mature functioning cell had reverted to a more embryonic stage as the result of ligament stimulation. The observations of osteoclast activities associated with the compression side of the tooth undergoing orthodontic movement were provocative because there seems to be significantly more inflammation associated with the osteoclastic activity at the apex of the tooth than at the interradicular alveolar crest of bone.

Previous findings reported by investigators at the Medical College of Georgia Dental School showed that a preexisting acidosis made rats sensitive to acute fluoride toxicity whereas a preexisting alkalosis rendered the animals more resistant. These findings were consistent with evidence that the acid-base status of the animal was the principal factor that controls fluoride excretion from the kidneys. Further studies with rats have shown that during the course of a toxic fluoride episode, the imposition of a metabolic alkalosis is an effective means of increasing the resistance to fluoride toxicity. The alkalotic rats survived the lethal fluoride challenge nearly twice as long and tolerated twice as much fluoride. At any given time or dose of fluoride after the intravenous fluoride infusion had begun, plasma fluoride concentrations were lower and mean arterial blood pressures, glomerular filtration rates, fluoride glomerular excretions, clearances and fractional clearances and blood and urine concentration values were higher in the alkalotic animals than in the control animals, which were in normal acid-base balance. Furthermore, at termination, the ratios of tissue plasma fluoride concentration for heart muscle were lower in the alkalotic animals.

A companion study, reported by the same investigator, examined the effect of mild chronic acid-base disturbances on the fluoride concentrations in the plasma and developing enamel of rats on high and low fluoride drinking water. An acid or base disturbance was induced for 30 days, long enough for nearly the entire length of the incisor to be renewed. These disturbances did not affect the percent ash or the percent phosphorus in either the developing maxillary or mandibular incisors. In rats on low fluoride water the average fluoride levels in the plasma and enamel of the acidotic group were significantly higher than in the alkalotic group. Of the groups drinking water with 50 parts per million fluoride, the total fluoride intake was higher in the alkalotic group and lower in the acidotic group. Nevertheless, despite the lower intake, fluoride concentrations in the plasma and enamel of the acidotic group were higher than in the alkalotic group. This study suggests that fluoride levels in plasma and developing enamel can be either independent of or inversely related to fluoride intake, depending on the acid-base status.

#### FUTURE PLANS

New findings which show how viruses can increase a cell's susceptibility to certain bacterial infections and how the application of antiviral drugs to local viral infections can be improved provide opportunities for additional progress. The Program will therefore continue to expand research on the etiology, prevention and treatment of oral viral diseases



through studies designed to develop suitable vaccines or other antiviral agents and to devise the most effective means of administering such agents. Research on nutrition, salivary secretions and mineral metabolism will be maintained. The role of specific nutrients in the development, function and maintenance of oral and para-oral structures during all stages of life is of particular interest. Saliva research efforts will stress the need to identify and characterize the constituents of saliva and to define their functions in maintaining oral health. Basic mineralization studies will continue to seek an understanding of normal and abnormal mineralization mechanisms for the purpose of preventing or controlling diseases involving calcified tissues. In order to increase fluoride research, staff will publish early in FY 1981 a second announcement that animals from a colony of mice maintained on high and low fluoride diets are available for study. The program maintains the colony for investigators to use in studying the long term effects of different levels of fluoride consumption on important biological processes. The program will continue to emphasize the training of scientists for research careers in the areas of nutrition, salivary secretions and oral soft tissue diseases.

#### SUMMARY

During FY 1980 the Soft Tissue Stomatology and Nutrition Program Branch expended over \$5 million for 88 research grants and almost \$0.5 million for 6 contracts and one interagency agreement. Funds were also provided for 4 institutional training grants supporting 19 trainees (\$327,799), 6 individual fellows (\$282,196), 8 career development awards (\$208,682) and one career award (\$32,508).

#### Nutrition

When hamsters were fed a high level of dietary protein (36% casein) they excreted large amounts of calcium which reduced the weight and calcium content of the long bone diaphyses; similar findings are seen in osteoporosis. Rats deprived of Vitamin A when nursing developed deep dentinal carious lesions of the molars later in life. These lesions were located in structural defects presumably caused by interference with the normal calcification process as a result of the deficiency. In several small animal experiments, low dietary zinc caused alveolar bone loss, retarded tooth and bone development, increased numbers of mast cells in the buccal mucosa, and prolonged retention of formed elements by buccal epithelial cells.

#### Soft Tissue

The chance of developing a secondary bacterial infection is enhanced by the ability of pathogenic staphylococci to adhere to virus infected cells. The antiviral drug acyclovir prevented ganglionic latency when applied topically to a herpesvirus infection in a mouse model up to three hours after infection. The application of iontophoresis (weak electrical current) to the antiviral drug Idoxuridine (IDU) aided penetration of the drug into the skin of patients with herpes labialis infections so that the lesions caused less discomfort and healed more rapidly than in controls. The flow of soluble materials into and out of the pulp chamber

of the dog tooth was found to be affected by certain vasoconstrictors used in anesthetic solutions. Thus during operative procedures on deep carious lesions bacterial toxins could build up to damaging levels in the pulp tissue.

### Salivary Secretions

The postnatal development of the three major salivary glands in rats occurs at different times and at different rates. A specific protein normally produced only by developing salivary glands can be induced or increased in glands of older animals by the drug isoproterenol; thus, the cells producing the protein may be "proacinar" cells which are stimulated directly by isoproterenol to a higher level of protein synthesis. Investigators recently developed a new method of studying the neuroglandular junction. In this method electrical activity in a stimulated tissue is monitored optically in a noninvasive fashion using potentiometric dyes rather than microelectrodes. The biochemical phenotype of salivary gland cells has been found to be stable in cell culture systems for studying secretory cell development and function. Gingival crevice fluid from dog canine teeth was found to contain the same fluoride concentrations as plasma, a finding which may explain why high fluoride levels are present in enamel toward the gingival margin and in plaque. Thus, gingival crevice fluoride may be important in preventing dental caries.

### Mineralization

Parathyroid hormone may act on bone cells by altering membrane permeability and allowing calcium to enter the cell as a mediator of hormone action. A comprehensive freeze fracture study of the cartilage growth plate of long bones showed that the extracellular matrix vesicles observed in mineralizing tissues by conventional electron microscopy are not artifacts. When incubated in calcifying solution, these matrix vesicles isolated from epiphyseal plates of rachitic rats formed clusters of apatite crystals similar to those observed in in vivo systems. Bone forming cells on the tension side of the periodontal ligament of teeth undergoing orthodontic movement contained intracellular vesicles loaded with collagen microfibrils. Many of these cells were undergoing mitosis suggesting that a mature functioning cell may have reverted to a more embryonic stage as a result of ligament stimulation. The imposition of a metabolic alkalosis in rats was an effective means of increasing their resistance to fluoride toxicity. In rats on drinking water with different fluoride levels the fluoride content of plasma and of developing enamel was independent of the fluoride intake but dependent upon the acid-base status of the animal.

Prepared by P. D. Frazier and D. A. Wolff





# ANNUAL REPORT FY 1980

## PAIN CONTROL AND BEHAVIORAL STUDIES PROGRAM BRANCH

### INTRODUCTION

The ultimate mission of the Pain Control and Behavioral Studies Program is to ameliorate the suffering caused by dental and oral facial pain and to increase understanding of the behavioral factors involved in oral health and disease. To achieve these purposes, the Program supports research and research training on dental and oral-facial pain problems and on sociopsychological factors that affect oral health. Support is provided for specific studies on: behavioral factors influencing the prevention of dental disease; fear and anxiety associated with dental treatment; the psychosocial concomitants of malocclusion and facial disfigurement; dental anesthesia and sedation; pain and discomfort associated with the temporomandibular joint; and tooth grinding (bruxism). In addition, the program supports studies in other areas of oral motor and sensory function such as gustation, olfaction, mastication, and deglutition.

### ADMINISTRATIVE REPORT

During FY 1980, the Program awarded a total of \$2.54 million for 46 research and training projects. Funds amounting to \$2.18 million were provided for 27 regular research grants, one program project, 7 new investigator awards and one conference grant. Research training funds (\$363,000) were provided for 7 individual fellowships, one senior fellowship and two institutional training grants. The following table shows the FY 1980 distribution of research grant funds by major subject area.

Research Area	No. of Grants	FY 80 Funds (\$000's)	Percent of Total
Basic (Neurophysiological, etc.)	18	\$ 924	42
Behavioral	12	723	33
Clinical	5	330	15
Combined	1	203	10
<hr/>			
TOTALS:	36	\$2,180	100

### STAFF ACTIVITIES

Working with Dr. Donald Kruper and two panels of nationally recognized authorities on pain and behavioral science, staff completed the preparation of formal statements of the program's research objectives. They defined specific goals and evaluation criteria for the pain, behavioral, and sensory-motor components of the program area. These statements will be useful both for immediate program development efforts as well as for the formal program evaluation to be completed later.

During FY 1980, Program staff made visits to institutions, monitored grants, communicated with researchers and professional organizations, and participated in scientific meetings. Through these professional activities they were able to maintain close communication with scientists working both in orofacial pain research and social-behavioral research relevant to oral health.

#### Participation in Scientific Meetings

American Sociological Association	August 1980
American Psychological Association	September 1980
American Association of Dental Schools	March 1980
American Association for Dental Research	March 1980
Society of Behavioral Medicine	December 1980
Association for Advancement of Behavioral Therapy*	December 1979
Second National Conference on Behavioral Dentistry*	October 1979
Academy of Behavioral Medicine Research*	May 1980
National Conference on Oral Health Behaviors*	April 1980

#### Monitoring, Evaluation, and Site Visits

University of Detroit School of Dentistry	May 1980
Maine State Health Department	May 1980
University of Illinois Dental School	April 1980
New Jersey Dental School	April 1980

#### Invited Lectures

Walter Reed Medical Center	September 1980
Howard University Faculty Retreat	July 1980
Uniformed Services University for the Health Sciences, Bethesda	March 1980, May 1980

#### Staff Development

EEO Training for Supervisors	September 1980
Capitol Hill Workshop	September 1980
Federally Employed Women Workshop	July 1980
DHHS Project Officers Training Course	June 1980
Contract Compliance Course	February 1980

#### Publications

Bryant, P., Gale, E., and Rugh, J., Oral Motor Behavior: Impact on Oral Conditions and Dental Treatment, DHHS/PHS/NIH Publication No. 79-1845, August 1979.

Bryant, Patricia S., "Behavioral Dentistry: Concept and Challenge", in Clinical Research in Behavioral Dentistry, Proceedings of the Second National Conference on Behavioral Dentistry, W.Va. University, Morgantown, W.Va., Oct. 8-9, 1979, (ed. B. Ingersoll and W. McCutcheon).

\* Paper presented.

## RESEARCH HIGHLIGHTS

### Treatment of Dental Post-operative Pain

Clinical investigators at the University of California at San Francisco are studying the factors that affect postoperative dental pain, especially endorphins, natural opiate-like substances found in the brain. Their most important finding so far is that pain relief obtained by placebo administration is mediated by endorphin release which was more effective in alleviating severe rather than mild pain. These observations challenge the popular notion that placebos affect imaginary pain more than real pain.

Another major finding relates to the palliative effects of morphine. By focusing on the results achieved in individual patients instead of analyzing the combined data of groups of patients, the San Francisco investigators have demonstrated that patients suffering from postoperative dental pain respond to morphine in an all-or-none fashion. Once a certain threshold of pain relief is achieved in each individual, no further increment of morphine will effect greater relief. This study, therefore, provides a scientific basis for titrating the dosage of morphine until the maximal relief for each patient is reached.

### Sensory Innervation of Rat Molars

Investigators at the University of Washington have studied the sensory innervation of rat molars at various stages of development. They found that dentin innervation has already begun before tooth eruption and that subsequent pulp and dentin innervation gradually increases over many months and parallels increases in molar sensitivity. Each section of coronal dentin becomes innervated during the production of the last 100 to 150 um of dentin and retains this innervation for as long as that section of dentin is viable. When coronal attrition destroys tubules, the innervation of that section is lost, and new, more apical sections of dentin become innervated. Thus nerve locations gradually shift from the tip of the pulp horn in newly erupted teeth to the apical crown and upper root areas in the molars of older rats. These studies provide detailed evidence that dentin contains sensory nerve endings which are an important part of the sensory apparatus in developing and mature teeth. These results are consistent with the theory that sensory nerves are the principal sensory transmitters in teeth, not secondary to odontoblast receptor activity. They should eventually lead to improved testing of tooth vitality and to better knowledge of sensitivity changes as teeth mature and age.

### Surgical Techniques for Oral-facial Pain Relief

Investigators at Harbor General Hospital in Los Angeles have explored surgical approaches to relieve the intractable oral and facial pain resulting from cancer of the head and neck. Working on monkeys, the investigators have found that, contrary to their expectations, trigeminal tractotomy (severing of the trigeminal nerve tract) did not relieve pain



from teeth. Even the facial analgesia effected by trigeminal tractotomy was incomplete and could be reversed by such pharmacological agents as strychnine or L-Dopa. This finding is contrary to the general assumption that the trigeminal nucleus caudalis, part of the trigeminal brain stem complex, is the exclusive brain stem relay station for impulses transmitting oral, dental and facial pain. The similarity between the trigeminal apparatus in both human and nonhuman primates suggests that these findings are also relevant to dental and facial pain in human patients.

These results, though negative, will help investigators in the future to design surgical procedures that will be effective against intractable pain. For example, they have clearly shown that trigeminal tractotomy, even when combined with extensive upper cervical rhizotomy (interrupting the roots of spinal nerves within the spinal canal), cannot effectively relieve oral-facial pain even though these procedures sometimes relieve pain in the neck and peripheral parts of the face. As a result of these findings, investigators are now using behavioral and electrophysiological techniques to test new surgical procedures for pain relief.

#### Dental Fear and Anxiety

Investigators at the SUNY at Buffalo are comparing practical procedures for allaying patient anxiety during routine dental treatment. They had already shown that tape-recorded instructions designed to teach subjects how to relax each part of the body reduced patient anxiety and stress. More recently, the scientists have compared the relative effectiveness of musical programming with that of the relaxation instructions. The subjects were college students who received similar treatment (Class II amalgam restorations) at two separate sittings. The first visit served as a baseline control for all patients. During the restoration procedure, skin resistance, an indicator of anxiety, was recorded continuously, and the subjects completed several rating scales to record their reaction.

On the second visit, the patients were randomly assigned to one of four groups: 1) routine dental treatment (control); 2) relaxation instructions before treatment; 3) musical programs with a choice of one of eight different programs; and 4) musical programs with a patient-controlled volume dial on the chair arm during treatment.

Preference ratings indicated that all three experimental groups preferred the second visit, but that only the relaxation instructions consistently lowered the indices of anxiety or stress below the baseline levels established during the first visit. Thus, this study confirmed previous findings that brief relaxation instructions provide an effective method of actually reducing patient anxiety. Musical programming, on the other hand, apparently produced a distracting placebo effect, but did not actually reduce anxiety.

Investigators at Kent State University have also studied behavioral interventions to reduce patient anxiety during dental treatment. In one project adult patients in a private practice setting were used to evaluate the effectiveness of electromyographic (EMG) biofeedback training and of relaxation instructions. In the biofeedback training, surface electrodes were placed on the forearm extensor muscle and a continuous read-out of the activity of this muscle was shown on an electronic monitor. While observing the read-out, each subject tried to learn how to exert voluntary control over the muscle. For the experiment, each patient first filled out a report to rate his individual level of dental anxiety and expected pain, and was then assigned to one of four different groups. The first group received EMG biofeedback training for eight minutes before treatment and continuous biofeedback during dental treatment. The second group also received biofeedback training for eight minutes before treatment but no feedback during treatment. The third group received an eight-minute program of verbal instructions in progressive muscle relaxation before treatment, and the fourth group, a no-training control group, merely adapted to the dental chair for eight minutes before treatment. The EMG muscle activity levels for all groups were monitored and recorded continuously during the dental treatment, and ratings of anxiety and "expected" and "experienced" pain were obtained.

Compared with the no-training control group, both EMG feedback and relaxation groups showed significantly lower EMG levels in the forearm extensor during dental treatment. High-anxiety subjects exposed to continuous EMG feedback showed the greatest reduction in stress and in experienced vs. expected pain. Since these beneficial effects occurred after only brief training in procedures which can be administered by dental auxiliaries, the authors concluded that these methods are potentially useful in routine dental practice.

#### Children's Perceptions of Dento-facial Appearance

Since improved appearance is often a major objective of orthodontic treatment, investigators at the University of Michigan studied approximately 500 Caucasian school children 10 to 13 years of age to determine what factors influence how individuals perceive or judge facial appearance. Each subject first completed a questionnaire in which he described his own teeth, rated his level of self-satisfaction with his teeth, and rated the importance of dental appearance in overall facial appearance. Each child then underwent a comprehensive examination in which the investigators measured teeth position, dental arch relationships, and recorded deformations of the teeth, including cracks and fractures, discolorations, and other variables that might affect esthetic judgments. These physical variables were then compared with the questionnaire responses to determine which dental variables could be correlated with the questionnaire answers.

A broad range of dental conditions were seen as acceptable by these children. In general, they made objective evaluations of the "straightness" of their own teeth, with only 20% judging their own teeth unattractive. When the dental variables were correlated with dental self-



satisfaction ratings, definite sex differences were revealed. Over-jet (the degree to which the upper teeth extend outward beyond the lower teeth) and its influence on facial features were the most important to the female subjects whereas tooth crowding was the major cause of dental dissatisfaction for the male subjects. These responses may result from the fact that the girls had more definite standards for acceptable facial form than the boys and thus that deviations in dental form which negatively affect facial outline are more important to girls than to boys.

The investigators observed with special interest that the girls who were satisfied with their teeth practiced better oral hygiene than those who were not. Scores on gingival health for girls with positive evaluations of their own teeth were higher than those of girls with negative evaluations. Thus, in girls, positive perceptions of one's own dental esthetics may prove a powerful incentive in preserving this self-perceived positive attribute. Male subjects with positive evaluations did not score higher on gingival health than those with negative evaluations.

#### FUTURE PLANS

In FY1981 program development will proceed in accord with the expanded objectives established by staff and consultants at their meetings during FY1980. Efforts will be made to stimulate more basic research on dental anesthesia and sedation, to develop multidisciplinary studies on the etiology, diagnosis and treatment of oral-facial pain syndromes; and to initiate coordinated basic and clinical studies on endorphin neuropharmacology. In the behavior area, support will be maintained for research in dental fear and anxiety, a major program initiative. In addition, efforts will be made to expand sociobehavioral research in promising areas judged to be appropriate for rapid development. These areas include research on how new preventive technologies can be made more acceptable to individuals and to the community, epidemiologic studies to correlate sociobehavioral factors with the incidence and prevalence of oral diseases, prospective studies on the effects of altered dento-facial appearance on behavior, and investigations of selected psychophysiologic responses with potential etiologic or therapeutic significance.

Post-doctoral research training in sociobehavioral dental research will be an important aspect of these program development efforts. The FY1981 goal is to initiate two institutional training programs to develop outstanding investigators in this area, and to increase the number of individual post-doctoral fellows.

To accelerate the implementation of these program plans during FY 1981, staff will prepare and publish two major documents which are expected to stimulate interest in behavioral aspects of oral health. The first of these publications, to appear in a special issue of the Journal of Behavioral Medicine in early 1981, will consist of major review papers



from the National Conference on Oral Health Behaviors sponsored by the program area in April, 1980. The Journal of Dental Research will also publish a summary of these papers together with the conference recommendations. The second publication will contain abstracts of all social and behavioral research the NIDR has supported. It will be distributed to dental researchers and to sociobehavioral investigators in the wider health research community. In the future this compendium will be updated on a regular basis.

Staff will also initiate state-of-the-art reviews on the major research objectives identified in the preparation of the program area's formal evaluation plan referred to above. It is hoped that these efforts, along with presentations at professional meetings and program announcements, will help to achieve the goal of soundly-based expansion in research on pain and sociobehavioral aspects of oral health.

#### SUMMARY

During FY 1980, the program area made 36 research grant awards totaling \$2.18 million, and awarded funds of \$363,000 to support two institutional training programs, 7 individual fellowships, and one senior fellowship. Highlights of the research are summarized below.

#### Treatment of Post-Operative Dental Pain

Following the demonstration that pain relief obtained by placebo administration is mediated by endorphin release, it was shown that this placebo-induced pain relief was more effective in patients whose pain was rated as severe than in patients whose pain was rated as mild. These observations challenge the popular notion that placebos affect imaginary or mild pain more than real or severe pain. Investigators also showed that patients suffering from post-operative dental pain respond to morphine in an all-or-none fashion. Therefore, the dose for each individual should be titrated so that maximal relief is obtained with the minimum amount of morphine.

#### Surgical Techniques for Oral-Facial Pain Relief

Investigations to develop surgical procedures to relieve intractable oral-facial pain, particularly in patients with head and neck cancer, led to the unexpected finding that severing the trigeminal nerve tract in monkeys did not produce dental analgesia. As a result, the authors concluded that the trigeminal nucleus caudalis is not the exclusive brain stem relay station for the transmission of oral, facial and dental pain impulses. In view of the similarity between the monkey and human trigeminal apparatus, these results appear to be relevant to man.

#### Sensory Innervation of Rat Molars

Studies on the innervation of rat molars at different stages of development provided strong evidence that the dentin contains sensory nerve

endings which function in developing and mature teeth. The results indicate that sensory nerves, and not odontoblastic receptors, serve as the primary sensory transmitters in teeth.

#### Dental Fear and Anxiety

Behavioral techniques for reducing anxiety during routine dental treatment were tested. Presentation of tape-recorded relaxation instructions prior to treatment reduced physiological indicators of anxiety, as well as the patient's self-ratings of anxiety. Musical programming during dental treatment, even when controlled by the patient, had no effect on these same anxiety indices. Electromyographic (EMG) biofeedback training, another anxiety reduction procedure, was also tested. In this training, subjects learn to control muscle tension by observing a visual display of their muscle activity. Eight minutes of EMG biofeedback training prior to dental treatment enabled patients to reduce muscle tension and pain during dental treatment. Biofeedback training procedures were more effective in reducing anxiety and pain than the relaxation instructions.

#### Children's Perception of Dento-facial Appearance

Investigators studied 500 school children 10-13 years of age to determine how they judged facial appearance and whether they were satisfied with the appearance of their own teeth. The children found a broad range of dental conditions acceptable and showed objectivity in judging their own teeth. Only 20% found their own teeth unattractive. Over-jet (the degree to which the upper teeth extend out beyond the lower teeth) was the most important reason for dissatisfaction among girls, whereas tooth crowding was the major cause of dental dissatisfaction in males. Girls who were satisfied with their teeth practiced better oral hygiene than girls who were not. Boys did not show this difference.

Prepared by A. Ganz and P. Bryant











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